Probabilistic models for Risk assessment of viral infection associated with contaminated food consumption : public health impact of norovirus or hepatitis A virus contamination in oysters

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Modélisations probabilistes du risque d'infection virale associé à la consommation d'aliments contaminés: impact pour la santé publique de la contamination d'huîtres par Norovirus ou par le virus de l'Hépatite A

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En guise de préambule ou de conclusion ....

"Alors, ouvrant le Livre, tu promenais un doigt usé entre les prophéties, puis le regard fixé au large, tu attendais l'instant du départ, le lever du grand vent qui te descellerait d'un coup, comme un typhon, divisant les nuées devant l'attente de tes yeux". Saint John Perse, 1904. Images à Crusoé.

"But the jungle is large and the cub is small. Let him think and be still." The Jungle Books. Rudyard Kipling.

"Qui serait assez insensé pour mourir sans avoir fait au moins le tour de sa prison ? (..) Je sais que je ne sais pas ce que je ne sais pas, j'envie ceux qui sauront davantage, mais je sais qu'ils auront tout comme moi à mesurer, peser, déduire et se méfier des déductions produites, faire dans le faux la part du vrai et tenir compte dans le vrai de l'éternelle admixtion du faux". Marguerite Yourcenar. L'oeuvre au noir.

"Mais au creux de ce destin mineur, j'avais connu la fièvre de m'interroger, la joie d'apprendre, la fécilicité de comprendre sans rien prendre, et sans rien altérer". Patrick Chamoiseau. Les neufs consciences du Malfini.

« Laisse ce chien, cela n'est pas cruel (...) Accepter cette fortune cela m'est impossible si je dois pour cela abandonner ce chien (..) » Le Mahabharata.

“In consequence of what I said, the handle of the pump was removed” John Snow. 1855. Londres.

Résumé

Norovirus (NoV) et le virus de l'hépatite A (VHA) ont le cout sanitaire des virus par voie alimentaire en termes de morbidité et de mortalité le plus élevé. Ces virus, dont la voie de transmission est orofécale, se transmettent aussi par une voie interhumaine. L'ingestion de coquillages contaminés constitue une source de cas groupés fréquente pour VHA et NoV en France. L'objectif de cette thèse est d'évaluer le risque lié à la consommation de coquillages contaminés par VHA ou NoV et d'évaluer l'efficacité de quelques mesures de gestion pour une population côtière, par une modélisation probabiliste. Une appréciation quantitative des risques (AQR) sur VHA a montré l'intérêt de mesures préventives visant à diminuer les apports contaminés et l'intérêt d'une surveillance virologique. Pour NoV, les paramètres de dose-réponse ont été évalués sur des données de TIAC documentées, par inférence bayesienne. Les résultats montrent une infectiosité très forte de GI et GII chez les individus secréteurs. Ces résultats ont été pris en compte dans l'élaboration d'un modèle dynamique stochastique compartimental sur NoV. L'impact d'une fermeture de zone dans une situation d'épidémie hivernale, prenant en compte une transmission interhumaine, à GI, GII ou les deux ensembles ont été évalués. Les premiers résultats montrent que la fermeture de zone peut avoir un impact sur le nombre de cas total, et expliquer un plus grand nombre de cas d'origine alimentaire pour GI que pour GII. Ce travail montre l'intérêt d'une approche AQR pour évaluer l'efficacité de mesures de gestion sur les cas d'origine alimentaire, mais aussi d'un modèle dynamique prenant en compte la transmission par voie alimentaire.

Abstract

Norovirus (NoV) and hepatitis A virus (HAV) are most important foodborne viruses considering morbidity and mortality disease burden. The main transmission pathways of these viruses are orofecal, involving inter-human transmission. Contaminated shellfish are identified as an important source of human viral outbreaks in France. The study of a coastal situation allows exploring complete feco –oral pathway. The aim work is to estimate the risk of infection for a coastal population, by consumption of contaminated oysters and to evaluate the effect of some mitigation strategies. The benefit of reducing the contamination of sea waters and of viral monitoring was show by Quantitative Risk Assessment (QRA). The dose-response parameters were estimated for GII and GI, based on shellfish outbreaks data, by bayesian inference. Nov GI and GII for secretors have been shown to be highly infectious. QRA approach was included into a stochastic compartmental dynamic model for NoV. The aim of this model was to estimate the global health impact of closure of shellfish area, with two ways of transmission (by food and inter-human), whenever a winter epidemic occurs with GI or GII or both. First results demonstrated the potential interest of closure of the area, and the main importance of food transmission for GI cases. This work show the benefit of QRA for estimating foodborne cases but also dynamic modeling involving foodborne pathway for humans.

Résumé substantiel en Français

<u>Chapitre I</u>: Introduction générale

Le coût sanitaire des infections virales d'origine alimentaire est, à quelques exceptions près, difficile à estimer avec précision. La liste des agents concernés est variable selon la prise en considération de virus rarement mis en cause par voie alimentaire ou potentiellement émergents (WHO-EFSA). Les estimations globales par famille de virus mettent en tête les norovirus, par voie alimentaire, qui seraient responsable de 119 cas/100.000 habitants par an en France, associés à une consultation médicale. Le virus de l'Hépatite A arrive en seconde position, avec un nombre de cas bien moindre (entre 0.7 et 0.08/100.000 habitants) mais avec des symptômes, en général plus sévères. Certains cas peuvent évoluer jusqu'à une hépatite fulminante, avec un taux de létalité pour les cas symptomatiques de l'ordre de 4 à 5 pour 1000 cas. Les estimations annuelles d'incidence pour les virus diffèrent notamment selon la valeur de la part attribuable à l'alimentation prise en compte dans l'estimation globale du nombre de cas. Par exemple, la part alimentaire des infections à rotavirus est suivant les pays, estimée comme pouvant être négligeable ou pas.

L'estimation du nombre de cas, quelque soit le mode de transmission, lié à chaque virus, pour la population générale, repose sur un certain nombre d'étapes. Pour le virus de l'Hépatite A, la déclaration obligatoire permet d'estimer le nombre de cas dans la population générale à partir des cas confirmés par le laboratoire. Pour Norovirus, des enquêtes ponctuelles permettent de réestimer les cas liés à Norovirus à partir des gastroentérites recensées. L'estimation du nombre de cas attribuable à la voie alimentaire repose sur des études ponctuelles de TIAC, d'études épidémiologiques ponctuelles (enquêtes rétrospectives cas-témoin), d'élicitation de dire d'expert ou s'effectue par une modélisation de l'exposition et du risque. Le mode de transmission des deux principaux virus d'intérêt sanitaire (Norovirus et VHA) n'est pas seulement alimentaire. Il n'existe pas, pour ces deux virus, de réservoir animal connu pour l'infection humaine (non zoonotique). La transmission interhumaine est plus importante que la transmission par voie alimentaire, ce qui différencie ces virus des autres agents microbiens (bactéries, parasites), pour lesquels la voie alimentaire est prépondérante (par exemple Salmonella, Toxoplasma, Listeria). Cependant les deux formes de transmission (alimentaire et interhumaine) peuvent coexister et se succéder au cours des épidémies, rendant l'estimation précise de leur contribution respective au nombre de cas délicate à mener au cours d'enquêtes épidémiologiques.

La voie féco-orale est la principale voie de transmission et peut intervenir directement entre personnes, par contact ou par aérosols de vomissures (pour norovirus), indirectement par des supports physiques contaminés, ou par des aliments contaminés directement ou indirectement par des selles humaines. Cette transmission orofécale explique le type d'aliment contaminé, que ce soit par des eaux d'irrigation contaminées (les végétaux), par un rejet d'eau usé insuffisamment traitées en zone côtière (les coquillages), ou par une contamination de l'aliment par des mains souillées (les produits élaborés-complexes). L'estimation du nombre de cas attribuable par type d'aliment repose le plus souvent, sur des études rétrospectives des causes de TIAC et d'elicitation de dires d'expert.

Ces études mettent en avant, pour les norovirus, les aliments composés, les coquillages et les produits végétaux pour l'ensemble des cas en population générale (données USA). Pour l'Hépatite A, vis à vis des épidémies observées, les coquillages sont la source alimentaire la plus souvent impliquée (données françaises). Les coquillages sont fréquemment associés à des TIAC à Norovirus observées en France et font l'objet de recommandations spécifiques récentes au niveau européen. La région côtière, avec la consommation de coquillages permet d'explorer la voie orofécale de façon complète, la contamination des eaux côtières venant, le plus souvent, d'un défaut de traitement des eaux usées de la population humaine côtière.

L'objectif de cette thèse est donc d'évaluer le risque attribuable, pour le virus de l'Hépatite A et de Norovirus, à la consommation d'huîtres contaminées, pour une population côtière, proche d'une zone de production, et d'évaluer l'efficacité potentielle de quelques mesures de gestion visant à diminuer ce risque.

Le virus de l'hépatite A et norovirus se distinguent par des caractéristiques biologiques distinctes. La durée d'incubation est plus longue pour VHA (de l'ordre de 4 semaines), la sévérité plus forte et plus longue pour les cas symptomatiques à VHA (durée des symptômes en mois). La sévérite des symptomes pour VHA est clairement liée à l'âge, avec une plus grande proportion de cas asymptomatiques chez l'enfant. Le nombre de cas a considérablement diminué au cours des vingt dernières années, et est beaucoup plus faible que le nombre de cas à Norovirus. L'immunité est longue et durerait toute la vie, pour un individu immunocompétent. Du fait de la diminution du nombre de cas et de la séroprévalence, la proportion d'individus de plus de quarante ans sensible à VHA augmente, avec un risque de symptômes graves en cas d'infection. L'étude de VHA est donc justifié par un risque de réémergence ou de TIAC impliquant un grand nombre d'individus sensibles. Les caractéristiques biologiques de norovirus sont clairement différentes, avec une durée d'incubation courte (24-48h), une durée courte des symptômes (48 heures) et une faible sévérité (sauf pour des populations fragilisées). L'âge ne semble pas être un facteur discriminant d'acquisition de l'infection. L'immunité acquise après l'infection est de courte durée (en mois) et peu connue. Une sensibilité individuelle innée liée à des caractéristiques génétiques est décrite, liée à l'existence d'un statut sécréteur et, avec de plus grandes variations vis à vis des souches de norovirus, au groupe sanguin. La grande diversité des souches impliquées dans les infections humaines, est une des caractéristiques des norovirus, et permettrait d'expliquer la faible immunité acquise contre la réinfection et les épidémies récurrentes hivernales. Le cluster (qui comprend plusieurs souches) GII.4 est fréquemment impliqué ces dernières années dans les épidémies (forte transmission interhumaine) tandis que la transmission par voie alimentaire implique GII.4, mais aussi d'autres clusters comme GII.3 ou le genogroupe GI.

Le nombre de cas attribuable à norovirus en France est de l'ordre de 1 à quelques % de la population française, dont une partie, entre 40 et 14 % (suivant les estimations et les pays) serait d'origine alimentaire. Pour HAV, le risque attribuable à une voie alimentaire serait de 5 à 7%.

Les différences entre les caractéristiques biologiques et épidémiologiques de ces virus, qui ont en commun une transmission orofécale d'origine humaine, et un certain nombre de TIAC liées à des

coquillages contaminés par ces virus justifie l'intérêt d'essayer de les maintenir, en tant que modèle contrasté des virus concerné par la voie alimentaire, dans une même étude.

La difficulté d'estimation des contributions relatives entre la voie alimentaire et les autres voies de transmission ne permet pas non plus d'estimer ni de comprendre facilement le gain relatif en terme sanitaire d'une mesure de gestion visant à réduire la contamination des aliments pour une situation donnée. **La méthode choisie, afin de répondre à cet objectif, est celle d'une modélisation probabiliste, permettant de prendre en compte la variabilité inhérente à tout système biologique.** L'objectif de cette modélisation est aussi de mieux comprendre les mécanismes mis en œuvre. L'appréciation quantitative des risques alimentaires est reconnue utile pour l'estimation et la mesure de l'efficacité de la gestion du risque alimentaire par la FAO et l'OMS. Cependant cette approche est peu utilisée pour le risque viral, probablement du fait de l'absence de mesure de l'infectiosité (pas de culture cellulaire possible) et de dose-réponse fiable pour les deux principaux virus d'intérêt, VHA et norovirus (Genogroup I sur des données de volontaires). Deux publications récentes apportent des éléments pour une dose-réponse sur VHA et sur norovirus (GI sur une étude sur volontaires). L'appréciation quantitative des risques et ses concepts seront donc utilisés pour estimer le nombre de cas d'origine alimentaire. La dose-réponse de norovirus, a été évaluée pour GI et GII, sur des données de TIAC à coquillages, ce qui correspond au cadre de notre étude. Enfin l'approche AQR sera intégrée dans un modèle dynamique afin d'étudier les relations possibles entre la transmission interhumaine et la transmission alimentaire.

<u>Chapitre II</u>: Apport de l'appréciation quantitative des risques vis à vis du risque lié à la contamination des coquillages

La première situation étudiée répond à un contexte particulier. Au regard de deux épidémies d'Hépatite A, survenues dans une même population côtière (baie de Paimpol), à quelques années de distance (1999 et 2007) et associées, dans les deux cas, à une contamination des huîtres, la Direction Générale de la Santé et la Direction Générale de l'agriculture ont sollicité l'avis de l'ANSES. Les questions portaient sur l'efficacité du système de surveillance des huitres et/ou sur une origine de contamination particulièrement défavorable sur ce site. Afin d'apporter une contribution à l'expertise, une approche par Appréciation Quantitative des Risques a été menée afin d'évaluer l'efficacité relative de différents scenarios de surveillance et de gestion, ce travail s'insérant aussi dans ce travail de thèse. L'appréciation quantitative des risques repose sur des données de contamination quantitatives dans les aliments, sur des données de consommation, et sur des données de dose-réponse. Deux situations de contamination théoriques ont été explorées, à savoir une situation de contamination rare, de courte durée, mais forte, correspondant à un scénario 1 et une situation de contamination chronique des coquillages, correspondant à un scénario 2. En absence de données de contamination quantitatives suffisantes (1 seule disponible sur Paimpol) pour ajuster une distribution de contamination, une distribution plausible de contamination théorique des coquillages a été attribuée, au regard des données de la littérature disponibles.

La consommation d'huîtres est une consommation hétérogène : la consommation est plus forte en zone côtière que dans les autres zones, la consommation différente suivant les saisons et les jours de la semaine, et même liée à certains jours de fête (Noël, nouvel an). La consommation est aussi

différente suivant le sexe et l'âge. Ce dernier facteur a été cependant négligé, pour s'intéresser à une population adulte de plus de 18 ans. La variabilité temporelle de la consommation, pour des consommateurs adultes, vivant en région côtière a été prise en compte, en croisant les informations obtenues par trois bases de données différentes, pour l'élaboration d'une base de données reconstituée théorique de 1000 consommateurs. La dose-réponse utilisée, ainsi que l'hypothèse portant sur l'infectiosité des génomes détectés par RT-PCR, est issu d'un travail portant sur l'analyse d'une épidémie d'hépatite A en Espagne, lié à la consommation de coquillages contaminés.

Les systèmes de surveillance et de gestion sont inspirés de ceux existant ou de ceux envisagés dans l'avenir : d'une part la surveillance microbiologique, basée en routine sur un suivi mensuel de *E. coli* dans les coquillages et d'autre part la surveillance basée sur la détection de VHA par RT-PCR, mise en place sur quelques zones de pèche à pied. Une autre approche concerne la diminution de la contamination des coquillages en amont, par une diminution des apports côtiers ou la recherche de sites moins exposés, et en aval, par une ré immersion ou une attente de commercialisation, associée ou non à des analyses virologiques

L'efficacité de la surveillance par la détection directe de VHA, tenant compte des performances du test, et en l'absence de réglementation spécifique, a été envisagée à deux fréquences de suivi, mensuel et bimensuel. A chaque épisode de contamination fécale, une contamination en E.Coli et en VHA est concomitante. La différence de demi-vie dans les coquillages entre *E. coli* et VHA, fait disparaître, pour le scénario 1 en quelques jours le niveau d'alerte prévu dans la surveillance environnementale, tandis que VHA subsiste à des niveaux importants des semaines après l'épisode de contamination fécale. Différents systèmes de gestion sont associés aux différents systèmes de surveillance, plus ou moins rapides pour la prise de décision de fermeture et de réouverture, avec nécessité d'une analyse de confirmation (ou pas) pour décider de la fermeture de zone (et de mise sur le marché de produits conchylicoles contaminés), et d'une ou plusieurs analyses négatives pour ré-ouvrir la zone. Le risque alimentaire annuel est diminué dès que la zone est fermée par l'arrêt de l'exposition durant la période considérée. La durée correspondante de fermeture de zone, constituant un coût préjudiciable pour les conchyliculteurs a été évalué pour tous les scénarios de surveillance et de gestion. Le risque annuel de référence pour chaque scénario est une situation d'exposition en l'absence de tout système de surveillance et de gestion. Le risque annuel est ensuite évalué pour chacun des systèmes de surveillance et de gestion. Le pourcentage de cas évités est ensuite évalué (1-rapport entre risque évalué/risque de référence). L'approche de modélisation choisie est une approche stochastique séparant incertitude et variabilité (AQR de second ordre). C'est la première approche d'appréciation des risques de second ordre concernant un risque alimentaire viral. La seule approche utilisant cette méthode et préalablement publiée s'arrête à l'exposition. Les résultats indiquent l'absence d'efficacité du système de surveillance microbiologique, si une alerte précoce n'est pas enclenchée (scénario1), et la potentielle absence d'efficacité si *E. coli* reste globalement conforme à la réglementation (scénario2). Le système de surveillance virologique est bien plus efficace pour prévenir les risques, pour les deux scénarios, en particulier si le suivi est bimensuel. Le système le moins couteux et le plus efficace reste celui de la diminution de la contamination en amont (de 2 log10). Les systèmes de surveillance envisagés négligent l'hétérogénéité spatiale, qui devraient être pris en compte, mais qui dépendent de la

configuration des sites conchylicoles. La dose-réponse utilisée, qui est fondamentale dans l'estimation d'un nombre de cas, repose sur une publication. Or la validité de cette dose-réponse est basée sur l'acceptation d'un certain nombre d'hypothèses sur l'infectiosité, l'efficacité de cuisson, la consommation. Pour une prédiction du nombre de cas « plausible » il faudrait préalablement valider par d'autres études les résultats obtenus, et prendre en compte la variabilité et l'incertitude associés à l''estimation des paramètres de dose-réponse. Mais même avec ces limites, L'appréciation quantitative des risques constitue un précieux outil d'aide à la gestion. Les travaux de ce travail ont été repris dans l'expertise de l'ANSES et ont fait l'objet d'une publication scientifique dans le Journal of Food Protection. La dose-réponse en constitue un élément critique, facteur limitant pour VHA et Norovirus. Enfin, l'estimation du nombre de cas par une AQR seule ne permet pas d'évaluer les conséquences en termes de transmission secondaire, avec extension d'une TIAC par une épidémie avec une transmission interhumaine.

<u>Chapitre III</u>: La dose- réponse facteur critique de la transmission alimentaire

La revue bibliographique des modèles de dose-réponse existant en microbiologie des aliments montre que les modèles les plus utilisés pour décrire le risque d'infection à partir d'une dose ingérée sont le modèle exponentiel, qui suppose l'absence de variabilité dans la relation hôte-pathogène et le modèle beta-poisson, qui assume l'hypothèse de l'existence d'une telle variabilité. Ces deux modèles ont en commun l'absence de seuil, l'absence de coopération entre agent pathogène, et la possibilité, pour un agent infectieux unique, d'être à l'origine d'une infection ou d'une maladie, avec une probabilité non nulle. Le risque de maladie est considéré comme conditionnel à l'infection. Le plus souvent le risque de maladie, à l'inverse du risque d'infection n'est pas considéré comme lié à la dose reçue, notamment pour le risque viral. Cependant pour l'ajustement de données obtenues sur des volontaires, sur Norovirus, avec le genogroup I (souche Norwalk), l'effet de la dose a aussi été pris en considération pour le risque de maladie. La revue des données disponibles sur les dose-réponse sur les virus transmissibles par les aliments montre que la plupart des données disponibles sont anciennes, basées sur des outils de quantification peu utilisés actuellement car remplacés par des outils de biologie moléculaires, et menés sur des volontaires ou sur des souches vaccinales. L'analyse de la dose réponse pour VHA a été vue dans la partie précédente. Pour norovirus, il semblait intéressant d'apporter des informations, sur la base de données observées dans les TIAC, en particulier celles concernant des coquillages. Des premiers résultats étaient publiés sur GI (virus de Norwalk), sur volontaires, avec une dose ingérée diluée dans de l'eau. La disponibilité de données de TIAC, avec la mesure de contamination dans les coquillages, avec une quantification par Real-Time RT-PCR, avec suivant les cas une contamination en GI, GII (ou les deux ensembles) nous permettait d'envisager d'évaluer une dose réponse pour GI et GII. Aucune étude n'avait jamais été publiée pour GII et en particulier GII.4, impliqués dans les épidémies inter-humaines hivernales. Seules les TIAC où les mêmes souches ont été identifiées dans les selles de malade et les coquillages ont été prises en considération. Les critères de Kaplan ont été pris en considération, vérifiant l'adéquation de l'incubation et des symptômes avec une infection d'origine alimentaire à norovirus pour définir les cas. De surcroit, pour plusieurs de ces TIAC, des données individuelles de consommation, ou un ordre de grandeur de la consommation (repas commun en restauration) étaient disponibles. Enfin le statut individuel sécréteur (ou pas) pour l'une des TIAC et une estimation du pourcentage de sécréteurs

dans la population générale française (même si d'effectif limité) pour les autres TIAC étaient connus. L'usage d'un modèle Beta-Poisson était justifié par le fait que les contaminations, par exemple, associaient parfois plusieurs souches, dont on ne peut exclure a priori une variabilité d'infectiosité. Le modèle a aussi gardé l'hypothèse retenue, au cours des essais sur volontaires, d'un effet de la dose sur le risque de maladie. L'hétérogénéité des données disponibles nous a amené à effectuer l'ajustement du modèle de dose-réponse par inférence bayesienne. L'espérance de la probabilité d'infection avec un seul virus infectieux est liée linéairement au statut sécréteur et au genogroupe. Les priors ont été choisies de façon à être peu informatives, sauf pour l'estimation de la probabilité d'être sécréteur.

La recherche d'interaction entre ces deux facteurs(effet sécréteur et génogroupe) n'était pas réaliste au regard des données disponibles. Les résultats obtenus sur GI sont en accord avec ceux menés au cours de l'étude sur volontaire. Il n'a pas été possible de démontrer une différence entre GI et GII mais, par contre, la différence entre sécréteur et non sécréteur est extrêmement importante, comme cela avait été constaté dans l'étude sur volontaires sur GI. Pour les sécréteurs, le risque d'avoir une chance sur deux d'être malade ou 50% de la population malade (DI_{50}) est en médiane de 32 copies pour GI et de 5 copies pour GI, la probabilité de maladie de 0.13 [0.007-0.39] pour GI et de 0 .18 [0.017-0.42] pour GII. L'infectiosité de GI et GII pour les sécréteurs est une des plus élevées décrite pour un virus à transmission alimentaire. Comme l'unité de contamination est mesurée en génomes, l'infectiosité associée au génome est élevée quand mesurée dans les coquillages de ces TIAC. Pour les non-sécréteurs, les estimations d'infectiosité à dose égales chutent fortement d'un facteur 100 à 1000. Ces résultats mériteraient d'être validés sur d'autres TIAC, en particulier pour celles où la consommation et le statut sécréteur sont connus individuellement, les souches identifiées dans l'aliment et les selles de malades et quantifiées dans l'aliment. L'information pourrait être alors être complétée, et l'analyse de la dose-réponse pourrait être effectuée à l'échelle du génotype ou de la souche virale en cause, et permettrait du côté des individus, de prendre en compte des facteurs liés au groupe sanguin ou à toute autre caractéristique (âge, co-morbidité).

L'ajustement réalisé est comparé aux données existantes, et l'intervalle à 95% des estimations basées sur les posteriors des paramètres comprend, dans tous les cas, les fréquences de malades pour chaque groupe de même exposition moyenne. Avec cette dose réponse il devient possible d'envisager une appréciation quantitative des risques liée à la contamination des coquillages sur GI et GII. La publication de ces travaux de dose-réponse de Norovirus a été acceptée dans le journal Epidemics.

<u>Chapitre IV</u>: un modèle dynamique pour la compréhension des mécanismes impliqués et du rôle de la transmission alimentaire au cours d'une épidémie hivernale à Norovirus

Les modèles dynamiques décrivant des infections humaines et prenant en compte une transmission alimentaire et interhumaine sont peu nombreux. On peut citer, cependant les travaux sur Cryptosporidium et les enterovirus comme des travaux ouvrant la perspective de combiner un modèle épidémique interhumain et une approche AQR, par consommation d'eau contaminée (eau de boisson ou de baignade).

De récentes publications sur les épidémies de choléra et d'Hépatite A étudient le rôle de la voie alimentaire (l'eau de boisson et les coquillages) et leur impact sur les épidémies humaines. Cependant la consommation humaine n'est pas évaluée par une enquête spécifique, et la dose-réponse utilisée est de type logistique (pour le Choléra). Pour l'Hépatite A, étudiée dans une région endémique côtière en Italie, la voie alimentaire (moules contaminées) augmenterait la durée inter-épidémique et jouerait un rôle important dans le nombre de total de cas de l'ordre de 50%. Cependant la diminution du risque alimentaire ne suffirait pas, dans l'étude par modélisation mené sur la situation italienne, en l'absence de campagne de vaccination sur les populations endémiques, à diminuer de façon significative et à long terme le nombre de cas de VHA en Italie (pour les populations côtières et non côtières).

Pour norovirus si des modèles dynamiques de propagation de l'infection existent, ces modèles décrivent la transmission inter-humaine dans des environnements semi fermés comme des hôpitaux ou des colonies de vacances. La voie alimentaire n'est pas prise en compte explicitement dans ces approches, visant à comprendre si les vomissures sont une source d'infection, le rôle des mesures d'hygiène dans la diminution de la transmission au cours d'une épidémie, l'impact de la durée d'hospitalisation des patients sur le maintien au stade endémique de l'infection à norovirus dans les hôpitaux, si l'infection des asymptomatiques joue un rôle important dans la propagation de l'épidémie et le rôle respectif des patients et des malades dans la transmission des infections nosocomiales. La Direction Générale de l'alimentation, en 2011, a saisi l'ANSES pour une évaluation du risque lié à la réouverture d'une zone conchylicole, préalablement fermée pour contamination avérée de norovirus dans les coquillages, dans une situation d'épidémie hivernale côtière. Les TIAC liées à la contamination des coquillages ne sont pas un phénomène nouveau et peuvent être abordées par une AQR pour une population sensible. L'impact sur une population dans laquelle une épidémie à norovirus sévit est moins évident, et l'exemple de l'étang de Thau nous a servi comme modèle d'étude pour évaluer l'impact de la transmission alimentaire dans une épidémie à Norovirus dans une zone côtière, ou au moins, à en voir quelques impacts potentiels et les mécanismes impliqués. Un même phénomène s'est répété, à quelques variations près sur l'étang de Thau (hiver 2002/2003 ; 2005/2006 ; 2009 et 2010/2011). Après le démarrage de l'épidémie de gastroentérites hivernales dans la population humaine, de fortes pluies ont occasionné un dysfonctionnement du traitement des eaux usées, avec un relargage de courte durée d'eau contaminée dans l'étang, où les huîtres sont produites. Les huîtres consommées quelques jours à quelques semaines après ont été à l'origine de TIAC pour la population côtière et non côtière. Un modèle dynamique stochastique, a pris en compte la voie alimentaire par une approche AQR comme décrite dans le chapitre II, mais sans prise en compte de l'incertitude. Le modèle choisi a été de type compartimental pour le volet humain et environnemental. Compte tenu de la rapidité d'évolution de l'épidémie, un modèle « hybride » a été élaboré, à pas de temps continu pour décrire la transmission interhumaine (algorithme de Gillespie) et à pas de temps discret (le jour) pour décrire le changement du taux de transmission par voie alimentaire. Le modèle de transmission interhumaine est de type SEIR, avec une durée de période infectieuse courte, de l'ordre de 2 jours, et du même ordre que la période latente, de l'ordre de 1.5 jours. Deux populations humaines interagissant entre elles ont été définies suivant leur consommation, ou pas, d'huîtres. Les caractéristiques de transmission interhumaine ont ensuite été calibrées de façon à générer une importante épidémie interhumaine de quelques

semaines. Le volet environnemental a pris en compte les données publiées d'excrétion (mesurées en génomes) humaines, le poids des selles pendant les périodes symptomatiques et asymptomatiques, le volume d'eau associé pour chaque individu, l'abattement dans la station de traitement des eaux usées en fonctionnement normal, la durée de résidence dans la station de traitement, et l'abattement de charge virale qui en résulte, lié à la survie du virus dans l'environnement. Un facteur de dilution dans les eaux marines a été pris en compte pour les eaux traitées relarguées. En cas de dysfonctionnement, ni l'abattement lié au traitement, ni la durée de résidence ni la dilution n'est pris en compte. Pour les huîtres un facteur de bioaccumulation est pris en compte, et de répartition de la contamination dans les tissus a été pris en compte sur la base de données publiés, permettant d'estimer la contamination moyenne des huîtres. La dose-réponse utilisée est celle élaborée dans le chapitre III, les données de consommation celles du chapitre I. Le modèle décrit le cas d'une épidémie à GI, GII et le cas d'une épidémie associant GI et GII. La chronologie des événements tente de correspondre à celle décrite sur l'étang de Thau, et explore différents scénarios de période de fermeture de l'étang. La comparaison de GI et de GII.4 vise à explorer si les connaissances actuelles permettent d'expliquer les différences épidémiologiques observées, à savoir l'association forte de GII.4 aux épidémies interhumaines, et l'association moins spécifique au genogroupe GII.4, en cas de TIAC, avec détection de GI et de GII.3. Quatre facteurs ont été choisis différemment entre GI et GII.4 : le taux de contact (plus élevé pour GII mais sans données en regard), l'excrétion (plus élevée pour GII), la bioaccumulation et la répartition dans les huîtres et les paramètres de dose-réponse. En cas de co-contamination, deux scénarios d'immunité croisée, forte et faible ont été testés. Les résultats préliminaires montrent que le nombre de cas augmente si un risque alimentaire se surajoute à l'épidémie humaine, en particulier pour la population de consommateurs mais pas seulement. La durée et la précocité de la fermeture limitant le risque alimentaire pourrait donc avoir un intérêt même pour une population côtière. Ce résultat est d'ailleurs en accord avec d'autres résultats portant sur l'étude de la grippe aviaire, d'oiseaux sauvages, avec la prise en compte d'un réservoir environnemental. La calibration a été choisie sur la base de données publiées, sauf pour la transmission interhumaine. Les premiers résultats, sur la base de cette calibration, sont en faveur d'un plus grand rôle de la part alimentaire pour GI que pour GII. En cas de co-contamination, la transmission inter alimentaire semble favoriser le nombre de cas lié à GI, et de coïnfections (successives ou simultanées), en cas de forte ou de faible valeur prise en compte pour décrire l'immunité croisée. L'étude menée ici doit être poursuivie par un plus grand nombre de simulations, par un ajustement sur des données réelles, et une analyse de sensibilité visant à identifier les paramètres les plus critiques de la modélisation. Cependant ce travail montre que la combinaison d'une approche AQR et d'un modèle dynamique, permettent de prendre en compte de façon plus précise la voie alimentaire et son impact direct et indirect dans le nombre de cas.

<u>Chapitre V</u>: limites, perspectives, conclusion

L'extension de ce travail au virus de l'hépatite A devrait être poursuivi par une amélioration de la précision sur la dose-réponse, par un ajustement sur des données de TIAC, tenant compte du niveau d'information (incertitude sur la consommation, sur l'effet de la cuisson) et évaluant l'effet de la dose (avec son incertitude) sur la durée d'incubation, la sévérité des symptômes, ou l'excrétion virale. Dans les zones où des épidémies de VHA ont été associées à la contamination de coquillages

dans la zone de production à proximité, et lorsque les abattements des stations de traitement sont insuffisants pour prévenir la contamination virale des coquillages, il serait intéressant d'évaluer l'efficacité d'une surveillance virale à la sortie des stations de traitement ou des effluents, voir dans les coquillages, en particulier dans les zones les plus exposées. L'incubation de la maladie étant longue, de nombreux cas asymptomatiques et infectieux (excréteurs), ce système pourrait être précoce pour détecter le démarrage d'une épidémie, et éviter la consommation de produits contaminés. Ceci s'inscrirait dans la continuité d'un travail mené sur l'efficacité d'un système de surveillance de Poliovirus dans les eaux usées, mais qui serait étendu aux coquillages et à leur éventuelle consommation.

Un premier modèle dynamique sur VHA pour évaluer cette efficacité dans une région côtière, nécessiterait, a minima de disposer d'une estimation du statut sérologique et des taux de contact efficaces par classe d'âge, avec prise en compte des asymptomatiques, pour une population côtière. Pour le virus de l'Hépatite A, comme pour norovirus, la prise en compte d'une transmission environnementale, nécessiterait de mieux connaître les caractéristiques de l'excrétion humaine, en terme de quantité émise (en génomes) au cours du temps, en relation avec les symptômes, les doses ingérées, pour les asymptomatiques et les éventuels porteurs chroniques. La survie de ces virus dans les coquillages a été étudiée pour norovirus, mais on ne dispose pas de telles données publiées dans les autres compartiments environnementaux (sédiment, station d'épuration), et on dispose d'encore moins de données sur le virus de l'Hépatite A.

La question de l'infectiosité du génome est délicate, mais les résultats exprimés en génomes, donnent, à minima une dose maximum infectieuse potentielle. En fonction des connaissances, des niveaux d'abattement de l'infectiosité peuvent être pris en compte dans la modélisation (effet d'un traitement par exemple). Cette question n'a pas été un frein à la modélisation qui a été menée, mais l'apport d'information sur l'infectiosité pourrait être intégré à de futurs travaux. L'infectiosité n'est pas actuellement mesurable, sur des échantillons de l'environnement pour le virus de l'hépatite A, et il n'existe pas de culture cellulaire pour norovirus.

Pour norovirus, une meilleure connaissance des mécanismes de l'immunité acquise, même de courte durée, et sur les mécanismes de l'immunité croisée sont des éléments qui nous ont manqué. L'ajustement d'un modèle dynamique à des données observées en zone côtières, l'analyse de sensibilité du modèle, conforterait (ou pas) notre analyse et seraient à mener dans la continuité du travail ébauché. Au delà, une analyse plus longue dans le temps, et plus large dans l'espace, serait intéressante pour étudier les conditions d'émergence ou de réémergence de certaines souches, liées à des réservoirs environnementaux mais amènerait aussi des modifications structurelles du modèle. L'extension de ce travail pourrait s'intéresser à évaluer d'autres stratégies au niveau des populations humaines, comme la vaccination pour l'hépatite A, ou l'application de recommandations d'hygiène pour norovirus, ciblées ou globale, qui n'ont pas été abordées ici. La prise en compte des autres sources d'infection humaine vis à vis de VHA ou norovirus, concernant les aliments (produits végétaux, eau de boisson, aliments préparés) ou des sources environnementales (eau de baignade, surfaces souillées) constituerait une extension naturelle du travail mené. Enfin d'autres virus d'origine alimentaire nécessiteraient des travaux spécifiques, comme le virus de l'hépatite E, avec ses

réservoirs zoonotiques, ou d'autres virus, dont les effets sont peu étudiés ou peu connus, qui comporteraient un risque d'émergence ou de réémergence.

Ce travail montre l'intérêt de combiner dans une même approche, l'Appréciation quantitative des risques en tant que maillon d'un modèle dynamique, permettant une prise en compte plus précise du risque viral par voie alimentaire pour l'homme, et de mieux évaluer l'impact de mesures de gestion sur ce mode de transmission. Il est possible d'imaginer pour les agents zoonotiques, notamment pour le virus de l'hépatite E, une approche combinant dans un même modèle dynamique, l'appréciation quantitative des risques pour l'homme au regard d'une surveillance des épizooties dans les populations animale.

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ACCRONYMS AND ABBREVIATIONS

AFSSA: French Food Safety Agency

ANSES: French Agency for Food, Environmental and Occupational health and safety

CDC: Center for Disease Control (USA)

DAG: Directed Acyclic Graph

DR: dose response

DT: digestive tissues

EFSA: European Food Safety Authority

FAO: Food and Agriculture Organization

GI: genotype I

GII: genotype II

HAV: hepatitis A virus

HRV: human Rotavirus

IC: confidence interval

IFREMER: French Research Institute for Exploration of the Sea

INRA: French National Institute for Agricultural Research

INSERM: French National Institute of Health and Medical Research

InVS: French Institute for public health surveillance

NoV: Norovirus

France AGRIMER (inclusion OFIMER): Etablissement National des Produits de l'Agriculture et de la mer.

PAR Population Attributable Rate

PCR : Polymerase Chain Reaction

QRA: Quantitative Risk Assessment

REMI: réseau de surveillance microbiologique de zones conchylicoles

Real-Time RT-PCR: PCR quantitative

RT-PCR: Reverse-Transcription PCR: RNA to DNA before PCR

TBEV: tick-borne encephalitis viruses

VLP: Virus Like Particles

WHO: World Health Organization

WTP: water treatment plant

CHAPTER I: GENERAL INTRODUCTION

I.1. PUBLIC HEALTH IMPACT OF FOODBORNE VIRUSES

Foodborne viruses are regularly involved in outbreaks and are taken into account in foodborne disease burden. This PhD thesis focused on the risk associated with oyster consumption potentially contaminated with Hepatitis A Virus (HAV) or norovirus (NoV). However it seems useful, in this first part, to present other viruses and other food products to better consider if this work can be extrapolated or not to other situations, and also to better evaluate, in a general context, the relative sanitary importance of these viruses and of this food product.

Sanitary risk ranking between infectious agents is classically based on mortality and morbidity data (Mead *et al*., 1999). Risk ranking are also dealing with different sources of uncertainties (Vaillant *et al.*, 2005).

In this context, a short description of the epidemiological data and methods used for sanitary risk ranking are given, and in particular, for NoV and HAV.

I.1.1. IDENTIFICATION OF FOODBORNE VIRUSES

- The first step is the identification of the list of viruses of potential interest in food and water.

Foodborne viruses were identified as a subject of concern in AFSSA (2007): rotaviruses (Human Rotavirus, HRV), norovirus (NoV), Hepatitis A virus (HAV), hepatitis E virus (HEV), sapovirus, astrovirus, adenovirus 40 and 41, enterovirus, parechovirus et reovirus (AFSSA, 2007).

The working group of experts decided only to consider viruses which have been shown to carry a risk of transmission to humans through ingestion of contaminated food or water and which posed problems in the food processing and water supply sectors (AFSSA, 2007; FAO/WHO, 2008). Subsequently viruses belonging to 10 families, associated with foodborne illness, classified with their site of replication in the human body, were identified at international level (FAO/WHO, 2008). The criteria for prioritizing between viruses were (FAO/WHO, 2008):

- The high incidence, estimated worldwide, based on the currently available data.

- The severity of disease including significant mortality level worldwide.
- The potential for foodborne transmission and raise a significant threat to public health (including emerging viruses).

The ranking, for FAO/WHO report, was done qualitatively. All viruses of the AFSSA report were included plus Aichi virus and emerging viruses, where the transmission by food can be exceptional or occasional, such as SARS-Cov (Acute Respiratory Syndrom-causing Coronavirus) or HPAI virus H5N1.

The viruses listed are reported in Table 1, together with the available quantitative data of morbidity and mortality.

For each virus, in the USA and France, the overall estimated number of cases is given (morbidity, mortality), and, in a second step, restricted to the number cases attributable only to food transmission (the number of reported cases includes foodborne outbreaks and sporadic ones).

The method used for estimating the attributable part of cases linked to those viruses, and among them, the relative contribution of food, is far from being simple, and is described in two steps:

(i) the global estimation of the number of cases (morbidity/mortality)

(ii) The percentage attributable to a foodborne origin.

Site of infection	Virus	cases (worldwide)(deaths)	estimated cases (USA)/100,000 [IC 95] for 2006[2]	Foodborne % (USA)	Foodborne cases (USA) /100 000 [IC 95]for 2006 (deaths)[2]	estimated cases (France) /100 000 **no conf.interval.** for 1990-2000	Foodborne % (France)	Foodborne cases (France) /100.000 (deaths) for 1990-2000
intestinal system	**Norovirus (Nov)**	**ND**	**7026[4151-10689]**	**40%** [1]**-26%** [2]	**1826[1079-2779] (0.05[2])**	**850**[3] **no conf.interval.** with medical seek	**14%**[3]	**119**[3] with medical seek
	Rotavirus (HRV)	**ND**	**1032[373-1782]**	**0.5%**	**2[5-9]**	**271**[4]**(0.01)**	**Négligeable**	**ND**
	Sapovirus	ND	**1032[373-1782]**	**0.5%**	**2[5-9]**		ND	ND
	Astrovirus	ND	**1032[373-1782]**	**0.5%**[2]	**2[5-9]**		ND	ND
	Adenovirus 40-41	ND					ND	ND
	Aichi virus[2]	ND					ND	ND
Liver	**HAV**	**ND**	**7.5[3-14.5]**	**5%**[1]**-7%**[2]	**0.53[0.23-1] (0.003)**	**13.7**[3]**-1.6**[5]	**5%**[3]	**0.68** [3]**-0.08**[5,3]**(0.00032**[3]**)**
	HEV	**ND**		**ND**		**0.36** [6]		
Neural tissue and nervous system	Echovirus/Coxsackie	ND		ND		3.043[7]	0	ND
	Nipah virus	South East Asia: 2007-2008: 10-20 cases/years (10-20)[8]		ND	0	0		0
	Poliovirus	around 650 cases [9]			0	0 in Europa since 2002		0
	Parechovirus	ND				ND		ND
	Tick-borne encephalitis virus (TBEV)	Europe and Russia: 1990-2007: 8755 [10]				some cases in (?-5)[11]		0
Respiratory system	HPAI-H5N1	2003-2012 602 cases [12](355)[12]				0		0
	SARS-Cov4,5[13]	no cases since 2004;		0			0	0

TABLE 1: ESTIMATED NUMBER OF ANNUAL CASES (DEATHS) IN THE WORLD, USA (DOMESTICALLY ACQUIRED), AND FRANCE, CAUSED BY FOODBORNE PATHOGENS.

Legend: Source of data: [1] (Mead *et al.,* 1999), [2] (Scallan *et al.*, 2011), [3](InVS, 2004), [4] 160,000 severe acute diarrhea in children/ 59 millions French population estimates 1999-2000 (Melliez *et al.,* 2005); [5] (InVS, 2012, Hepatitis A 2011), [6] 218 cases of confirmed diagnosis in 2008 (AFSSA, 2009) with 59 million French population estimates; [7]8978 cases with strain confirmation during 5 years (Antona *et al.,* 2005), [8](WHO, 2009), [9]data 2011 (Polio eradication initiative, 2012), [10](Mansfield *et al*., 2009), [11](Chastel and Heller, 2012), [12](FAO/EMPRESS, 2012), [13](CDC, 2012). ND: No data. In parenthesis: annual number of deaths.

I.1.1. Estimate of number of cases linked to specific viruses

For estimating the number of cases associated with an infectious agent two modeling approach are available, depending of the available data (Mead *et al*., 1999; Vaillant *et al*., 2005) (**Figure1**):

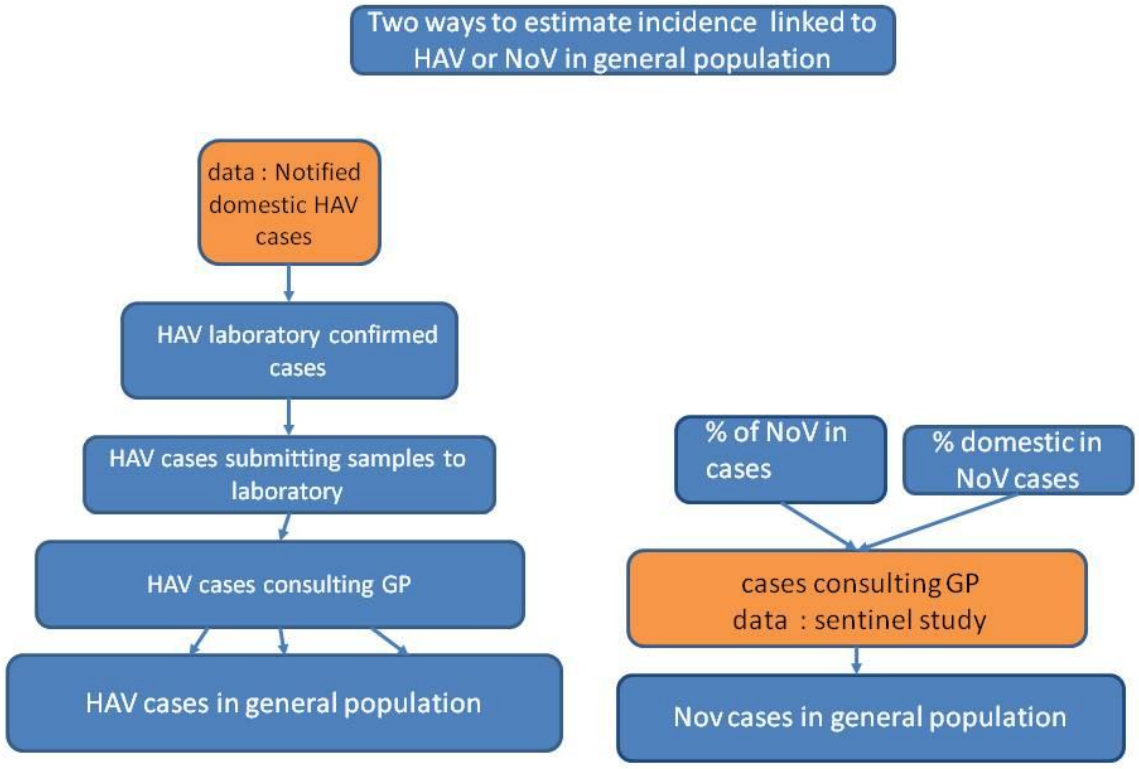

Figure 1: Ways to estimate incidence using the example of Hepatitis A virus and Norovirus

Legend: in orange the step were the data are available (Nov: Norovirus, HAV: hepatitis A virus) from Vaillant (2005); Mead (1999) and Scallan (2011) approaches; In blue the level to be estimated; the basis of the pyramid is representing all symptomatic cases in the population of interest, arrows are describing the different step from the data to the needed estimate.

1.1.1.1 Estimate of incidence with counts of laboratory confirmed illnesses (pathogen based data)

These estimates (**Figure 1**) are adjusted for undercounts (because of underreporting and underdiagnosis). An example of this method can be given with Listeriosis (even if it not a virus) in France. In 1999, exhaustivity of the mandatory reporting system was reported to be around 85% IC 95[82-89] with the capture-recapture method on EPIBAC data (Goulet *et al*., 1999). However other parameters, described as under-reporting factors, have also to be taken into account, because all symptomatic cases are not seeking medical attention, not all cases are investigated, and examination are not exhaustive of all pathogens.

At the laboratory level, sensitivity of analysis is not perfect, and reporting to national reference center (NRC) depend of the type of laboratory (private, hospital) and of the pathogen (Gallay *et al.*, 2006; InVS, 2004; Vaillant *et al*., 2012 a). The different parameters of reporting have to be estimated, in specific studies, in order to be extrapolated to general population (Gallay *et al*., 2006; Kemmeren *et al*., 2006; Couturier *et al.,* 2007). The sensivity of reporting varies according to the severity of cases and has an impact on the representativeness of the surveillance. This approach could be appropriate for hepatitis A cases, because since November 2005, acute hepatitis A became a mandatory notifiable disease (InVS, 2012). The part of domestic cases has to be taken into account (InVS, 2004; Scallan *et al*., 2011). The Table 1 gave the latest number of notified cases (including those linked to foreign travels). In the nineties period, the percentage of domestically acquired cases was around 23% (InVS, 2004).

In the estimate from USA in 2011, HAV estimate takes into account domestic cases in the population (Scallan *et al.*, 2011) (Figure 1). Some of these reported estimates, expressed in percentage, are linked to the severity of symptoms. Only 70% of the cases are considered to be associated with severe symptoms, such as jaundice (Scallan *et al*., 2011). For severe infections with hepatitis A virus, rates of medical care-seeking was assumed to be around 90%, (for mild infections 18%), and the specimen submission rate to the laboratory at 100% (19% for mild). The laboratory testing for HAV was estimated around 97%, with perfect sensitivity and specificity of analysis. The proportion relative to foreign travel related was estimated around 41% (Scallan *et al.,* 2011). These percentages were used as mode values in Pert distribution, used to describe the uncertainty around those estimates (Scallan *et al*., 2011).

1.1.1.2. Indirect estimate of incidence at national level (symptom based data)

The data of symptomatic cases (such as acute gastroenteritis) are estimated for the population level. The data are scaled down when attributing those cases to known pathogens (Figure 1). This is the way to estimate noroviruses cases (Scallan *et al*., 2011; InVS, 2004; Vaillant *et al.,* 2005).

For estimating annual number of acute diarrhea (AD) in France (InVS, 2004), data of acute gastro-enteritis were taken from the French Sentinel surveillance network (sentiweb, 2012). When estimating the norovirus contribution in those acute-enteritis cases, data were taken from an epidemiological study, nested in the French Sentinel surveillance network (sentiweb, 2012) during the winter of 1998 to 1999. A physician-based (103 general practitioner) case-control survey of Acute Diarrhea (defined as losing at least three soft or aqueous stools per 24 h for a duration less than 2 weeks), with virological screening in stools, gives estimates of the proportion of acute diarrhea linked to norovirus detection about 19.2% (Chiki-Brachet *et al.,* 2002) at the practitioner level.

New data are now available in order to estimate the number of people with AD (Acute Diarrhea) who are consulting general practitioners around 33% (27-40) (Van Cauteren *et al*., 2012).

Recent data give an estimate of the proportion of AD cases associated with norovirus in France associated with other viruses, in particular influenza (Arena *et al.,* 2012). For norovirus, alternative approaches focus on medical sales (drug reimbursement data from the national health insurance system) to improve outbreak detection (Beaudeau *et al*., 2008; Pelat *et al*., 2010). For other pathogen sentinel surveillance results are linked to infectious disease transmission dynamics (Dorigatti *et al.,* 2012).

Consequently, more precise estimates of noroviruses annual incidence cases, including sporadic cases (not consulting a practitioner), could be probably estimated in the near future. For USA, all domestic cases of gastroenteritis due to norovirus in the general population are supposed to be estimated (Scallan *et al*., 2011) (1% non domestic for norovirus).

The differences in reported estimates among pathogens can be explained by the severity of their respective symptoms. Rare pathogens or not severe were unlikely to be detected. By example the reporting estimates were different, between pathogens causing bloody diarrhea or not (or in %) (Mead *et al*., 1999) (Norovirus is associated with non-bloody diarrhea).

The number of cases estimation at the population level can be found in French studies (Gallay *et al.,* 2000, 2003) and in other recent international studies (Scallan *et al.,* 2011). Uncertainty estimates are given in recent literature taking into account the different sources of uncertainty (Powell *et al*., 2001; Scallan *et al.,* 2011) and in particular with bayesian inference (Albert *et al*., 2011; Presanis *et al*., 2009).

When the estimate of the total number of cases attributable to a specific virus is known, the second step is to estimate those linked to food transmission.

1.1.2. Attributing illness to food source

Foodborne is for several viruses one of the possible pathways of transmission. For feco-oral transmission, indirect pathway can occur due to the environment, such as carpets, or toilets. Direct transmission from human to human can occur via droplet, fomites, contacts, in particular for HAV, NoV, and Rotavirus. General approaches can be found from different methodological published

papers (Mead *et al.*, 1999; Batz *et al*., 2005; Vaillant *et al.,* 2005; Kemmeren *et al.,* 2006; Havelaar *et al.,* 2007; Mangen *et al*., 2010; Havelaar *et al.,* 2012).

Four broad categories of methods can be defined as source of information for attributing illness to food origins.

(1.1.2.1) Outbreak studies

Outbreaks are used to estimate foodborne part but also case-fatality rate. The principle of food attribution is to estimate attributable risk from the outbreak data. Criteria were used for selecting outbreaks. For waterborne outbreaks, restricted criteria were requested to better established causal link with etiological infectious agent, setting the identification of the pathogen strain in water and stools (Beaudeau *et al*., 2008) as a criteria or setting minimum level to RR (Relative Risk) to 2 or p value =<0.05 (Blackburn *et al*., 2004).

For estimating the first % attributable to food (Mead, 1999), the % of outbreaks in which the mode of transmission were known to be foodborne was used (Mead, 1999). Same approach was made for Hepatitis A virus (Mead *et al.*, 1999). For Noroviruses data were taken from a retrospective study on 348 NoV outbreaks, between 1996 and 2000: 3% were waterborne, 12% person-to-person, 39% foodborne, and 46% unknown (MMWR 1st June 2011 data from Fankhauser (1998) and CDC, between 1996-2000 (CDC, 2001). The attributable foodborne part to foodborne was at 40% (Mead *et al*., 1999).

For 2011 estimate, each outbreak is attributed to an etiological origin, taking into account the population concerned in each kind of outbreaks. Among 13,944 persons illness cases, 3,628 (26%) were in foodborne classified outbreaks (CDC, unpublished data) (Batz *et al.,* 2011; Scallan *et al.,* 2011).

However, analyses of outbreaks are difficult to extrapolate to general and sporadic cases. Combination of mode of transmission is possible for HAV and NoV (first cases foodborne and secondary cases inter-human transmission) makes the real attribution of cases to each way difficult, even when focusing on primary cases (Matthews *et al*., 2012). Biological characteristics make the attribution difficult for NoV, with short incubation period, and for HAV, because the investigation for food contamination is delayed by a long incubation period. Food contamination or exposure can be higher in outbreaks than in the average level of the population, explaining exceptional events such as food outbreaks. For Norovirus, because genogroups I and II seem to be linked with different ways of transmission and severity in outbreaks (Desay *et al*., 2012; Matthews *et al*., 2012), it could be more effective to distinguish food attribution, hospitalization rate and case fatality ratio of genogroup I and II.

(1.1.2. 2) Population based and retrospective case control studies

For France estimates (Table 1), food attribution in NoV cases was estimated from data established in Netherlands, in a community-based prospective cohort study and particularly in a nested case-control study in 1999 (De Wit *et al.,* 2001; 2003). The cohort study was followed to estimate the

incidence of gastroenteritis. The nested case-control study was used to identify risk factors and determine etiology (De Wit *et al*., 2003).

Foodborne source is commonly involved if the food contamination occurred before the household. In order to exclude direct transmission, in particular for norovirus, criteria were added (De Wit *et al*., 2003). In this study a case can be counted as suspected foodborne origin if there were no contact with an ill person seven days before symptoms. All persons who reportedly had contact with someone with gastroenteritis were, by hypothesis supposed infected by that person. The estimate of PAR (Population Attributable Rate) for contaminated food's entering the household was 16%. The food-handling hygiene effect is a separate factor from foodborne and includes both the effect of poor food-handling hygiene in the household favoring indirect person-to-person transmission and food cross contamination within the household. For rotavirus, in the same study the estimate of PAR for contaminated food's entering the household was 4% of all rotavirus gastroenteritis cases (De Wit *et al.,* 2003).

These studies were considered to be more representative of sporadic cases than outbreak data. However, unlike the latter, food vehicles are not laboratory confirmed (Mangen *et al*., 2010). Surveillance system, and any epidemiological studies have their own limitations (Hardnett *et al.,* 2004; Vaillant *et al.,* 2012 a).

(1.1.2. 3) EXPERT ELICITATION

Expert elicitation is not explicitly used in estimates of Table1; however this approach is used in the disease burden of foodborne pathogens in the Netherlands 2009 (Havelaar *et al.,* 2012). The detailed method is published (Havelaar *et al*., 2008; EFSA, 2008).

(1.1.2.4) FORWARD APPROACH BY MODELING.

ATTRIBUTION MODELS BASED ON MICROBIAL SUBTYPING

Microbial subtyping (based on genetic subtyping or serotyping) is based on the difference of microbial fingerprintings between different food sources, between species of animal population, in comparison with microbial fingerprintings in human cases population. The interest is to identify the animal reservoir, taking into account human consumption data. It was found to be adapted for Salmonella and Campylobacter, using a Bayesian framework to propose an estimate attribution fraction (Hald *et al.,* 2004; Hald *et al*., 2007, Mullner *et al.,* 2009, Strachan *et al.*, 2009; Ranta *et al.*, 2011; David *et al.,* 2012). However because human source is the main source of virus for HAV and NoV human cases, this method is not appropriate, except in the case of an emerging zoonotic strain. For HEV, because of zoonotic transmission by pigs or other mammals, geographical and transmission pathways seem linked to the source. For rotavirus, main transmission is inter-human; however some animal strains can infect food and can be source of infection for humans.

QUANTITATIVE RISK ASSESSMENT

Potential contribution of risk assessment for estimating the relative sources among food products is a quite complex task. Semi quantitative methods were used in particular for fish products (Ross and

Sumner 2002; Guillier *et al.,* 2011). Quantitative and relative risk exposure assessment was tried for Campylobacter and Listeria (Evers *et al.*, 2008; Endrikat *et al.*, 2010). Data needed, in particular in numerous food contamination possibilities, can be difficult to obtain, in particular in a representative way. Quantitative risk assessment alone is not adapted to investigating relative contribution of food with direct inter-human transmission.

1.1.3. Public health impact estimates of foodborne viruses

The results of Table 1 show that with the available data, NoV and HAV are the main foodborne concerns in France, including morbidity and mortality criteria. Secondary HEV could also be of concern (newly diagnosed).

The rotavirus rank is linked to the estimated percentage of % of cases attributable to foodborne origin, which varies between countries (France and USA Table 1). Human Rotavirus (HRV) is the leading cause of gastroenteritis in infants and young children worldwide, but cases are particularly severe in developing countries. Outbreaks of HRV gastroenteritis in day-care centers and hospitals can spread rapidly among non-immune children, presumably through person-to-person contacts, airborne droplets, or contact with contaminated toys (Gleizes *et al*., 2006). The use of a vaccine seems efficient in the USA (CDC, 2009). Although person-to-person transmission is most common, there is evidence of the potential for virus transmission via drinking water and water used for food preparation (Villena *et al*., 2003). In France, among ten waterborne outbreaks, only one waterborne outbreak is suspected to be associated with rotavirus contaminated water (also contaminated with Campylobacter) (Beaudeau *et al*., 2008). In France, and for other high income countries (WHO, 2008) the foodborne contribution (excluding water) is negligible (InVS, 2004).

NoV and HAV highest ranking are in agreement with other ranking conclusion (ANSES, 2007; FAO/WHO, 2008; EFSA, 2011). Norovirus are the first foodborne origin of acute gastro enteritis (AGE) (bacteria and parasites included), with 95% of non bacterial AGE (Karst, 2010) and more than 50% of AGE by itself (Batz *et al*., 2012).

This is motivating the interest to focus this work to norovirus and HAV. The precision of foodborne sanitary impact is not well estimate for many foodborne viruses, even for HAV nor NoV. The foodborne pathway is not the only way of transmission, and different pathways can co-exist and interact with each other.

I.2 Transmission of foodborne viruses

Two levels of foodborne pathways are described here: the first one compares the food pathway to other pathways of transmission, and the second evaluates the impact of different food matrix for attributing virus infection in humans inside the foodborne pathways.

1.2.1. Pathways of transmission of foodborne viruses

Foodborne, is for several viruses one of the possible transmission pathway. Foodborne viruses are different, for this factor, from other groups of foodborne pathogens like parasites and bacteria. The estimate proportion of foodborne origin in cases is more than 50% for Toxoplasma (excluding water transmission), around 95% for Salmonella spp., 99% for Listeria, 80% for Campylobacter and 68% for STEC 0157. For these pathogens human-to- human transmission is not feasible or negligible.

In comparison, for NoV and HAV, the food-borne part is only between 4% to 26% (Scallan *et al.,* 2011), and the second pathway of transmission is mainly from humans to humans. For these two pathogens, the contamination of food and water is made by human stool contamination (feco-oral pathway). The contamination step in the food process is variable and can occur at the primary production step, or taking into account poor hygienic practices, at the food preparation step (EFSA, 2011; Mokhtari and Jaykus, 2009).

Feco-oral indirect transmission from humans to humans can occur through the environment, via toilets, carpets. Direct transmission from human to human (or from animal to humans) can be also caused by contact, and in more exceptional cases, via droplet or fomites (ANSES, 2007; FAO/WHO, 2008; EFSA, 2011). The different known pathways and their relative contribution, qualitatively evaluated for each virus listed in Table 1, are given in **Table 2**. Some pathways are not taken into account bloody transfusion risk, is not considered here).

Because of the rare foodborne infections, some viruses were excluded, at this step, from further analysis. However their inclusion in WHO (2008) and EFSA (2011) lists needs some explanations. Outbreaks associated with foodborne transmission of newly emerging viruses are a low probability event but have a potentially high impact.

- SARS coronavirus epidemiological investigations have shown that the major mode of transmission of the SARS virus is through close person-to-person contact, in particular exposure, to droplets of respiratory secretions from an infected person. However, due to the close phylogenetic relationship it is plausible that at the origin the virus was spread into the human population through the preparation and consumption of food of animal origin, Chinese ferret badgers (*Melogale moschata*), masked palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*). Those animals are suspected to catch the infection from another reservoir, probably bats (Guan *et al.,* 2003; Martina *et al.,* 2003; Drexler *et al*., 2010). SARS coronavirus have been found in bat populations, and even in Europe, but does not represent a significant risk for foodborne transmission (Drexler *et al.,* 2010; EFSA, 2011). More over no human case was detected around the world till 2004 (CDC, 2012).
- H5N1 infections of humans are rare, and not transmitted from human to human and are associated with direct contact with ill poultry (FAO-WHO, 2008; EFSA 2011). However, infectious H5N1 avian influenza virus has been cultured from duck meat, and the exceptional consumption of duck blood has resulted in the infection of humans (Tumpey *et al*., 2003). Food contaminated with H5N1 does not appear to be a significant risk for infection in humans (FAO-WHO, 2008; EFSA 2011).

Infection site	Virus	animal origin (zoonotic) (food or contact transmission to human)	Feco(animal origin)-oral (food, water or contact transmission to human)	feco (human origin)-oral transmission involving food or water contamination	feco-oral : short interhuman transmission: direct-indirect (environmental contamination)	other inter-human transmission fomites, aerial transmission
	Norovirus (Nov)	negligible	*not demonstrated*	++	++++	++ aerolized vomitus particles, fomites
	Rotavirus (HRV)	possible	*pig, cattle, cats (contact)*	+ (accident of treatment of water)	++++	+
	Sapovirus	-		?		
	Astrovirus	-	-	+	+++	
	Adenovirus 40-41	-	-	+		
	Aichi virus[2]	-	-	+		
Liver	HAV	-	*neglible (primates)*	+(vegetables)	++++	
	HEV	++ +: food +/-: contact (pig pets)	?	++(water for endemic geographical areas)	+(not demonstrated to be important in Europa)	-
Nervous system	Echovirus/coxsackie	-	*primates**/pigs***			?
	Nipah virus	+	contamination of fruits by urines/ bats saliva	-	-	-
	Poliovirus[5]	-	*primates***	++	+++	
	Parechovirus	-	-			
	Tick encephalitis vius (TBEV)[1]	vectorial disease transmission by ticks	Unheated raw milk (cows, goats, sheep)	-	-	-
Système Respiratoire	HPAI-H5N1[3]	+ ++: contact with ill birds +/-: duck blood or food	poultry	-	-	-
	SARS-Cov4,5[4]	+/-: original cases by food or contact transmission (civets)		-	+++	+++ (Honk Kong)

TABLE 2: TRANSMISSION PATHWAYS OF IDENTIFIED FOODBORNE VIRUSES

Legend: *Number of cases per year or during the period **rare or not based on epidemiological data. [1] Mansfield *et al.,* 2009; [2] Drexler *et al.*, 2011; [3]FAO/EMPRESS 2012; [4]Martina *et al.,* 2003; the number of + is the relative importance, qualitatively evaluated.- not observed.

- Human infections with NIPAH virus were occasionally observed, following consumption of contaminated fruits which were contaminated through the saliva of fruit bats (Luby *et al*., 2006; WHO, 2009, EFSA, 2011). The biggest concern is the possible adaptation of these viruses to humans (EFSA 2011). This disease is not present in France.

- In Europe, tick-borne encephalitis viruses (TBEV) (flaviviruses) are transmitted from their natural hosts, mostly rodents, from ticks (*Ixodes sp*) to humans, or to cows, sheep, and goats. Viruses can be excreted in their milk. The consumption of contaminated raw milk can lead to human infection and disease, such as "biphasic milk fever" (Mansfield *et al*., 2009). Infectious TBEV can also be found in yoghurt, butter and cheese (EFSA, 2011). However in France, the human cases detected are not reported or very rare.

The feco-oral pathway is the most common pathway of transmission of foodborne viruses listed, in particular for HAV and Norovirus in Table 2.

The different pathways (feco-oral and human to human) are not stochastically independent of each other. For NoV, or hepatitis A virus, if the inter-human transmission is so high that a human epidemic emerges, sewage or waste water (of human origin) can be highly contaminated. Then, in the case of inefficient treatment of sewage water, the risk of contamination of food or water increased (Ranta *et al*., 2001; Ajelli *et al*., 2008). On the other hand if food is highly contaminated, a high number of primary cases can provoke an inter-human transmission of secondary cases and an epidemic, such as in Shanghai, with several hundred thousands cases of HAV (Halliday *et al*., 1991). On a short term scale, 16% of norovirus outbreaks involved two or more mode of transmission (Mathews *et al*., 2012).

This is one of the fundamental aspects of this work, which is trying to investigate the impact of food contamination in relation with the epidemiological situation in the human population.

The relative importance among the different food products that can be contaminated was evaluated.

1.2.2. Relative importance of different food products in the foodborne pathways

Figure 2 gives an idea of what kind of products are concerned by the feco-oral pathway. For HAV and norovirus, the animal origin of contamination can be neglected.

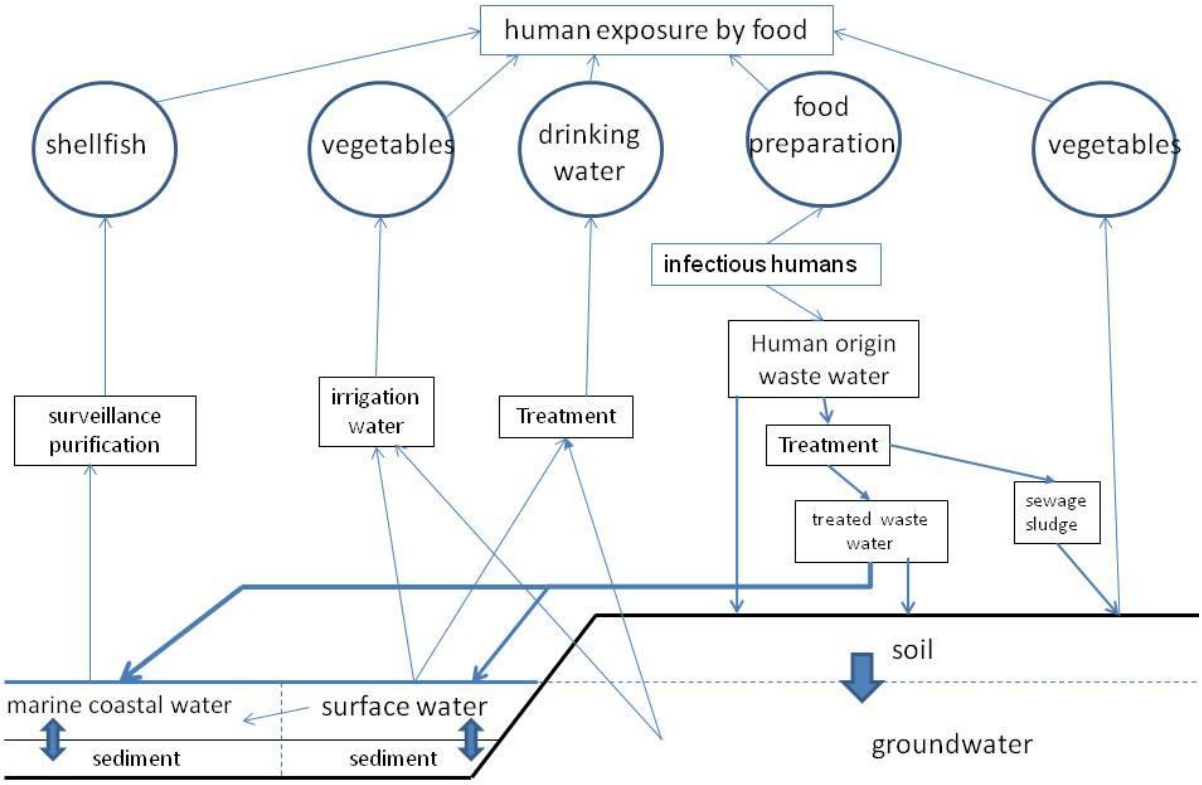

FIGURE 2: DIFFERENT SOURCES OF HUMAN EXPOSURE RISK VIA FOOD FROM HUMAN FECO-ORAL PATHWAY

Legend: the origin of contamination is from humans (box "infectious humans") food can be contaminated at the preparation step (hand contamination) or the environment by infected stools. Different pathways are possible to food products via sewage sludge or by sewage water contamination. Vegetables can be contaminated by sewage sludge or irrigation water. Arrows indicate the transmission pathway between each box.

Water can be contaminated by accidental sewage contamination or inefficient treatment of drinking water. Food products are contaminated via contaminated water (or in some cases sewage sludge) used for irrigation of fruits or vegetables, in particular salads, or raspberries. Food can also be contaminated by foodhandling and poor hygienic practice during the food preparation (Mokhtari *et al*., 2009).

Those viruses are not replicated outside of their living hosts, and in particular in the environment, food, and water. Excluding the case of zoonotic viruses (HEV and HRV), food products and drinking water can be seen as passive vehicle of contamination for Norovirus and HAV.

Shellfish are in a particular situation, because they filtrate and concentrate HAV and Norovirus (Atmar *et al*., 1995; Le Guyader *et al.,* 2009; Maalouf *et al*., 2010, b). Moreover it seems that they

keep it inside their own tissues for a duration expressed in several weeks, with conservation that depends on seasonality and of genogroup for norovirus (Le Guyader *et al*., 2006; Maalouf *et al.,* 2010 a and b). Specific receptors for human norovirus were recently found in oyster tissues (Le Guyader *et al*., 2006; Maalouf *et al*., 2010 b).

Considering the characteristic of shellfish bioaccumulation, it is not surprising to see the relative high contribution of shellfish products in outbreaks (of identified origin) nor to observe their ranking in sporadic and outbreak foodborne cases, among the different food products, as given by EFSA in 2011, or FAO/WHO report (2008):

From FAO/WHO 2008, food virus combination priority, based on expert elicitation:

- "For fresh produce, the main routes of contamination are through contaminated water (used for irrigation, agrochemical application or wash water); the use of human sewage as fertilizer; and manual (human) handling during and post-harvest. However, the relative contribution of each is not known.
- For bivalve molluscs consumed raw, the main route of contamination is through fecal contamination of the waters in which they are growing. The contamination most commonly occurs through sewage discharge, run-off from agriculture, and point source contamination of the immediate surrounding of the growing areas.
- For prepared ready-to-eat foods, the main route of contamination is via infected foodhandlers practicing poor personal hygiene during food preparation and serving."

From EFSA 2011: ordered priority setting for risk assessment, based on expert elicitation:

"NoV and HAV in bivalve molluscan shellfish

NoV and HAV A in fresh produce

NoV and HAV in prepared foods

Rotaviruses in water for food preparation

Emerging viruses in selected commodities"

Based on foodborne US outbreaks with known etiology and vehicle, from 1999 to 2008, foodborne cases of gastroenteritis by NoV are attributed in the respective ranking to complex foods, produce (vegetables) and seafood (Batz *et al*., 2012). Based on expert elicitation, the ranking is, when attributing NoV cases, in respective order, produce, seafood and complex food (Batz *et al.*, 2012). Around 10-34% of Nov foodborne cases are attributed to seafood in the USA (shellfish mainly) (Batz *et al*., 2012).

In France, from the data 2009, among 1255 outbreaks, 18.4% were confirmed from a pathogen, and 39.9 suspected (InVS, 2012). Among last category 12% were attributable to viruses. Among those outbreaks, when analyzing the food product, shellfish are involved in 85 outbreaks (drinking origin 6, complex food 269, egg products 84 and fish product 87). Shellfish are in the third position ahead of

egg products, meat (80), chicken meat (67), milk (50) or pig products (53), which is important considering that the consumption is limited in a general population in comparison to other products. For example in a study of consumption in general population study of 2492 individuals, INCA, 1999, only 56 consumers of oysters and 216 consumers of mussels were registered (AFSSA, 1999). "The analysis of data from systems contributing to the surveillance of foodborne illnesses and from published outbreak investigations shows that, in France, foodborne illnesses associated with shellfish consumption are mainly of viral origin, mostly due to NoV followed by HAV" (Vaillant *et al*., 2012b).

Thus our work focused on shellfish contamination with HAV and Norovirus, for three main arguments:

- Shellfish accumulate virus not passively, in comparison with other contaminated food vehicles.
- Different risk ranking classifies shellfish as one of the first level of concern for virus contamination.
- The shellfish contamination and coastal population close to shellfish areas of production can be studied, in order to investigate the relationship between food contamination and the human epidemiological situation.

1.3. Biological characteristics of norovirus and hepatitis A virus.

Norovirus and HAV have some features in common, in particular the transmission pathway. For other aspects, their biological characteristics, impact and immune response of their human hosts are different, thereby justify specific approaches.

1.3.1 Biological Characteristics of norovirus

Genetic diversity and molecular epidemiology

NoV belongs to the Family *Caliciviridae*, with a single-stranded positive sense RNA genome, non enveloped capsid virus, explaining its high resistance in the environment.
NoV can be divided into distinct genogroups (or genera), based on phylogenetic analyses of the capsid protein. To date, five NoV genogroups (G) have been recognized (GI-GV). Viruses of GI, GII, GIV are known to infect humans (Zheng, 2006). GII viruses have additionally been detected in pigs. GIII viruses infect cattle and sheep and GV viruses infect mice. Human Infection from viruses of animal origin is not feasible or rare. GI and GII are the most frequent genogroups associated with human cases, in particular GII in human epidemics. The recombination among viruses from different genogroups is rare, suggesting that genogroup constitutes a species level in taxonomy (Zheng, 2006). Norovirus display a wide degree of genetic variability between genogroups (44.9-61.4%). Inside

genogroups they are subdivided into clusters or genotypes (variability between clusters 14.3-43.8), for example the genotype GII.4. Finally, clusters or genotypes are subdivided into strains (variability between 0 and 14.1%) (Zheng *et al*., 2006). Since 2002, GII.4 is an emerging cluster, involved every two years in pandemics, (2002, 2004, 2006, and 1974 or 1995-6) (Lopman *et al*., 2004; Karst *et al*., 2010; Siebenga *et al.,* 2009). The GII.4 cluster is perhaps more severe, spreading rapidly from inter-human transmission and associated with AG (Acute Gastroenteritis) epidemics in winter (Lopman *et al*., 2004; Lindesmith *et al*., 2008; Rohayem *et al*., 2009). The mutation rate is high and is of importance when explaining regular epidemics in human populations (Dingle *et al*., 2004; Lindesmith *et al*., 2008; Bull *et al*., 2010).

CLINICAL DISEASE AND EXCRETION

The initial description of large Nov outbreaks called the "winter vomiting disease" (Mounts *et al*., 2000). In immunocompetent adults, the incubation period is 24-48 hours (min 10-max 50) and the duration of symptoms is within 12-72 hours (Karst *et al.,* 2010). The symptoms include vomiting and unbloody diarrhea with or without nausea and abdominal cramps (Kaplan, 1982). Norovirus can be much more severe and prolonged in specific risk groups, in the elderly (>=65), even resulting in death (Lopman *et al.,* 2003; 2004; Van Asten, 2011; Gustavsson *et al.,* 2011; Rondy *et al*., 2011; Verhoef *et al*., 2012). Case fatality ratios were recently estimated from German surveillance data to be around or less between0.02-0.04/ 100,000, changing a little with age and susceptiblity; 0.1/ 100,000 the first year of age, 0-0.001/ 100,000 between 2 and 11, 0.07/ 100,000 between 11 and 17, around 0.03% between 18-64, and 0.63/ 100,000 more than 65 (Verhoef *et al*., 2012). Norovirus disease can be also more severe in association with other infections such as inflammatory bowel disease (Khan *et al*., 2009). Norovirus is also implicated in gastro enteritis of children (Zintz, 2005), and is particularly involved in nosocomial infection (Gallimore, 2006; Lopman *et al*., 2004; Sukhrie, 2010, 2011, 2012; Greig and Lee, 2012). Other symptoms are also described in recent papers (Porter *et al*., 2012; Nelson *et al*., 2012).

Although the symptoms are usually gone after several days, virus particles can be shed from asymptomatic individuals up to several weeks after exposure. In a recent study, the median duration of shedding was 28 days after inoculation (range 13–56 days) (Atmar *et al*., 2006). The median peak amount of virus shedding was 95×10^9 (CI 95 0.5–1,640 $\times 10^9$) genomic copies/g feces as measured by quantitative RT-PCR (Atmar *et al.,* 2006). The peak period is during 3-10 days and decreases during the last 21 days.

With the antigen ELISA detection method, the mean duration of shedding was estimated to be around 10 days (median 7 days) after inoculation (Atmar *et al*., 2006).

EPIDEMIOLOGY

Globally, Noroviruses are the first foodborne origin of acute gastro enteritis (bacteria and parasites included), with 95% of non bacterial GE (Karst, 2010) and more than 50% of GE by itself (Batz, 2012). For the cost of illness, Nov are rank fourth in foodborne pathogen (after Salmonella, Toxoplasma and Listeria), taking into account the incidence, the severity of symptoms and the cost of treatment, in the US (Batz *et al*., 2012).

High attack rates are commonly reported in NoV outbreaks. Primary attack rates are around 50% of exposed individuals (Noda *et al*., 2008; Matthews *et al*., 2012). Norovirus outbreaks occur most commonly in semi-closed communities such as cruise ships, hospitals, schools, disaster relief/evacuation sites and military settings (Karst, 2010).
Epidemics with intense inter-human transmission occur mainly in winter time (sentiweb), in France and elsewhere in the world (Lopman *et al*., 2003).

IMMUNITY AND SUSCEPTIBILITY

- classical immunity

The duration of norovirus antibody responses has not been clearly determined. The lack of long term immunity is however recognized (Karst *et al*., 2010). Volunteer studies show that individuals are equally susceptible to primary and secondary exposure when there is at least a six-month interval between challenges (Johnson *et al.*, 1990). The variability and evolution of strains involved could partly explain the phenomena (Lindesmith *et al*., 2008; Bull *et al*., 2010).

- Susceptibility-resistance in humans

High infectivity is described for norovirus infection for low doses of exposure in susceptible individuals (Teunis *et al*., 2008). However, the human noroviruses recognize histo-blood group antigens (HBGAs) that are expressed on the surface of mucosal epithelial cells. The glycosyltransferases that control their synthesis are encoded by the highly polymorphic ABO, Lewis, and secretor gene families (Marionneau *et al.,* 2005; Le Pendu *et al*., 2006, Tan *et al.,* 2008). The association of noroviruses with HBGAs has been demonstrated to be essential for NoV strains (Lindesmith *et al.*, 2003). This is best exemplified by the correlation of secretor status with Nov susceptibility (Teunis *et al.,* 2008). Secretor status is expressed in gut epithelial cells and then can be tested with saliva specimen (Lindesmith *et al*., 2003). Secretor status is linked with wild type FUT2 gene (referred to secretors) that represents around 80% in the Caucasian population (Marionneau *et al*., 2005). Blood type (A, B, O, AB) can also interfere with susceptibility to Norovirus (Le Pendu *et al*., 2006).

- Vaccine

Vaccines are still experimentally tested but not used to prevent cases in population (Atmar *et al*., 2011).

RESISTANCE IN ENVIRONMENT

Noroviruses are highly resistant in the environment. Experimental studies use of quantification of genomes, and not demonstrated infectious particles. Laboratory, commercial settings and known outbreaks history, have shown that depuration times are inadequate to remove viruses despite the rapid removal of indicator organisms (Lees, 2000; Mc Leod *et al*., 2009; EFSA, 2012). The NoV GII contamination was reduced from 2900 to 492 copies /g of DT after 17 days of relaying (Le Guyader *et al*., 2008). NoV were further reduced from 492 to 136 copies/g of DT in 4 days and <100 copies/g of DT in 6 days at 17 °C for NoV GII genotypes (Dore *et al*., 2010, EFSA, 2012, Flannery *et al.,* 2012).

DETECTION-QUANTIFICATION IN SHELLFISH

The quantification is done with real-time RT-PCR. The quantification of NoV is difficult, as the comparison of results between laboratory, without a harmonization of the way to quantify. In particular, before any comparison, some steps should be examined carefully because they could be considered as critical: (i) sensitivity of the technique should be high, in particular, the extraction efficiency should be above 10%, because low numbers of virus particles could cause disease, (ii) the necessity of searching with different primers because of the high variability of the virus genome, and (iii) the control of the possible presence of inhibitory substances (EFSA, 2012). Without any cell culture system nor animal model for human GI or GII, infectivity estimates cannot be classically estimated. The integrity of capsids should be an alternative to investigating infectivity, and experimental work was done to investigate persistence with artificial VLP (Loisy *et al*., 2005). However, the integrity of the capsid is not enough in itself to certify infectivity and cannot be used in routine surveillance.

1.3.2. BIOLOGICAL CHARACTERISTICS OF HEPATITIS A VIRUS

GENETIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY

HAV belongs to the genus Hepatovirus within the Picornaviridae family, and is a non enveloped capsid virus, with a single-stranded positive sense RNA genome. Six genotypes are described at the present time (Costa-Mattioli *et al.,* 2002; Lu *et al.,* 2004). Three out of these six (I, II and III) are of human origin while the others (IV, V and VI) are of simian origin. In France genotype IA is the most frequently discovered, and genogroup IIIA, less so, in 2008 and 2009 (ANSES, 2010).

CLINICAL DISEASE AND INFECTIOUS MATERIAL EXCRETION

Clinical symptoms are linked to age. Hepatitis A infection mostly develops asymptomatically or subclinically among young children (under 5), while for older children and in adulthood the infection usually develops with symptoms.

Only 10% of infected children under six years of age develop jaundice. Among older children and adults, the infection usually causes more severe symptoms, with jaundice occurring in more than 70% of cases (Poovorawan *et al*., 2005; WHO, 2000). Asymptomatic cases, in particular in children, are considered to be infectious (Armstrong and Bell, 2002).

The incubation period of hepatitis A ranges from 15 to 50 days, with a mean of 30 days.

Clinical illness usually does not last longer than 2 months, although 10%-15% of patients have prolonged or relapsing signs and symptoms for up to 6 months (Glikson *et al*., 1992; Sjogren *et al*., 1987).

The beginning phase prodroma is characterized by arthralgia, myalgia, fever and digestive symptoms (anorexia, nausea, abdominal pain). The typical case definition for hepatitis A is an acute illness with moderate onset of symptoms (fever, malaise, anorexia, nausea, abdominal discomfort, dark urine) and jaundice, in addition to elevated serum bilirubin and aminotransferases levels later on. Icteric phase duration is between 1 to 4 months (median 2 months).

Viraemia can be detected before clinical symptoms. Fecal excretion of viruses begins 3 to 10 days before the symptomatic phase. By RT-PCR, the duration is 80 days (range 57-127) (Tjon *et al*., 2006). Using Elisa screening the excretion is detected after symptoms and lasts 3 weeks in average (Polish *et al*., 1999; Hollinger *et al*., 2001). The quantity of virus should be maximum at the beginning of the clinical signs and is estimated around 10^9particles /g or 10^8 genomes copies/ml of feces. Sixty days after the beginning of symptoms, excretion should be around 2.10^3copies/ml of feces (Tjon *et al*., 2006). It is intermittent and viruses are detected in 50 to 94.5% of symptomatic cases (Yotsuanagi, 1996). Excretion is also expected to be detected in asymptomatic cases especially in children.

Because the clinical characteristics are the same for all types of acute viral hepatitis, Hepatitis A diagnosis must be confirmed by a positive serologic test for immunoglobulin M (IgM) antibody to Hepatitis A virus. In France, cases are defined by a positive IgM-anti HAV in blood serum. Cases are notified by the laboratory and the clinician to the district health department (DDASS), which transmits the results to InVS.

Occasionally the infection may evolve into a fulminant hepatitis, mainly among patients with underlying chronic liver diseases (Akriviadis and Redeker, 1989). Chronic Hepatitis A is exceptional.

The case fatality rate is calculated on the basis of symptomatic cases (hepatitis) and can be estimated between 0.2-0.4% for children and around 2% for adults over 40.

The risk of infection seems to be lower than for Norovirus for the same dose. Dose-response used in QRA was established with a surrogate virus, Echovirus 12 (Rose and Sobsey, 1993; Shuval and Fattal, 2003; Pinto *et al*., 2009). In this dose-response, a mean dose of 6 PFU is associated with 1% of the risk of moderate infection. The dose seems inversely correlated with the length of incubation (Istre *et al*., 1985)

EPIDEMIOLOGY

- Cases in the general population:

  Hepatitis A occurs worldwide and causes about 1.5 million cases of clinical hepatitis each year (WHO, 2000). Seroprevalence is highly correlated to hygiene and sanitary conditions, the socio-economic level and other development indicators (Jacobsen *et al.,* 2004). Moreover, the likelihood and severity of symptomatic illness are age-related (WHO, 2000). In a low endemicity area, HAV infection is more frequently observed in adults, who are more likely to show clinical symptoms, be hospitalized and occasionally die, while infants and young children are usually asymptomatic (*WHO, 2000)*. In Italy, a progressive reduction in the prevalence of infection in children has been observed over the last decade, and the percentage of patients with severe clinical presentations has progressively increased (Gentile *et al.,* 2009,). In France sero-surveys of French military recruits, at a mean age of 21.2 years, declined from 50% in 1978 to 11.5% in 1997 in young adults (Joussemet *et al.,* 1999). The surveillance of acute hepatitis A has been based on mandatory notification since November 2005 in France. The incidence of reported cases of hepatitis A (notification rate) was as low as 2.2/100,000 in 2006, 1.6/100,000 in 2007, 1.97/100,000 in 2010 (InVS 2012). Foreign travel is suspected to contribute to 30% of cases. In 2010 33% cases were linked with outbreaks. A new serological survey confirmed the low level of seroprevalence (Lepoutre *et al.,* 2011). In developing countries with very poor sanitary conditions and hygienic practices, most children (90%) have been infected with the hepatitis A virus before

the age of 10; In France seroprevalence around 10 years is around 6%. A seasonal pattern of incidence can be described with the return of foreign travelling in Autumn (InVS, 2012).

- Outbreaks:

Foodborne outbreaks are found to be on average, between 5-6 % of all outbreaks (Mead *et al*., 1999, Scallan *et al*., 2011). Raw seafood is a known source of hepatitis A outbreaks in France (Guillois *et al*., 2009) and abroad (Desenclos *et al., 1991,* Conaty *et al., 2000;* Shieh *et al., 2007*). Epidemics related to contaminated food or water can erupt explosively, such as the epidemic in Shanghai, attributable to the ingestion of raw clams in 1988 that affected about 300 000 people (Halliday *et al*., 1991). In France, two hepatitis A outbreaks linked to oyster consumption were identified in 1999 (33 identified cases) and 2007 (111 identified cases) (InVS, 2007; Guillois Becel *et al*., 2009) in the same location in Brittany. However, other contaminated food products can be involved, linked to the oro-fecal pathways, in outbreaks such as fruits (dried tomatoes) or vegetables (Gallot *et al*., 2011; Calder *et al*., 2003; Dentinger, 2001).

Cooked foods also can transmit HAV if the temperature during food preparation is inadequate to suppress the infectivity of the virus. From frozen imported clams (Peroo), slightly cooked, two outbreaks were associated in Spain (Bosch *et al*., 2001). One in 1999 affected 184 patients (lasting 3 months). The second one in 2008, lasting 7 months affected 100 patients (Pinto *et al*., 2009).

Food can also be contaminated after cooking, as it occurs in outbreaks associated with infected food handlers (Chirona *et al*., 2004, Rowe *et al*., 2009). Waterborne HAV outbreaks are infrequent in developed countries with well-maintained sanitation and water supplies. In the USA, Hepatitis A (22%) and Shigella (16%) were the two most frequently identified etiologic agents of waterborne outbreaks in the 1960s (Craun *et al*., 2006). During 1971–1990, the relative contribution decreased for hepatitis A (4%) and Shigella (6%), and for 1991-2002, HAV relative contribution to waterbone outbreaks was around 2/207 (less than 1% with 56 cases in total) (Craun *et al*., 2006).

IMMUNITY AND SUSCEPTIBILITY

A single serotype of HAV has so far been reported. Immunity is protective whatever the genotype (among the 6 reported) is of the first infection, and immunity is considered lifelong.

Susceptibility-resistance

No particular genetic resistance or susceptibility has been detected.

Vaccine

Vaccine is used to efficiently protect an exposed population. Some new variants detected in a population at risk could escape the protective effect of available vaccines (Perez-Sautu *et al*., 2011).

RESISTANCE IN ENVIRONMENT

HAV was found to be very resistant. Because a laboratory strain can be cultivated, the survival of infectivity was confirmed experimentally on this particular strain.

DETECTION-QUANTIFICATION IN SHELLFISH

Cell culture is feasible but very difficult and in particular is not efficient routinely for wild types of HAV. The main way to detect (and quantify) is real-time RT-PCR, without any confirmation of infectivity.

1.3.3. Comparison between HAV and NoV biological characteristics

In summary, HAV and norovirus have some characteristics in common:

- The fecal oral transmission is the main pathway for HAV and NoV, highly resistant in the environment, with same food products at risk, in particular shellfish, vegetables and drinking water.
- Lack of cell culture, makes the evaluation of contamination classically made by real-time RT-PCR. The contamination is expressed in genomes, and no way is available to estimate the relative infectivity of these genomes
- In comparison to foodborne bacteria, no multiplication is feasible in food.
- Both have a seasonal pattern peak of incidence. In winter for NoV and in autumn for HAV (linked to travel acquired cases) (InVS, 2011).

HAV and NoV have some important differences:

- High diversity of strains and no long protective acquired immunity for NoV.
- Lesser diversity of strains and one serotype for HAV, causing life-long or quasi life-long protective immunity, and making vaccine efficient for HAV.
- Difference of mechanism of susceptibility in humans, for NoV linked to the affinity and presence of specific receptors, linked to classical antibody immunity for HAV (seroprevalence in the population).
- High infectivity at low doses is suspected for NoV but not for HAV.
- The annual incidence is very high for NoV, not for HAV.
- Excluding global immunodepression situations, susceptibility is different with age for HAV, in general less for NoV.
- Asymptomatic excreting cases are identified and important source of transmission for HAV, but not for NoV.
- Case fatality rate is stronger for HAV than for NoV.

These differences can explain why both viruses were kept for modeling purpose, with their own characteristics.

1.4. Conceptual Framework and modeling approach

Globally foodborne public health impact cannot be easily and precisely estimated from epidemiological data, and can vary in space and time (Hardnett *et al*., 2004; Vaillant *et al*., 2012a).,

2004. Moreover the foodborne pathway can be correlated to from other transmission, by example person to person or environmental transmission (contact with contaminated surface. Then, it is not feasible to easily predict the public health impact when setting quantitative limits of contamination in food or waste water.

Environmental studies give the level of contamination in oysters observed with foodborne outbreaks (Lowther *et al*., 2012a and b), but do not explain the mechanisms involved (how oysters were contaminated, what was human consumption, is there other transmission pathway possible).

A modeling approach in a limited context can explore easier these mechanisms. A quantitative risk assessment can give an idea of the link between the food contamination and foodborne cases. Dynamic modeling can take into account inter -human transmission.

Our efforts focused on a coastal situation, with HAV and NoV contaminating shellfish, because all the parameters we need to describe were present.

The conceptual framework we tried to study in this PhD is summarized, for a HAV situation, in **Figure 3**, but can be easily extrapolated to NoV.

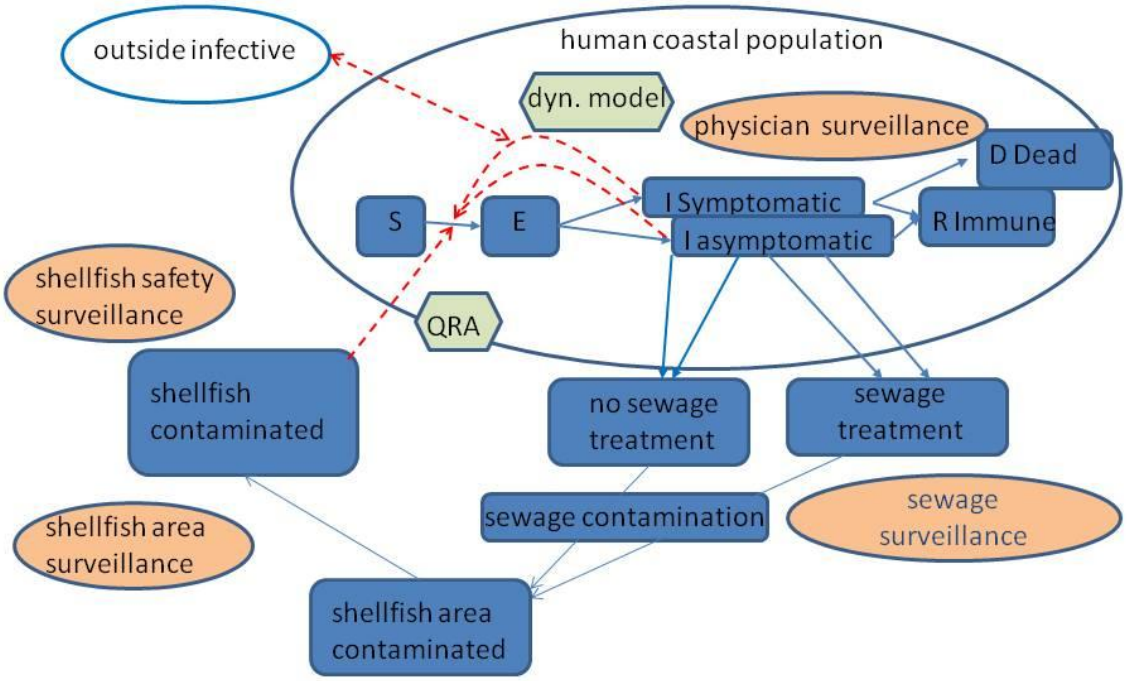

FIGURE 3: CONCEPTUAL FRAMEWORK OF THE PHD, ILLUSTRATED FOR HAV SITUATION

Legend: In blue the transmission pathway in human and in the environment under study; in orange the surveillance; in green, the modeling proposal

The Figure 3 illustrated the conceptual framework, with the inter-human and foodborne transmission pathways. Only one foodborne pathway is considered, the oysters contamination and consumption. The feco-oral contamination of other products such as water supply or vegetables is considered negligible. The consumption of shellfish by the coastal population is supposed to be local. The contamination of shellfish is considered to originate from the coastal population. Thus the system is quite closed, except for some population moves, for touristic, travelling or migrating purposes, which can export or import the disease.

The different states of individuals are described as "S" for susceptible, "E" for exposed or infected, "I" for infectious (symptomatic or not for HAV) "R" for Immune, and "D" for Dead (HAV situation), in a classical SEIRD dynamic system. Infection can occur from direct or indirect transmission with a symptomatic or asymptomatic case or from consumption of contaminated food. The description of real outbreaks shows that this framework can be based on real observations (Guillois-Becel *et al*., 2009; Halliday *et al*., 1991). Modeling this situation was done for HAV (Ajelli *et al*., 2008). The role of environmental reservoir with feco-oral pathway is described for other pathogen, such as wells or water for cholera by dynamic modeling (dyn. model. in Figure 3) (Hartley *et al*., 2006; Righetto *et al*., 2012). However, the pathway between infectious individuals, rejecting contaminated stools, to shellfish contamination and the risk of infection through food consumption is generally a blackbox, not taking into account, in particular, the classical triad data of contamination, consumption, and related dose response used in QRA (Ajelli *et al*., 2008) (QRA in Figure 3). It is partly justified by the objective, focusing the safety management approach on the interruption of interhuman transmission through early detection (physician surveillance), improving hygiene practices, social distancing or vaccine uses (Ajelli *et al*., 2008). Only one paper, relating to foodborne viruses, describes the cycle from infectious humans to sewage contamination, in order to investigate the efficiency of monitoring Poliovirus in sewage water, with different situations of epidemics in the population (Ranta *et al*., 2001).

Eisenberg describes and estimates using a dynamic deterministic approach, the overall cycle for enterovirus and Cryptosporidium for a drinking water or a bathing situation (Eisenberg *et al*., 1998; 2004; 2005). On the other hand, a quantitative risk assessment for viruses linked with contamination of food products, is rarely done using a probabilistic approach (see part II).

This is the methodological innovation of this work in order to investigate the improvement of using QRA within an epidemiological context. Then, the efficiency of management strategies such as the monitoring of the shellfish production area or the monitoring of shellfish safety (Figure 3) can be investigated more precisely. The first part of this PhD will deal with that approach.

Dose-response is a crucial step in QRA. In particular it is needed in food safety assessment for setting maximum limits of contamination in food products. No validated dose-response with observed human cases was available for QRA for NoV, genogroup II, and the most frequent in human population. Available data from oyster outbreaks were used, in a Bayesian network, in order to fill in this data gap.

Finally, the impact of forbidding oyster consumption, whenever abnormal contamination is detected, can be estimated with a predicted attack rate, as it is usually done in QRA analysis. But it is possible to further investigate trying to evaluate the public health impact including secondary inter-human transmission, using a stochastic dynamic modeling. This is the purpose of the third part of this PhD.

1.5. Overall aim of this study

The main objective of this study is to propose a better estimation of the oyster contribution of viruses, and to suggest ways to limit their impact. Norovirus is first cause of foodborne gastro enteritis (Karst *et al.*, 2010, Batz *et al.*, 2012). HAV can be a re-merging problem, in high income countries, in particular for human outbreaks, involving elderly people less immunized than before. Those two viruses are considered to be main foodborne viruses to consider (InVS, 2004).

In France, shellfish as food product is identified as the third contributor to foodborne outbreaks, after complex food, and other seafood product. Furthermore, for studying the potential impact and mechanisms involved in foodborne outbreaks and potential epidemic impact, we chose to focus our efforts to describe the situation in a coastal human population, potentially concerned by HAV and Norovirus epidemics, close to a shellfish production area. The consumption of contaminated shellfish is considered to be a plausible pathway of transmission of the disease for this targeted population. This situation is not only theoretical. Two coastal epidemics of HAV were linked to local consumption of contaminated shellfish in Brittany (France) (InVS, 207). In Italy and Australia the potential impact of HAV contamination in shellfish was investigated (Conaty *et al*., 2000, Lopalco *et a*l., 2005). For norovirus, the detection of contamination in shellfish areas is also problematic for risk managers. Lastly from initial outbreaks with the first cases linked to food contamination, secondary cases and epidemics may occur (Halliday *et al*., 1991).

Considering the feco-oral transmission mean from coastal population to shellfish, we also consider management strategies and their potential impact on epidemiological situations. The Feco-oral pathway of transmission, involving food and inter-human transmission is not limited to foodborne viruses, and concerns other pathogens, such as cholera. Because pathways of transmission are not independent of each other we will also take into account the contamination of food as well as the epidemiological situation in the population and the secondary cases in the third part of this PhD

We chose a quantitative and probabilistic approach in order to quantify as much as possible, the variability effect (and uncertainty for part II and III). A modeling approach enables us to better understand biological mechanisms involved and to investigate their plausibility and consequences for management strategies. Specifically, among the different management strategies, the effect of setting a maximum limit of contamination in shellfish, the usefulness of monitoring and management of shellfish safety and the potential impact of forbidding the consumption of shellfish during a particular period will be investigate with a probabilistic analysis.

CHAPTER II: QUANTITATIVE RISK ASSESSMENT OF FOOD BORNE VIRAL CASES

II.1. INTRODUCTION

The need for an objective tool to improve food control system and harmonize international trade in foods was at the origin of quantitative risk assessment, giving numeric values to compare risk (FAO/WHO 1995; Codex alimentarius Commission, 2003; Vose 1998; Haas and al., 1999).

Risk is defined as the probability and severity of an adverse event. For food safety, it can be identified as the probability of illness conditionally to the exposure, by contaminated food with an identified hazard[1] (Codex Alimentarius Commission, 2003).

To summarize, organize and harmonize the procedure, the main different steps, from farm to fork were defined classically by (Haas *et al.,* 1999):

> Hazard identification: "to describe acute and chronic human health effects associated with any particular hazard, including toxicity (...)"
>
> Exposure assessment: "to determine the size and nature of the population exposed and the route, amount and duration of the exposure".
>
> Dose-response assessment or Hazard characterization: "to characterize the relationship between various doses administered and the incidence of the health effect."
>
> Risk characterization: "to integrate the information from exposure, dose-response, and health steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty".

The Figure 4 shows the connections that can be established between these different steps.

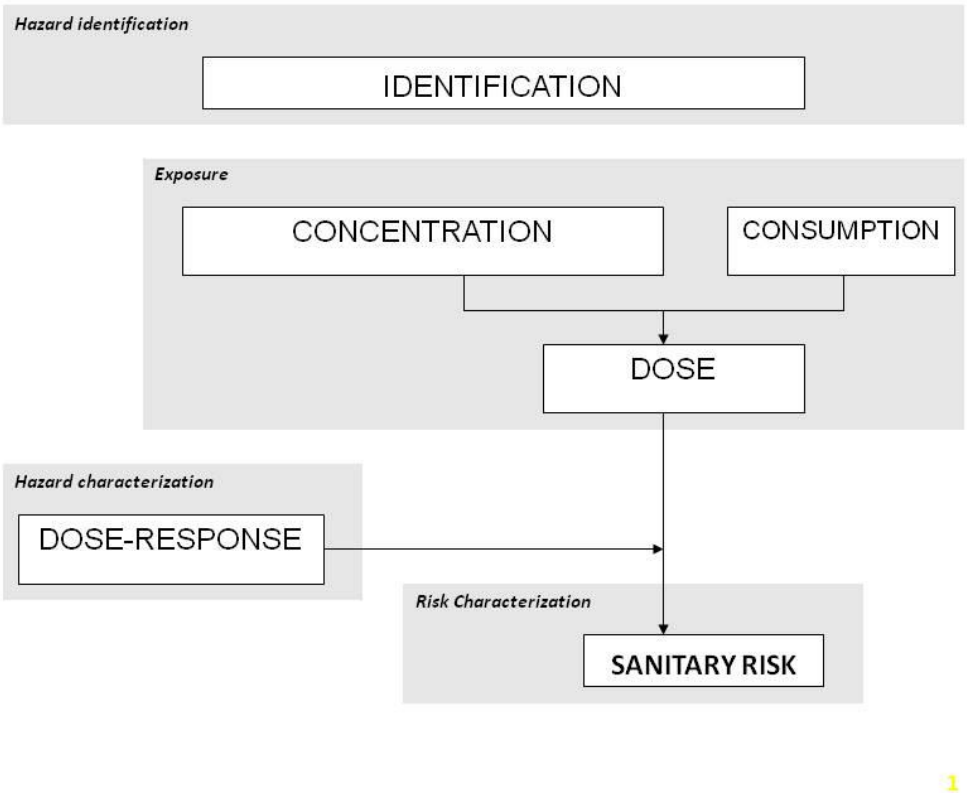

FIGURE 4: DIFFERENT STEPS OF QUANTITATIVE RISK ASSESSMENT

Legend: The product of consumption by concentration gives the ingested dose, that is the result of exposure assessment, using the dose in dose-response relationship (given by hazard characterization), the probability of infection or disease can be evaluated (risk characterization).

To protect consumers and compare sanitary effect of different management strategies, microbial risk assessment provides a comprehensive and reliable scientific prospective tool (WHO, 2001; WHO, 2011).

Increasing number of studies using Quantitative microbial risk assessment (QMRA) were published during the last decade (Delignette-Muller *et al*., 2008; Mokhtari *et al*., 2009; Pouillot *et al*., 2004). However most of them are dealing with bacteria or parasites, and few with viruses (EFSA, 2011; Mokhtari *et al*. 2009) even if, one of the older published paper of quantitative risk assessment was dealing with viruses (Regli *et al*., 1991). Bacterial growth is a sensitive subject for agro industrial sector, with the preparation and storing of complex or fresh food products (Bemrah *et al*., 1998; Havelaar *et al*., 2004; 2007; Albert *et al*., 2005; Rieu *et al*., 2007; Albert *et al*., 2008; Pouillot *et al*., 2009, 2011, 2012; Nauta *et al*., 2012).

The pertinence of quantitative risk assessment lies also in its ability, like any other modeling to reflect the complexity of the phenomena under study, in its necessary (essential) components (Vose, 1998, 2000).

Variability is irreducible and represents the heterogeneity of the studied biological population. By example, the consumption of oysters for human French population can be defined with its variability between consumers. However if the population under study changes, variability changes also.

Probabilistic approach is then needed to estimate this variability (Vose, 1998). Point estimates, taking into account mean (or median) value at each step or risk assessment, are not in general used with the generalization of monte-carlo simulation, making easier probabilistic approaches. The variability is sometimes not well described by a normal distribution. In this case median and mean can be not similar (by example for Log-normal distribution). Moreover, if relationships are not linear, point estimate of the resulting mean value is not correctly estimated.

> If time before consumption of a food product can be described by a normal distribution
> t ~ N (mean=15, sd=3)
> If initial concentration of a pathogen in this product can be described with a Poisson distribution of parameter, mean,
> C_0 ~P(10)
> If decreasing of the concentration in time is described by the relation C_0.exp(-(T-To)/T90) wit To =0 and T90=3
> With point estimate the mean concentration at consumption is :
> Mean (C)=10.exp(-15/3)=0.067
> With 10 000 sampled values of t and of C_0 in their respective distribution the mean concentration at consumption is :
> mean (C)=0.011
> If values of initial contamination are linked with the duration before consumption, the mean of c , again, would be different.

Finally it can be interesting to investigate extreme percentiles of exposure, or to compare results with observed data (which is difficult with point estimate), or to take into account correlations between variables. For all these reasons, probabilistic estimates are needed in particular for complex QRA.

Uncertainty is defined as a lack of perfect knowledge of a given variable. This lack of knowledge can be reduced by further experimental (or field) data, or by increasing the sampling size of investigations. Uncertainty can also be estimated by probabilistic distributions (Vose, 2000).

With probabilistic approaches, and the use of Monte-carlo simulations, the variability and the uncertainty of the results can be evaluated in the same QRA (Vose et al.1998).

The separation of uncertainty and variability of model parameters is now recommended in risk assessment and is described as second order risk assessment (Nauta *et al.*, 2000, Vose, 2000, Pouillot *et al.*, 2004, Rimbaud *et al.*, 2010). The result of such modeling is informative because it gives information of what can be reducible (uncertainty) by future research, and also separate and show quantitatively what we know and what we don't know.

The way to do this is first to determine what parameters are linked to variability and uncertainty. Sometimes it can be complex: by example the infectivity of virus in a food matrix can be a parameter describe by a variability distribution, by example by a Beta (α, β) distribution, but the parameters of this beta can be not well known. The uncertain distribution of α and β parameters that can be defined as hyperparameters. The method and the way to present the method were inspired from other work by (Pouillot, 2006) and (Crepet, 2007).

The number of virus in genome, $N_{g,}$ in a meal is described by a Poisson distribution:

N_g~P(10).

The percentage of infectious viruses is given by a Beta distribution

Pr_{inf}~Beta(α, β)

The resulting number of infectious virus for a known value of α and β is described by a Beta-Binomial distribution, the number of trials is given by N_g and the probability of success by Pr_{inf}:

Ng_{inf}~Bin (N_g, Pr_{inf})

Hyperparameters distributions of α and β can be estimated by Bayesian inference (there's other way to do, not detailed here). Then joined posterior distributions can be sampled. An example of hypothetical sampled values of those hyperparameters, describing their uncertainty is given in the Table above:

simulation	α	β
1	10	30
2	5	10
k	2	8
..	2	10
N	3	4

For each simulation k, from 1 to K, α_k and βk are random values of hyperparameters α and β, for different distribution of variability of infectiousness Beta (α_k, βk).

For each simulation of uncertainty, a row is randomly selected, say k, sampling one value of the distribution for α, α_k and one value of β, βk are selected.

Then for each iteration u of variability, from 1 to N we sample randomly a value from $pr_{inf(u)}$ ~ $_{Beta}(\alpha_k,\beta_k)$, of $N_{g(u)}$, and of $N_{inf(u).}$

For one value of α_k, βk, by N monte-carlo simulations (usually thousands) of variability, we obtain a distribution for N_{inf}, and it is possible to calculate statistics, such as quantiles, or moments such as mean or variance.

To illustrate this we simulate one thousand simulations with α_k= 2, β_k=8. We obtain for those particular values of uncertainty, the distribution of concentration of infectious viruses (variability)

mean	2.5 th percentile	median	97.5^{th} percentile	α	β
1.97	0	2	6	2	8

Of course the sampled values of α and β should be changed in order to explore the effect of uncertainty on results. Then in a second step, new sample values for α and β are randomly selected, and with those new values of hyperparameters, new estimate of statistics of the distribution of

variability are calculated. Simulating K times this two level with enough Monte-Carlo simulations, (usually thousands), a two-dimensional table is obtained:

mean	2.5 th percentile	median	97.5th percentile	α	β
1.97	0	2	6	2	8
1.68	0	1	6	2	10
….	…	…	…	…	…
median, IC 95 of the mean	median, IC 95 of the 2.5 th percentile	median, IC 95 of the median percentile	median, IC 95 of the 97.5th percentile		

From results of this two-dimensional table, different statistics (in particular quantiles) can be calculated for the mean, and the different percentiles of the distribution representing variability.

The Figure 5 is showing the structure of the little example developed here

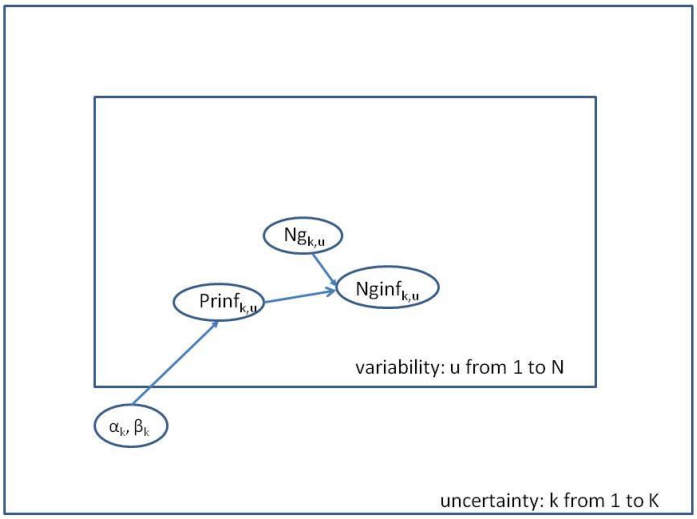

FIGURE 5: EXAMPLE OF SECOND ORDER MODELING

Legend: circles are describing random variables and arrows stochastic relationship between those random variables.

Usually 95% quantiles are representing the uncertainty credible interval (95% CI) about the mean or quantiles of the variability distribution. The median of the mean (and of quantiles) are given as usual estimators of results of second order risk assessment (Pouillot *et al.*, 2004; Crepet *et al.*, 2007, Rimbaud *et al.*, 2010).

In a more complex QRA different sources of uncertainties can be identified. In this case, each iteration of uncertainty is taking one value of each distribution of all uncertain hyperparameters, respecting, whenever it is known, correlations between them, if they are not independent.

However another dimension of uncertainty can also deal with model structure. The causality pathway is not always very well-known, and the biological mechanisms can be partly known. Other

hypothesis, other scenarios or other model can be compared in this case (known and described in what- if scenarios) (Vose, 2000).

Validation can be done, by comparison with other sources of information, and generally finding the same order of magnitude between prediction and observation, in a same context, is found satisfactory (Haas *et al*., 1999). However epidemiology is not an experimental and reproducible context, and then validation or reproducibility of an epidemiological model in another context is far to be trivial (Bonté, 2012).

Sensitivity analysis is done for establishing main factors (input) responsible of the main changes of the results (output) (Saltelli *et al.*, 2002). Different approaches are available (Mokhtari and Frey, 2005). Probabilistic models are characterized by difficult statistical constraints such as non linearities and interaction between inputs (Frey and Patil, 2002). Sobol's method is now one of the most used, because the importance of input is including interaction, non linear and non monotonic effect (Mokhtari and Jaykus, 2009; Lurette *et al*., 2009; Ellouze *et al*., 2010; Nauta *et al*., 2012).

II.2. Literature review of QRA for foodborne viruses

Published quantitative risk assessment dealing with viruses are given in the Table 3. The aim of this list is not to be exhaustive but to include main papers about virus risk assessment. There's no probabilistic risk assessment for shellfish viral risk, no complete risk assessment using second order risk modeling.

The few number of QRA for viruses is probably due to the lack of reliable dose-response for the main pathogen involved in food (NoV-HAV), the lack of the reliable and inexpensive way to measure the dose of pathogen (not indicator or surrogates) (RT-PCR technique were expensive), and the lack of feasibility of measurement of infectivity for Nov and HAV (EFSA, 2011, 2012; FAO/WHO; 2008).

Before the publication of Teunis (2008), the dose-response for norovirus or all viruses was substituted the use of Rotavirus dose-response (Regli *et al.,* 1991, Rose and Sobsey, 1993), and for HAV by the use of the surrogate Echovirus 1, 2 (see part III.2).

The question of aggregation or clumping of viruses is treated in some studies, more often with non homogenous distribution, negative –binomial use to describe the contamination in food matrix (Westrell *et al.*, 2006).

Then our work proposed in the quantitative risk assessment for HAV in shellfish is the second one dealing with shellfish since 1993 (Rose and Sobsey, 1993), using probabilistic, second order, risk modeling. Moreover, the proposed work includes the quantitative estimate of efficiency of different monitoring /management strategies of shellfish areas of production, in particular to avoid viral contamination in oysters, on the relative risk for human consumers, which was not done before.

Reference	Food	Virus	Part of QRA	stochastic approach		sensitivity analysis	validation*	remark
				probabilistic	second order	no		
Regli *et al.,* 1991	drinking water	rotavirus	complete QRA	no	no	no	no	
Haas *et al., 1*993	drinking water	rotavirus	complete QRA	partial	no	no	no	
Rose & Sobsey, 1993	shellfish	HAV and enterovirus	complete QRA	no	no	no	comparison with attack rate	
Gerba et al 1996	drinking water	rotavirus	complete QRA	no	no	no	no	
Crabtree et *al*., 1997	drinking water	adenovirus	complete QRA	no	no	no	no	
Petterson *et al.,* 2001	salad crops	rotavirus	complete QRA	partial	no	no	no	through wastewater irrigation, clumping exposure effect
Shuval *et al.,* 2003	shellfish / bathing	HAV and HEV	complete QRA	no	no			DALY cost
Hamilton *et al.,* 2006	raw vegetables (cucumber, lettuce	rotavirus	complete QRA	yes	no	yes(spearman)	no	through irrigation with reclaimed water
Masago *et al.,* 2006	drinking water	norovirus	complete QRA	partial	no	no	no	infection and DALY cost
Pinto *et al.,* 2009	coquina clams	HAV	complete QRA	no	no	no	partial	dose-response estimate
Mokhtari and Jaykus, 2009	retail food	Norovirus	Exposure model	yes	no	yes	no	
Mara and Sleigh 2010	lettuce	norovirus	complete QRA	yes	no	no	no	through irrigation with waste water (daly cost)
Schijven *et al.,* 2011	drinking water	rotavirus (and other campylobacter, giardia)	complete QRA	yes	no	no	no	neg binomial exposure

TABLE 3: PUBLISHED QUANTITATIVE QRA

II.3. QRA FOR HEPATITIS A VIRUS

II.3.1. PRESENTATION OF THE CONTEXT OF PAPER

The objective of the work is to investigate the relative efficiency of monitoring and management systems in case of contamination of shellfish by HAV. The method used is second order risk assessment, in order to propagate separately variability and uncertainty.

This subject emerged in France after two shellfish borne (highly suspected) outbreaks that occurred in the same area in Brittany in 1999 and 2007 (Guillois-Becel *et al.*, 2009). In order to prevent possible next outbreaks, a regular monitoring, based with HAV genomes, was done in the area of shellfish-fishing of the shore. Without any specific regulation, no particular monitoring was done for shellfish production, except the regular microbiological exams. In this context the signification of HAV genome detection and its effectiveness for monitoring and management purpose was asked by the ministry of agriculture to ANSES (ANSES, 2010).

The work realized for the group of expert was an opportunity to begin and illustrate the usefulness of the PhD work. However, this kind of question is not specific to the particular context in France, and in particular in Brittany, but could also be raised in other detected contaminated areas, in particular with HAV, such as in Australia or in the South of Italy (Puglia area) (Conaty *et al.*, 2000; Lopalco *et al.*, 2005).

II.3.2. PUBLISHED PAPER

Research Papers

Running title: risk management for hepatitis A contaminated oyster production

Title: Quantitative approach of risk management strategies for hepatitis A-contaminated oyster production areas

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Keywords: Hepatitis A virus, oyster, quantitative risk management, probabilistic model

ABSTRACT

It is not yet known whether using the new molecular tools to monitor the hepatitis A virus (HAV) in shellfish production areas could be useful for improving food safety. HAV contamination can be acute in coastal areas, such as Brittany, where outbreaks of hepatitis A have already occurred, and have been linked to consumption of raw shellfish. A quantitative probabilistic approach was carried out to estimate the mean annual risk of hepatitis A in a population of adult raw oyster consumers. Two hypothetical scenarios of contamination were considered, the first for a rare and brief event, and the second for regular and prolonged episodes of contamination. Fourteen monitoring and management strategies were simulated. Their effect was assessed by the relative risk reduction in mean annual risk. The duration of closure after abnormal detection in the shellfish area was also considered. Among the strategies tested, results show that monthly molecular (RT-PCR) monitoring of HAV is more useful than bacterial surveys. In terms of management measures, early closure of the shellfish area without waiting for confirmatory analysis was shown to be the most efficient strategy. When contamination is very short-lived and homogenous in the shellfish production area, waiting for three negative results before re-opening the area for harvest is time-wasting. When contamination is not well-identified or if contamination is heterogeneous, it can be harmful not to wait for three negative results. Finally, any preventive measures — such as improving sewage treatment or producing shellfish in safer areas — that can reduce contamination by at least two log10 units are more efficient and less costly. Finally we show that controlling and managing transferred shellfish is useful and can play an important role in preventing cases. Qualitative results from HAV monitoring can advantageously supplement other measures that improve the safety of shellfish products in exposed areas.

INTRODUCTION

Hepatitis A is generally an acute, self-limiting liver infection transmitted by a RNA picornavirus, the hepatitis A virus (HAV). Hepatitis A occurs worldwide and causes about 1.5 million cases of clinical hepatitis each year (*34*). Seroprevalence is highly correlated with hygiene and sanitary conditions, the socio-economic level and other development indicators (*13*). Moreover, the likelihood and severity of symptomatic illness are age-related (*34*). In a low endemicity area, HAV infection is more frequently observed in adults, who are more likely to show clinical symptoms, be hospitalized and occasionally die, while infants and young children are usually asymptomatic (*34)*. For example, in Italy, a progressive reduction in the prevalence of infection in children has been observed over the last decade, and the percentage of patients with severe clinical presentations has progressively increased (*9, 27*). In France, serological surveys of French military recruits show a decline in HAV seroprevalence from 50% in 1978 to 11.5% in 1997 (*14*). Surveillance of acute hepatitis A has been based on mandatory notification since November 2005 in France. The incidence of reported cases of hepatitis A (notification rate) was as low as 2.2 per100,000 population in 2006 and 1.6/100,000 population in 2007 (*5*).Therefore food borne outbreaks with severe cases can occur.

Infection is generally acquired via the fecal-oral route either through person-to-person contact or ingestion of contaminated food and water. Bivalve mollusks filter large volumes of water and thus may concentrate contaminants from polluted water receiving human sewage within their edible tissues (*15, 19, 21*). Even if raw sewage is treated, current water treatment practices are unable to provide virus-free sewage effluents (*20*). Regulations for shellfish production areas are based on acceptable levels of fecal indicators in shellfish tissues (European regulation No.854/2004/EC European and No 2073/2005/EC) such as *E. coli*. Unfortunately, the presence of *E. coli* is not tightly associated with the presence of viruses in areas classified in level A and B. This lack of association is due to differences in the survival-removal rate between *E. coli* and viruses in shellfish (*20*). The traditional purification duration of two days, appropriate for *E. coli*, is too short for virus decontamination (*17, 20*). Raw seafood is a known source of hepatitis A outbreaks in France (*10)* and abroad (6*, 11, 29*). However, other contaminated food products can be involved in outbreaks such as fruits or vegetables (*8*). In France, two hepatitis A outbreaks linked to oyster consumption were identified in 1999 (33 identified cases) and 2007 (111 identified cases) in the same location in Brittany (*10*). Since then, regular monitoring using quantitative real time RT-PCR to detect HAV has been added to the shellfish monitoring program for *E. coli* (*1*). Thus two questions arise: (1) how do shellfish monitoring and management strategies improve the safety of shellfish consumption in HAV-contaminated areas? (2) what is the cost incurred for shellfish farmers in terms of duration of closure of the shellfish production area ?

The aim of this study was to answer these questions, using a quantitative risk assessment approach (QRA), considering all available data and knowledge. The first quantitative risk assessment in shellfish with HAV was published in 1993 (*26*). Recent deterministic QRA (point estimates) have been done for HAV contaminated coquina clams as estimated from quantitative RT-PCR (*23*). In contrast, our approach is a probabilistic, second-order risk assessment that uses Monte Carlo simulations, thereby separately propagating variability and uncertainty of the model input variables (*25, 32*) to compare the efficiency of different management strategies.

MATERIALS AND METHODS

Baseline model parameters for a Quantitative Risk Assessment approach.

Four steps are necessary to assess a risk: (1) estimation of the contamination in food products; (2) estimation of consumption patterns; (3) exposure assessment by combining (1) and (2); (4) dose-response assessment. Parameter definition and the distribution and modeling of each variable are summarized in Table 4. For one individual and for a given day of the year, dose exposure can be evaluated by the product of oyster contamination times the total amount of edible oyster tissue eaten. The lapse of time between harvesting and consumption is most often short in coastal areas close to the production area and was not taken into account.

Estimation of contamination with a realistic number of genome copies per gram shellfish digestive tissue was done using two hypothetical scenarios, because observed quantified data of contamination of shellfish by HAV are rare. Values taken from an outbreak linked to coquina clams in Valencia, Spain, corrected for extraction and enzyme efficiencies, were around 230-1800 copies per gram of digestive tissue (*23*). The maximum uncorrected value observed in Brittany with monthly sampling, is around 1970 copies. Values corrected for extraction efficiencies (between 50% and 10%) and for enzyme efficiencies (around 90%) lie between 4,400 (1970*100/50*100/90) and 22,000 (1970*100/10*100/90) copies in grams of digestive tissue (*1)*. Monitoring of *E. coli* in either scenario gives results in agreement with European directives for Class B areas.

Parameter	Description/Value	Model/distribution	Reference
i	*index for individual i*		
j	*index for the day of the year*		
J_0	*First day of contamination in scenario 1*		
$C_{initVHA1J}$	*Incidental contamination for scenario 1 in genomes/g digestive tissue on day j* *T90 $_{VHA}$ =28 days* *C_{max}=25,000*	$C_{initVHA1j} = C_{\max} \times 10^{-(J-J_0)/T_{90VHA}}$	*17,23,1 assumption*
$C_{E.coliJ}$	*Incidental contamination by* E. coli *in scenario 1 per 100 g edible flesh day j* *T90 $_{E.coli}$ = 2 days* *Cmax=46,0000*	$C_{E.Colij} = C_{\max} \times 10^{-(J-J0)/T_{90E.Coli}}$	*24 assumption*
$C_{initVHA2J}$	*Contamination for scenario 2 in genomes/g digestive tissue on day j* *Min=Log10 (10)* *Max=Log10 (50,000)*	$C_{initVHA2} \sim 10^{\text{Uniform(Min,Max)}}$	*23,1 assumption*
$C_{E.coliJ}$	*Contamination by* E. coli *in scenario 2 per 100 g edible flesh on day j* *Min=log10(50)* *Max=log10(5,000)*	$C_{E.Colij} \sim 10^{\text{Uniform(Min,Max)}}$	*assumption*
No_j	*Noj: Number of copies in a single oyster* *$N_{initVHAj}$:Number of copies in digestive gland on day j* $\overline{Wdg}$ *: mean digestive gland weight* *R=5 % in tissues other than digestive gland*	$N_{initVHA_j} = C_{initVHA} \times \overline{Wdg}$ $No_j = N_{initVHA_j} + Negbin(N_{initVHA_j} + 1, R)$	*1* *1*
Nio_j	*Number of Infectious Copy In single oyster* *Inf:Infectiosity=1/60*	$Nio_j \sim \text{Bin}(\text{No}_j, \text{inf})$	*23*
$C_{gediblej}$	*Concentration of infectious genomes per gram of edible oyster tissue on day J for both scenarios* $\overline{Wo}$ *: mean oyster weight*	$C_{gediblej} = \frac{Nio_j}{\overline{Wo}}$	 *1*
C_{ij}	*Consumption for individual i on day j in grams of edible tissue*	*Empirical distribution (see text)*	*3, 2, 7*
P_{ij}	*Probability of hepatitis in individual i on day j* *α and β are correlated bootstrap replicates that vary with each uncertainty iteration* *α=0.373(maximum likelihood estimate)* *β=186.4(maximum likelihood estimate)*	*P_{ij}=BetaBin model($Dose_{ij}$, α ,β)* *With* $Dose_{ij} = C_{ij} \times C_{gediblej}$	*28, 23*
$P_{an(i)}$	*Annual probability of hepatitis in individual i*	$P_{an(i)} = 1 - \prod_{j=1}^{j=365} (1 - P_{ij})$	

TABLE 4: DEFINITION AND DESCRIPTION OF VARIABLES FOR QUANTITATIVE HEPATITIS A RISK ASSESSMENT

Oyster contamination scenarios.

The first scenario considers very short-term contamination, such as sudden and heavy rainfall and contaminated coastal land run-off, brief episodes involving the treatment of contaminated raw sewage, sewer overflows, or occasional contamination linked to tourist activities. Initial contamination, tides, currents, and environmental factors all contribute to the final concentration in oysters and are specific to each coastal area and situation. We chose not to set values of contamination from the land-based source, but set them directly in oysters with range values in the same order of magnitude as real observations (*1, 23*). We used initial contamination and maximum values of 25,000 HAV genome copies per gram digestive gland (Figure 6(A)). For *E. coli*, initial contamination and maximum values as tolerated for rare incidents in Class B areas are 46,000 genomes copies per100 g of oyster flesh (Figure 6(B)). Two incidents of less than 24 hours with fecal HAV contamination of the shellfish production area were set to occur during a winter and a summer period. We assumed that the maximum contamination level in shellfish is reached in 24 h or less, in particular for HAV (*1*). Then both values decay in agreement with their T90 (i.e. time necessary to inactivate 90 % of the original amount) of 28 days for HAV (*17)* and two days for *E. coli* (*24*). For *E. coli*, five days after the incident, concentrations per 100 g of edible flesh are described by a uniform distribution between 230 and 4,600 genome copies. This contamination scenario is plotted in Figure 6(A) (HAV) and in Figure 6(B) (*E.Coli*).

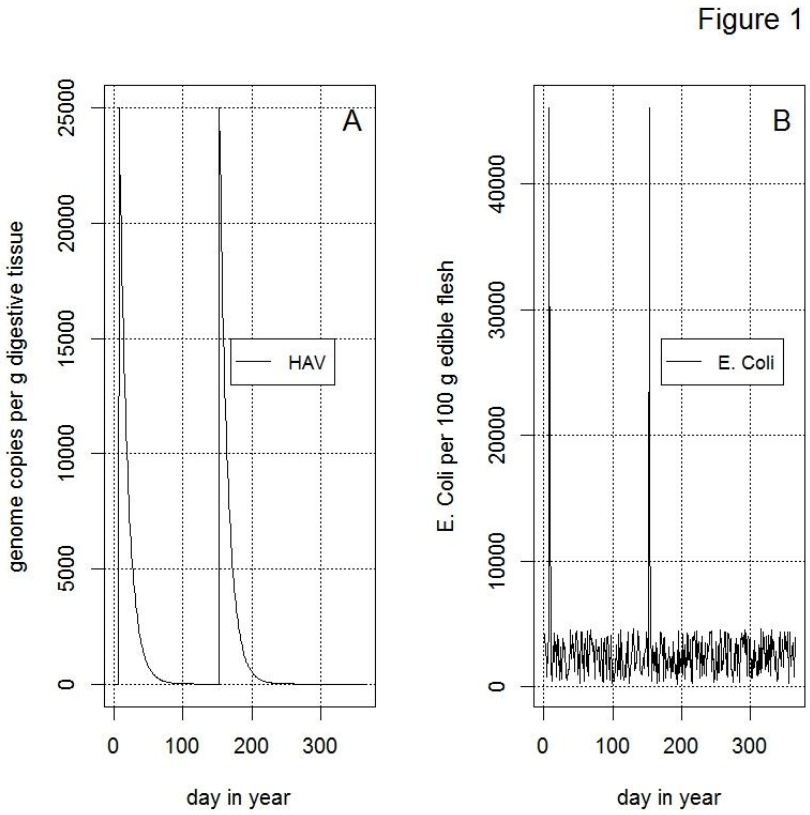

FIGURE 6. THEORETICAL LEVELS OF CONTAMINATION FOR HAV (A) AND *E.COLI* (B) FOR SCENARIO 1

The second scenario of contamination is related to an endemic situation, when, for example an autochthonous source of contamination of the coastal area is not detected for a long period of time. In this scenario, shellfish contamination by HAV is simulated twice a year, for longer periods and at higher levels than in the first scenario, with each episode lasting 90 days (Figure 7(A)). For comparison, excretion of HAV in feces is intermittent and its duration is estimated around 80 days by RT-PCR, for a single individual (between 57-127 days) (*1*). During the period of contamination, concentration per g of shellfish digestive tissue was chosen to vary between 10 and 50,000 genome copies (Figure 7(A), Table 4). Between contamination periods, HAV concentration is null. Concentrations of *E. coli* were set to within 50 and 5,000 genome copies per 100 g of edible flesh throughout the year (Figure 7(B)).

Figure 2.Theoretical levels of contamination for HAV (A) and *E. coli* (B) for scenario 2

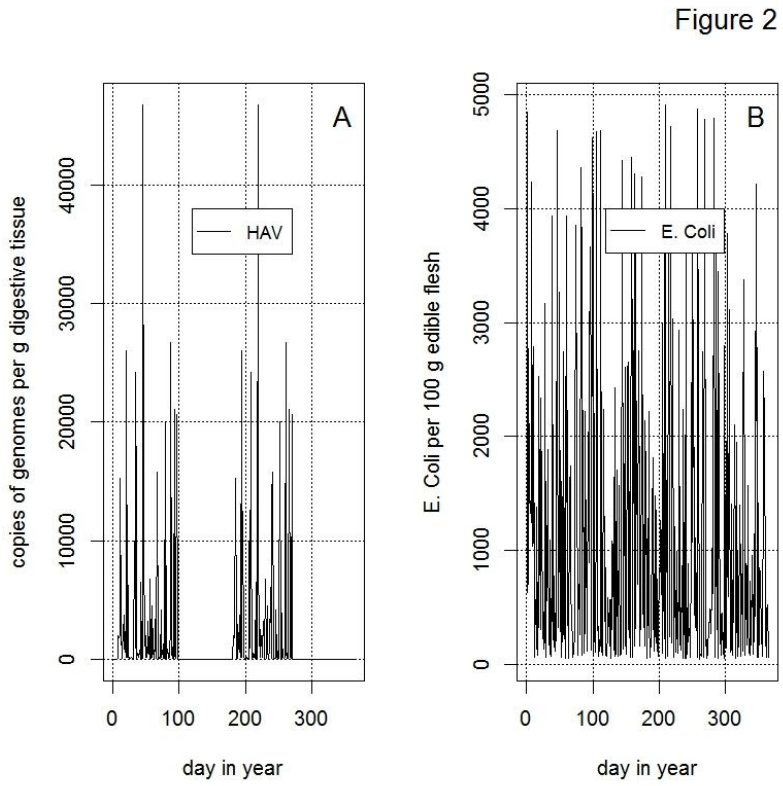

FIGURE 7: THEORETICAL LEVELS OF CONTAMINATION FOR HAV (A) AND E. COLI (B) FOR SCENARIO 2

For both scenarios, we assumed that contamination is homogeneous in the shellfish production area. The two episodes of contamination were modeled to occur in the winter and summer season, to account for the seasonality of consumption.

HAV, like other viruses, is concentrated in the digestive tissues, target of the method of detection-quantification (*23*). Ninety to 95% of HAV genome copies are estimated to be located in the digestive gland (*1*). Concentration in the digestive tissues is greater than any other edible oyster tissue (*15*). The relative weight of the digestive gland compared to all edible tissues is around 8% for typical commercial-sized oyster in France (*1*). The number of genome copies in the edible tissue of oyster was deduced from the number of copies in

digestive tissues (Table 4). The HAV risk assessment study published in 2009 estimated that one copy out of 60 genome copies is infectious (*23*).The same value was used here.

Consumption data.

Oyster consumption is localized spatially and temporally. Coastal areas close to shellfish production areas have higher consumption rates, particularly in adults. Three different data sets, CALIPSO (643 coastal consumers) (*3*), INCA (42 consumers) (*2*) and France AgriMer (oyster sales) (*7*) were combined into a single data set to obtain a daily representative sample of 1000 oyster consumers (men and women), older than 18 years, and residing in a coastal area. To illustrate this data set, the number of meals consumed per day for this population is given in Figure 8. Peaks of consumption occurred preferentially on weekends, during the year-end holidays (Christmas, New Year's Day), and, more generally, during the winter. In this population, the overall mean portion size (single meal of oysters) is 138.27 g of edible oyster flesh (one oyster weighs between 10 and 20 g). For a coastal population of shellfish consumers, the average size of a meal is between 6 and 14 oysters.

Figure 3

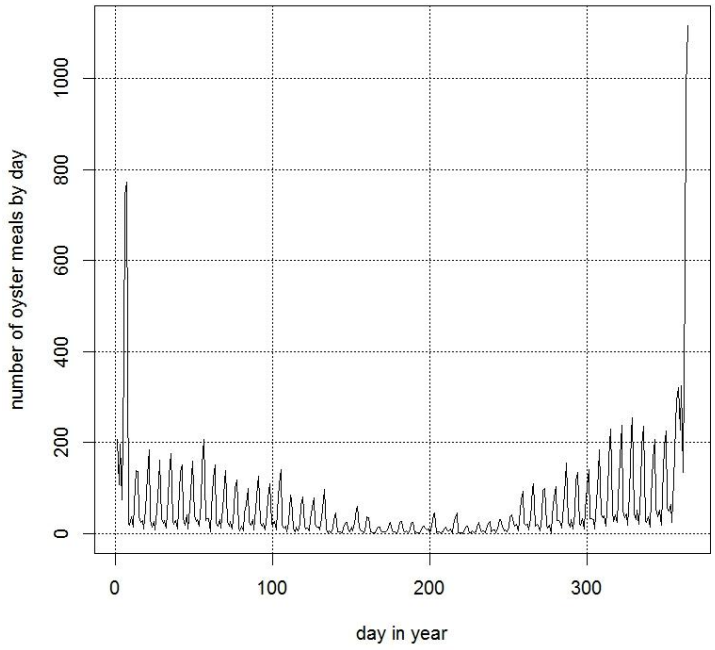

FIGURE 8: FREQUENCY OF CONSUMPTION BY DAY

legend: Distribution of oyster meals (maximum 2 per day for an individual) in a year, in a population of 1000 adults (older than 18 years, men and women) who frequently consume fish products and live in a coastal area

Hazard characterization.

The Beta-Poisson dose-response of Echovirus 12, based on published data (*28*), was assumed to have the same infectivity as HAV (*23, 26, and 30*). QRA-predicted attack rates based on the dose-response of Echovirus 12 gave similar results as observed attack rates during observed outbreaks in Spain, compared to other dose-responses (by example Rotavirus,Poliovirus1 and 3) (*23*). Consequently, we chose to fit the Echovirus 12 dose-response, using a Beta-Poisson model, to model HAV dose-response (*12, 31*); the uncertainty of parameters was estimated using a parametric bootstrap procedure (*12, 25*). The result is plotted in Figure 9 and maximum likelihood estimates for α and β are given in Table 4. Because we used simulated doses rather than the mean of a Poisson distribution dose, we used the Beta-binomial dose-response model with the parameter values of alpha and beta from the fitted Beta-Poisson model (*33*).

Figure 4

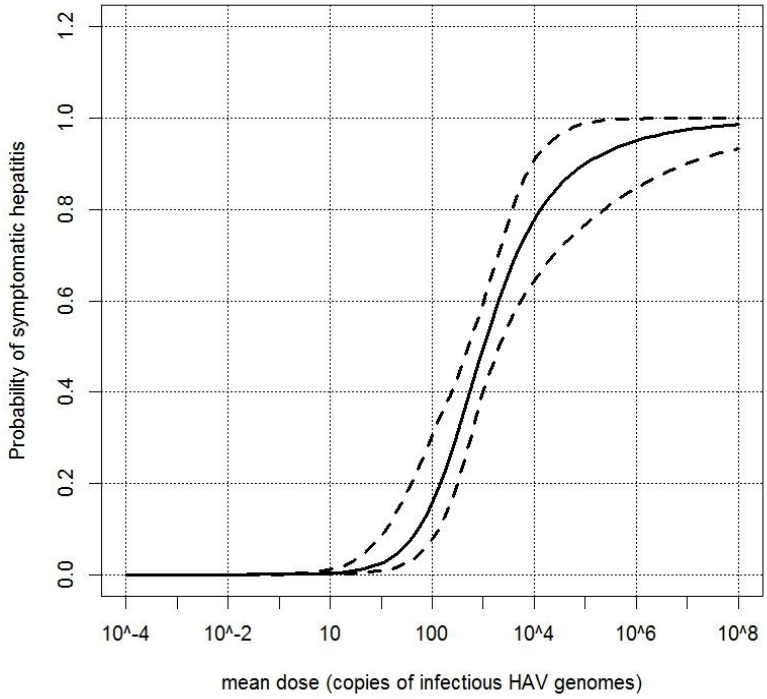

FIGURE 9:BETA-POISSON DOSE-RESPONSE MODEL FOR HEPATITIS A

management options

Different factors were taken into account: (1) sensitivity of different kinds of monitoring systems on shellfish; (2) time necessary to obtain analysis results; (3) time to application of management strategies (detailed below); (4) other management strategies. Some of the parameters taken into account were considered to be subject to improvement (human management); others were considered unalterable due to technical reasons (sensitivity of RT-PCR, time to result analysis). Assumptions made on the analysis technique for HAV detection were specific to RT-PCR. The different management strategies and their key parameters are summarized in Table 5.

Two types of monitoring systems were considered: one using *E. coli* enumeration and one with regular monitoring for detection of HAV in oyster digestive glands (Table 5).

Sensitivity of the monitoring system depends on the sensitivity of detection and the frequency of sampling. The sensitivity and specificity of *E. coli* enumeration techniques is assumed to be perfect. Abnormal *E. coli* levels were set to a value of more than 4,600 genome copies per 100 g of edible flesh. The result of the HAV analysis for management purposes was interpreted only qualitatively: abnormal levels are defined by any positive detection of HAV (whatever the quantity) The specificity of RT-PCR for HAV detection is considered to be perfect (there are no published cases of cross-reaction with other biological species). Sensitivity of HAV detection takes the following parameters into account: from the original sample of 1.5 g (Vs) extracted from six oyster digestive glands, after an extraction efficiency of 50% (p_{extrac}), enzyme efficiency of 95%, the final volume sample is 100 µl (V_{FS}); five µl (final volume V_F) is used for the RT-PCR reaction with a limit of detection of five copies (limit of detection, LD) (*1, 4, 16*)**.** We assumed that the sample is homogenously mixed. In comparison with extraction efficiency, the enzyme efficiency was considered to be almost perfect and is neglected. For each sample analyzed, the probability of detection P_{detect} is obtained from the HAV concentration ($conc_{HAV}$) per gram digestive tissue in the following way: N1 is the number of copies in 1.5 g of digestive gland, X number of copies in a final volume V_F.

$$N_1 \sim \mathrm{Bin}(\mathrm{conc}_{\mathrm{HAV}} \times V_s, p_{extract})$$

$$P_{detect} \sim 1 - \mathrm{CDF}(\mathrm{Binom},\ \mathrm{X} \le \mathrm{LD} - 1, \mathrm{N1}, \mathrm{p} = \frac{\mathrm{V_F}}{\mathrm{V_{FS}}})$$

Where CDF is the Cumulative Distribution Function

Then, event detection on a given day can be simulated using a Bernoulli distribution with P_{detect} as the parameter.

For each day of the year, the event (detection /no detection) is evaluated for HAV monitoring. The day of sampling is chosen according to the criteria described below. Whenever event detection is 1, the chosen management strategy is applied.

The monitoring for *E. coli* occurs monthly in agreement with European regulations.

The frequency of monitoring for HAV was monthly (Table 2) or twice monthly (s2-15 in Table 2). The start date was randomly chosen during the first month of the year (discrete uniform); thereafter, the frequency of sampling was systematically set to every 30 days (Table 5). The delay between sampling date and results was set to one day for *E. coli* and three days for HAV (short period). Then the delay between last sampling and confirmatory sampling was two days after the first sampling for *E. coli* and four days after the first sampling for HAV (when confirmatory analysis is requested). The next sampling date, delay between confirmatory sampling, and next sampling was five days for *E. coli* and set to four days for HAV (when confirmatory analysis is requested).

Different management strategies can be applied for closing a shellfish production area and re-opening it. For reopening, two negative analyses at weekly intervals are requested for *E. coli* monitoring (application of regulations) (REMI). Two hypotheses were formulated for re-opening the area with HAV monitoring, with confirmatory analysis (M1, M2, M3, L1, L2, L3) or without confirmatory analysis (S1,S2,S3) (Table 5).

The final result of analysis (confirmatory, if requested) should result in the closure of the area. This decision was set, with a delay estimated between one day for *E. coli* and seven or 14 days for HAV (Table 5). For HAV monitoring, to allow re-opening, three hypotheses were formulated, with one, two or three negative analysis results requested (Table 5). The time between the last negative requested sampling and re-opening of the area was set to two days for *E. coli* and four or 11 days for HAV monitoring (Table 5).

Management strategies can also be applied to shellfish that are transferred from the contaminated area to other production areas for growing or finishing. We set the duration of shellfish production in the finishing area to 15 days, by definition free of HAV. If abnormal levels are detected in the area of origin after confirmatory analysis (such as “M2” monitoring in Table 5), controls should be performed on transferred shellfish (‘monitoring transfer’ type of management in Table 5). All samples transferred between the sampling date for confirmatory analysis until the re-opening of the area were checked (after the closure of the area. If positive results are found, and whenever results are still positive (sampling every seven days), oysters cannot be sold. Two negative results and a period of five days were required between the last negative result and consumption (not detailed in Table 5). For transferred shellfish, two management practices were identified. The first was 15 days of “purification” with no special measures (“relaying 15 d”) (Table 5), with shellfish consumption 15 days after transfer to a non-contaminated area, with no special measures. The second management strategy was HAV controls (“monitoring transfer”) on transferred

shellfish before marketing and consumption (Table 5). For consumers, the real impact of this second management practice can be defined by the annual risk (AR) with controlled transferred shellfish (‘monitoring transfer’) divided by the AR “relaying 15 d”. For transferred products, the excess number of days (more than 15 days) of no selling is calculated and averaged for all samples transferred during the year (one every day of the year).

Other global management options, such as sewage treatment, or production of shellfish in safer areas, which can decrease contamination in shellfish on the order of two log10 units, were considered (‘type of management two log10 in Table 2). Each management option was simulated with the two hypothetical scenarios of contamination described in the QRA section.

Type of management	Type of monitoring	Frequency of sampling(days)	Confirmatory analysis requested for closing the area	Delay between last sampling and confirmatory sampling (days)	Delay between confirmatory sampling and next sampling (days)	Delay between last positive sampling and closing (days)	Number of negative results before re-opening	Delay between last negative results and re-opening (days)
REMI	*E. coli*	30	Yes	2	5	1	2	2
S1	HAV	30	No	--	0	7	1	4
S2	HAV	30	No	--	0	7	2	4
S3	HAV	30	No	--	0	7	3	4
M1	HAV	30	Yes	4	4	7	1	4
M2	HAV	30	Yes	4	4	7	2	4
M3	HAV	30	Yes	4	4	7	3	4
L1	HAV	30	Yes	4	4	14	1	11
L2	HAV	30	Yes	4	4	14	2	11
L3	HAV	30	Yes	4	4	14	3	11
S2-15	HAV	15	No	--	0	7	2	4
2 log10	None	--	--	--	-	--	--	--
Relaying 15 d	None	--	--	--	-	--	--	--
Mon. transfer	HAV	30	Yes	4	4	7	2	4

TABLE 5: MITIGATION STRATEGY DEFINITION

Model development and simulations

For each simulation of uncertainty, one sample value of the uncertain distribution of parameters was sampled. One thousand simulations were done for uncertain parameters: alpha, beta (parameter of dose-response) and the first date of sampling during the first period (month) of sampling. Then, depending on management strategy, every 30 days or 15 days, a sampling day was chosen for each month of the year. For each simulation of uncertainty, the annual probability of illness for each individual was calculated according to the formulae given in Table 1. Variability was taken into account for the population of 1000 consumers as was variability of contamination during the year. For each uncertainty simulation, the mean annual risk for this population was evaluated.

The baseline risk management option is no monitoring and no management at all.

If a management strategy is applied, the theoretical annual exposure and risk of each individual might change. Depending on the risk management strategy chosen, if there is abnormal level of *E. coli* (monitoring *E. coli*) or detection of HAV (monitoring HAV), there is no consumption of contaminated shellfish after a given period, for a given period (no exposure) of the year, for the entire exposed population. For two log10 units of lower contamination of oysters or relaying 15 days in a clean area, oysters are less contaminated, therefore, exposure is lower. We assumed that closure and reopening of the shellfish production area had no effect on shellfish consumption after the date of re-opening. The benefit of human intervention can be demonstrated by the risk reduction, 1 minus the ratio of mean risk with intervention to mean risk without intervention. The mean of the relative risk reduction is expressed in percentage, i.e. % of cases avoided, with the median and the 95% credible interval. Producers' cost is contingent upon the duration of closure of the area and can be simulated for each risk management option. All calculations and simulations were made in R language (version 2.12.2, R Foundation for Statistical Computing).

RESULTS

Incidental contamination

Results are shown in Table 6 (median and credibility intervals of the mean) (median of the mean) for accidental, twice yearly, short-term, homogenous contamination (scenario 1).

Type of management	Percentage of cases avoided Median [95% CI]	Number of days of closure of the area Median [95% CI]
REMI	0% [0-16.7]	0 [0-14]
No confirmation		
S1	20.62% [9.5-48.8]	103 [68-133]
S2	21.1 %[9.8-49.1]	119 [96-147]
S3	21.4% [9.9-50.9]	133 [110-161]
Confirmation and short		

delay to close the area		
M1	17% [7.1-43.8]	90 [53-125]
M2	17.4% [7.5-45.1]	111 [76-139]
M3	17.6% [7.5-45.4]	125 [90-153]
Confirmation and long delay to close the area		
L1	10.5% [4.5-29.4]	90 [53-125]
L2	10.7% [4.7-29.8]	111[76-139]
L3	10.8% [4.7-29]	125 [90-153]
Every 15 days		
s2-15	35.9% [23.1-52.2]	140[128-147]
non contaminated area of production		
2 log10	88% [69-94]	0
Transferred products		
Relaying 15 days	24.7% [16-33.7]	0
Monitoring transfer	69.5% [29.5-96.2]	6.5

TABLE 6: *RESULTS FOR CONTAMINATION SCENARIO 1*

Legend: "REMI": monthly *E. coli* monitoring; "S"," M"," L", "Monitoring transfer": monthly HAV monitoring; S (or M, L) 1, 2, 3: Number of negative results before re-opening; S2-15: every 15 days HAV monitoring, no confirmation; (details see Table 2)

The percentage of cases avoided by classical microbiological surveillance is close to zero. Classical microbiological surveillance cannot effectively indicate HAV contamination, because only a few days in the year show abnormal levels of bacterial contaminants. Therefore, this management strategy is not efficient.

By comparison, the percentage of cases avoided by direct monitoring of HAV is more effective, in particular with twice-monthly monitoring (s2-15), with a percentage of 35.9%; the probability that the monitoring system detects HAV contamination increases. The percentage of cases avoided with short delays for closing the area (no confirmatory analysis wait, quick decision to close the area) increases the efficiency of the management strategy when we compare it with, for example, the percentage of S3 (21.4%) to M3 (17.6%) or L3 (10.8%). In this situation, because contamination is homogenous and stopped quite rapidly, there is no particular advantage to waiting for three negative results to re-open the area, as shown by the percentage for one (% L1=10.5%) or three negative results (% L3=10.8%).

Controlling samples transferred to other areas for growing or finishing is very effective, with a percentage of cases avoided of 69.5%, because contaminated products can be detected and appropriate confinement (or destruction) measures can be taken before products are placed on the market. However, this system is not perfect, because the first signal is only based on monthly monitoring.

The best cost (duration of closure) benefit, with a percentage of cases avoided of 88 % was observed for management practices that can decrease contamination in shellfish, by example by two log10 units.

Endemic situation of contamination

Results are similar with those given for incidental contamination, except that waiting for three negative results, compared to just one; to re-open the area was an effective consumer protection measure (Table 7). For example, the percentage of cases avoided for S1 was 11.2%, compared to the percentage of cases avoided in S3, which was 37.2%.

Type of management	Percentage of cases avoided Median [95% CI]	Number of days of area closure Median [95% CI]
REMI	0% [0-0]	0 [0-0]
No confirmation		
S1	11.2[2-30]	41 [10-115]
S2	30.1 [8-75]	101 [24-175]
S3	37.2[9.7-77.4]	120 [82-199]
Confirmation and short delay to close the area		
M1	2 [0-20.7]	9 [0-64]
M2	9 [0-39]	51 [0-127]
M3	10 [0-49]	88 [0-148]
Confirmation and long delay to close the area		
L1	2 [0-24]	9 [0-64]
L2	9 [0-37]	51 [0-127]
L3	9 [0-46]	88 [0-148]
Every 15 days		
S2-15	51 [17.7-75.6]	161 [101-181]
non contaminated area of production		
2 log10	87 [72.5-93]	0
Transferred products		
Relaying 15 days	16 [10-26]	0
Monitoring transfer	10 [0-27]	3.5

TABLE 7: RESULTS FOR CONTAMINATION SCENARIO 2

Legend: "REMI": monthly E. coli monitoring; "S"," M"," L", "Monitoring transfer": monthly HAV monitoring; S (or M, L) 1, 2, 3: Number of negative results before re-opening; S2-15: every 15 days HAV monitoring, no confirmation;

In this scenario, the source of contamination is not identified or controlled, and variability of contamination among days (or samples) can give negative results that may be positive the following day. Controlling transfers after closure of the area, with monthly monitoring (M2 type monitoring) is less effective than in the incidental scenario with only 10% of cases

avoided. Efficiency is limited by the relative lack of correlation between two successive days of shellfish contamination in comparison with incidental scenario, and the lack of closure of the area (confirmatory analysis requested in M2) in comparison with incidental scenario. Again, the percentage of cases avoided by direct monitoring of HAV is more effective with twice-monthly monitoring (s2-15) giving a percentage of 51% and fecal monitoring is not efficient in this situation (0%).

Again, the best cost (duration of closure) benefit was observed for management practices that can decrease shellfish contamination; for example a decrease of two log10 units resulted in 87 % of cases avoided.

DISCUSSION

Quantitative data on HAV contamination are rare, particularly those that include monthly monitoring, and are sometimes difficult to compare (no standardization or harmonization of HAV quantification methods in shellfish). We therefore preferred to use hypothetical situations of contamination, designed after real situations and based on biological data (such as maximum level of contamination observed, T90).

Even in an open environment deemed safe, as a good microbiological quality area incidental and rare contamination can occur. In certain circumstances, these incidents can be rapidly identified and stopped or contained. The first scenario illustrated this situation of incidental contamination. Monitoring HAV, even with just qualitative detection and early management, was shown to be a useful complementary action to other preventive measures that can be applied to avoid contamination of the shellfish production area. Shellfish transfers for growing or finishing are very often neglected, because they are difficult to manage. However, we show here, based on simple assumptions, the importance of controlling shellfish and of rapidly informing producers of any detected contamination, so that they can take preventive measures on their production, in particular for transferred products.

The second scenario is more realistic whenever many little uncontrolled or identified sewage effluents are present. It is sometimes costly to take safety measures for each effluent event and sometimes difficult to identify the source of contamination. For example, more than 70 outfalls have been identified in the Bay of Paimpol, Brittany, a production area twice involved in outbreaks of hepatitis A (*1*). In this context, shellfish monitoring and better management strategies are obviously required. This implies the shortest possible time for making management decisions (no waiting for confirmatory analysis, because the assay specificity is almost perfect) and consideration of several negative results at different times before re-opening. For both scenarios, and generalizing to any exposed area (history of HAV outbreak or exposed to regular or high level of human microbiological contamination), HAV monitoring may be a useful complementary measure to microbiological monitoring, in particular when the frequency of sampling is every 15 days.

Among the assumptions made in the model, the one of spatial homogeneity seems crucial.

This is not a safe assumption, and is probably not the case in most situations of real contamination. Unfortunately, not enough data are available to model spatial heterogeneity. Also, although we did not model it here at each step it may be required to take several samples at different sites for each sampling step to evaluate spatial heterogeneity in the contamination.

Other assumptions are specific to this QRA for hepatitis A, such as the T90 used for HAV, dose-response analysis, data on bioaccumulation in oyster tissues, and real infectivity estimates (*22*). These parameters were simplified in QRA, such as T90 which depends on environmental factors, including temperature, oxygen concentration or physiological state of shellfish, as shown for *E. coli* (*24*). Because standardized methods and standards for real-time RT-PCR are not available yet, and because infectivity of genomes cannot be clearly assessed and may vary, we interpreted HAV RT-PCR results qualitatively.

However, we show that the best management strategies are those that can reduce contamination in sewage or reduce the exposure of shellfish (whenever it is possible). Treatment of sewage water should directly take viral risks into consideration. With this goal, the quantitative approach using real-time RT-PCR may be useful for non-cultivable viruses, the best being absence of detectable RNA. Another strategy is for shellfish producers to avoid using the most exposed areas or to decrease the time oysters spend in the most exposed part of production areas, and to apply preventive management practices, (e.g. HAV analysis in situations at risk), particularly for transferred products. The generic modeling approach developed here can be readily adapted to other data on contamination and other management strategies. For example, surveillance of sewage effluents and real- time information of cases in human populations in coastal areas could add overall safety of shellfish production (*18*).

Improving surveillance and quality of shellfish production area could help prevent outbreaks involving consumption of contaminated oyster in a large population susceptible to more severe symptoms, linked to the low levels of seroprevalence of hepatitis A in the adult population. Nonetheless real-world data would greatly improve and validate this model.

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II.3.3. COMPLEMENT TO THE PAPER

II.3.3.1. STRUCTURE OF THE MODEL

The structure of the model is given in Figure 10, illustrating the two dimensions of the QRA, uncertainty and variability. The effect of detection (day k) is affecting exposure for other days (day j).

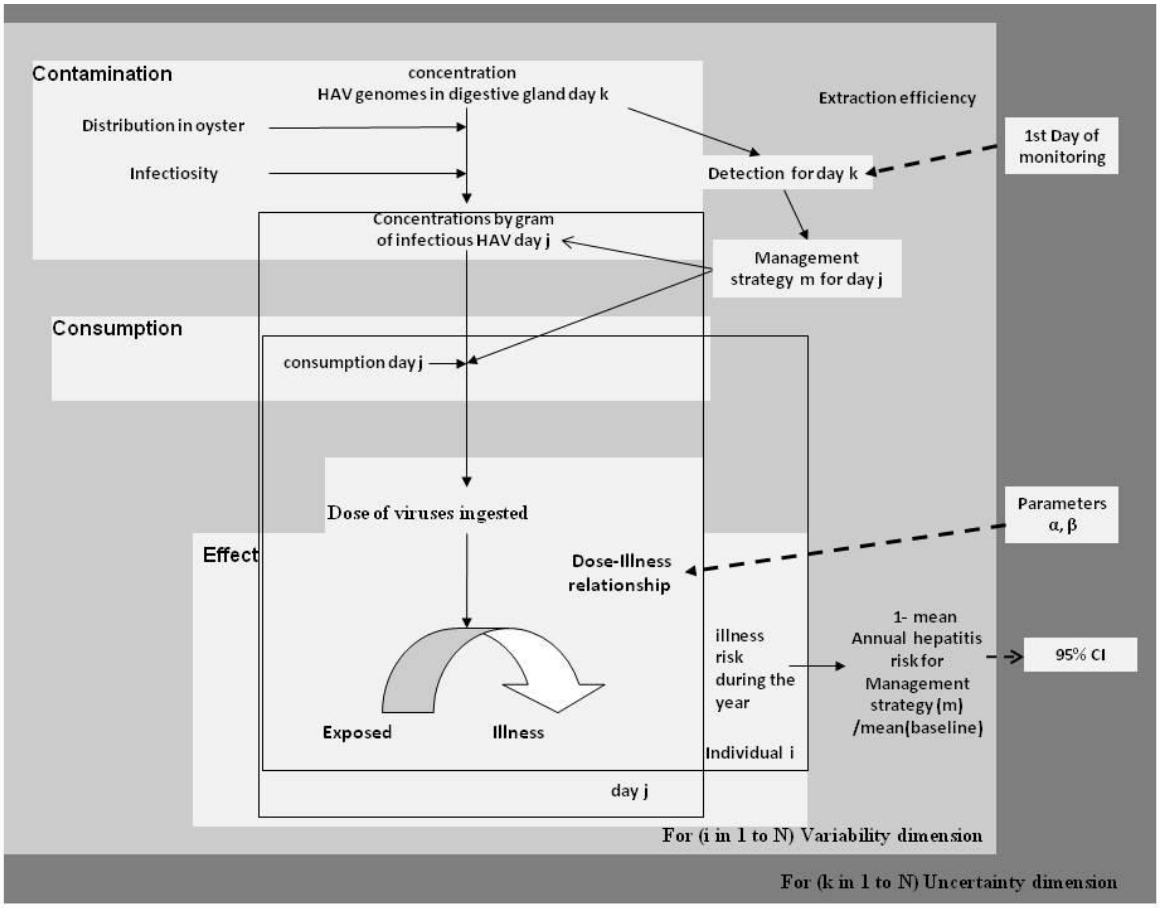

FIGURE 10: STRUCTURE OF THE QRA MODEL FOR HAV

Legend solid arrows link between variables inside variability dimension, hatched arrows link between variables between variability and uncertainty dimension

II.3.3.2 THE CONSUMPTION MODELING

The consumption for an adult population of seafood consumers, living in coastal areas is extracted from several data bases. Some details of the construction of the consumption data not provided in the paper, are given here, explaining how the variability of consumption during the year is modeled.

CALIPSO study is a frequency based questionnaire, established on 996 adults, seafood consumers of 5 coastal cities made in 2004 (Leblanc *et al.,* 2006, Bemrah *et al.,* 1998). Knowing that men are eating

more oysters than women, the ratio of male / female in CALIPSO study was established to over-represent woman population, with 71.4% of oyster consumers in CALIPSO are females. This study was made for estimating long term exposure to cumulative chemical risk (heavy metals). Data are giving the frequency of meal with oysters during the year, and the usual size of this meal, for all individuals included in this study. On this study we selected oysters consumers: 199 data of consumption of men and 443 data of women were taken into account.

INCA study, based on a representative sampling in general population (Volatier *et al.,* 2000) gives representative sex-ratio of oysters consumers, with 57.5% of males consuming oysters (with only 56, men and women, consumers on a sample 2492 individuals.

We sample by bootstrap of 1000 individuals from CALIPSO individuals (with replacement), respecting the sex ratio given by INCA database.

For one individual i of this sample (men or women), the total number of meals NTi during the year can be re estimated with the frequency of consumption (by example twice a month, or once a week) multiplied by the number of month (or weeks concerned) in the year.

From other studies made by SECODIP for OFIMER, data of selling show that the frequency of consumption is highly seasonal. Weekly data of selling were available and the relative proportion of each week (P_{sw}) was evaluated from SECODIP data (France Agri Mer, 2008).

Inside the week, we also know that the frequency of consuming is higher at the end of the week, during Friday, Saturday and in particular Sunday. Data from general population (INCA, 1999) give the frequency of consumption each day of a week. We used this source of data to estimate the relative proportion of the day, inside the week (P_{iw}).

The relative frequency of consumption each day k of the year is simulated by the product

$$P_{dayk} = P_{iw} \times P_{sw}$$

The two meals of the same day have the same probability to be chosen. There's no impact on risk, because the dose is evaluated daily.

If we consider an individual, consuming oysters, during the year there's 364*2=728 meals opportunities to eat oysters, suppressing one day of the year for simplification of calculations with 52 weeks. Between years this particular day can be Sunday or another day of the week. For simplification this last day is suppressed from further calculation. Because the model is comparing different strategies on the same yearly basis, there's no impact on comparing relative results.

For sampling the meals of an individual i, we sample without resampling with unequal probability a particular number of meals N_{Ti} of the year. Each meal is a category with a probability to be sample is $P_{dayk}/2$.

Knowing that the total number of meals (eq. Trials) is given by N_{Ti}, the corresponding distribution of probability is a Poly-Hypergeometric distribution.

For each day of the year, we attribute the total number of meals of each individual to the corresponding days of the year, taking into account the relative frequency of consumption of the week of the year and of the day inside the week.

The idea behind is that if an individual eat oysters two times a year, it's more likely to be at Christmas or at New Year day. And if you eat more often, it is more likely in winter in particular a Sunday.

The resulting number of meals of oyster/day for a given population is described in the Figure 3 of the paper.

Whenever an individual is sampled, the associate size of the meal (from CALIPSO data) is selected, and the different days (and inside 2 possible meals) of eating. Because the QRA is daily based, the sum of dose ingested each day is taken into consideration.

II.3.3.3. DOSE-RESPONSE CHOICE

The dose–response was taken from published paper (Pinto *et al*., 2009).

Dose in outbreaks is evaluated in this paper, with consumption of 60g, and for values of observed concentration corrected for extraction and enzyme efficiency of Real-Time RT-PCR, expressed in infectious dose (1 genome among 60 is considered to be infectious) and after light cooking.

Different predicted attack rates were evaluated with mode values of parameters of some viral dose-response, with an approximate Beta-Poisson model (for theoretical aspects of dose-response see part III). Those attack rates were compared to observed attack rates.

Reproducing results of the paper (Pinto *et al*., 2009), the comparison of observed and predicted attack rates were plotted on the Figure 11.

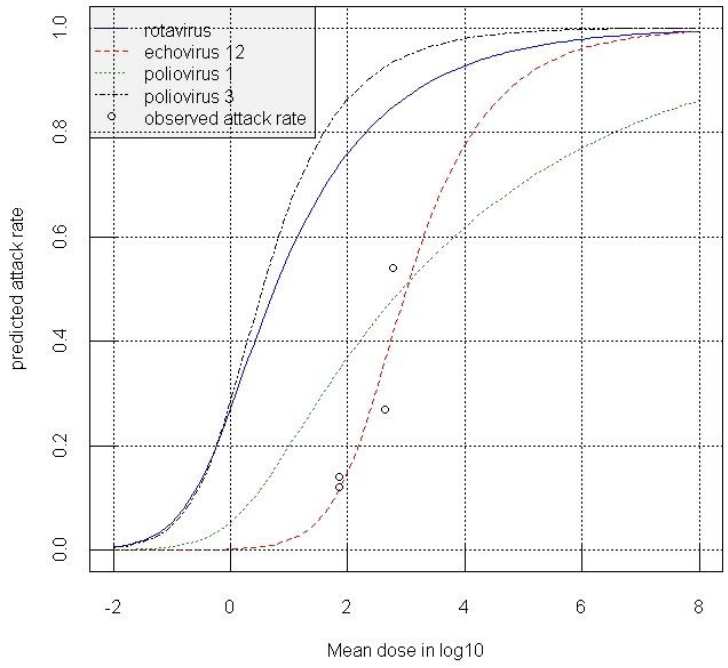

FIGURE 11: COMPARISON BETWEEN DIFFERENT MEAN DOSE (EXPRESSED IN INFECTIOUS GENOME) -RESPONSE AND ATTACK RATES OF HAV WITH THEORETICAL DOSES.

Legend: Doses, established from genomes were corrected (see text) (data from Pinto *et al.,* 2009)

The best fitting dose –response of HAV attack rates, shown in figure 11 is Echovirus 12

The quality of fitting and the choice of the best dose-response depend on hypothesis. If the mean individual consumption of clams is overestimated, or if another consideration is overestimating the concentration (% of infectivity, effect of cooking), the best-fitting dose-response could be different. Uncertainty about the dose and about the estimates can be done with other approaches (see part III).

However, trying to keep the same hypothesis with this paper (same % of infectivity) we assume that the dose-response of Echovirus 12 can predict cases of HAV not detailed in (Pinto *et al*., 2009) probably jaundice. For taking into account uncertainty distributions about the estimate of parameters, we used those that can be estimated from data of Echovirus 12, by maximum likelihood and bootstrap approach. The resulting dose-response with 95 % credible interval is shown in the Figure 9 of the published paper.

MANAGEMENT STRATEGIES

In order to prevent consumption of contaminated oysters and cases of hepatitis from foodborne origin, different ways of monitoring and management were explored. All of them have a theoretical impact on exposure, and ipso-facto, on the risk. Without accurate data, some simplifications have to be made. The negative impact of a closure to consumption is not taken into account. The effect of monitoring is a little over estimated, because we assume that all days in the month and every 30 days (for monthly based monitoring) can be days of sampling. It is not completely realistic because some days might be not considered, such as Saturday and Sunday, or national no working days are unlikely to be days of regular monitoring. Also oysters are unlikely to be sampled and put on the human market every day in same quantity (linked to the level of tide).

The relative gain in risk, (that can also be expressed in term of percentage of mortality) is explored for each scenario of contamination and for each monitoring and management system. The baseline risk for comparison is based with no monitoring at all ("B" in Figure 9).

Three kinds of management strategies are investigated:

- One of them is diminishing the level of contamination at the source ("2 log" in Figure 12).

- Most of other strategies are based on regular monitoring of shellfish in the (potentially) contaminated bay. One of the management strategy investigated is the actual regulation with *E. coli* monitoring ("REMI" Figure 12). Those different management strategies are forbidding the selling and consumption of shellfish, during a particular period, whenever an abnormal level of contamination is detected ("S","M","L"; Figure 12). During this period, consumers are protected of harmful exposure. This period should be chosen in order to wait enough for shellfish to eliminate naturally the contamination, without any uncontrolled source of contamination. Different strategies were tested, waiting for one to three negative results (made once a week), before re-opening the shellfish area.

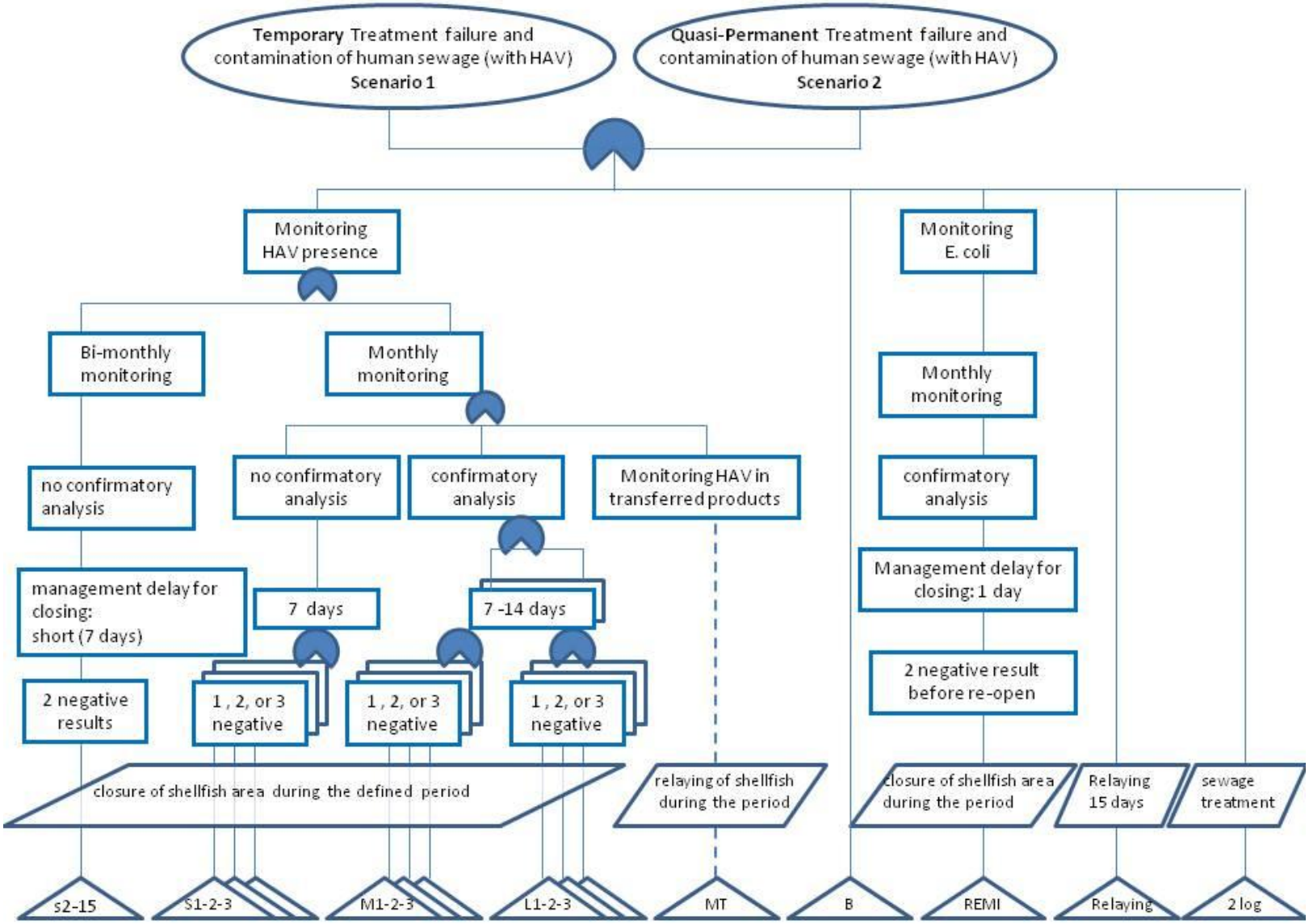
Temporary Treatment failure and contamination of human sewage (with HAV) Scenario 1
Quasi-Permanent Treatment failure and contamination of human sewage (with HAV) Scenario 2
Monitoring HAV presence
Monitoring E. coli
Bi-monthly monitoring
Monthly monitoring
Monthly monitoring
no confirmatory analysis
no confirmatory analysis
confirmatory analysis
Monitoring HAV in transferred products
confirmatory analysis
management delay for closing: short (7 days)
7 days
7 -14 days
Management delay for closing: 1 day
2 negative results
1 , 2, or 3 negative
1 , 2, or 3 negative
1 , 2, or 3 negative
2 negative result before re-open
closure of shellfish area during the defined period
relaying of shellfish during the period
closure of shellfish area during the period
Relaying 15 days
sewage treatment
S2-15
S1-2-3
M1-2-3
L1-2-3
MT
B
REMI
Relaying
2 log

FIGURE 12: MANAGEMENT STRATEGIES FOR HAV CONTAMINATED SHELLFISH IN AN AREA OF PRODUCTION

Legend: or (exclusive); triangle Relative Risk estimate for each management strategy, circles: contamination scenario, rectangles: monitoring strategy, parallelepipeds: management that could reduce risk, hatched lines: particular calculation for transferred shellfish (see Figure 9 and paper for details).

- The last kind of strategy investigated is concerning shellfish transfers, because most often production of shellfish can be transferred from contaminated area to not contaminated ones, and products can be consumed elsewhere. Producers that can be informed of bad results by official monitoring can then be concerned by checking the level of contamination of their transferred products, and wait for negative results before selling to the human market. We only test one way to do this monitoring, (Figure 13) however the efficiency is linked to the efficiency of the official monitoring in the shellfish area, creating an alert for shellfish producers whenever HAV is detected.

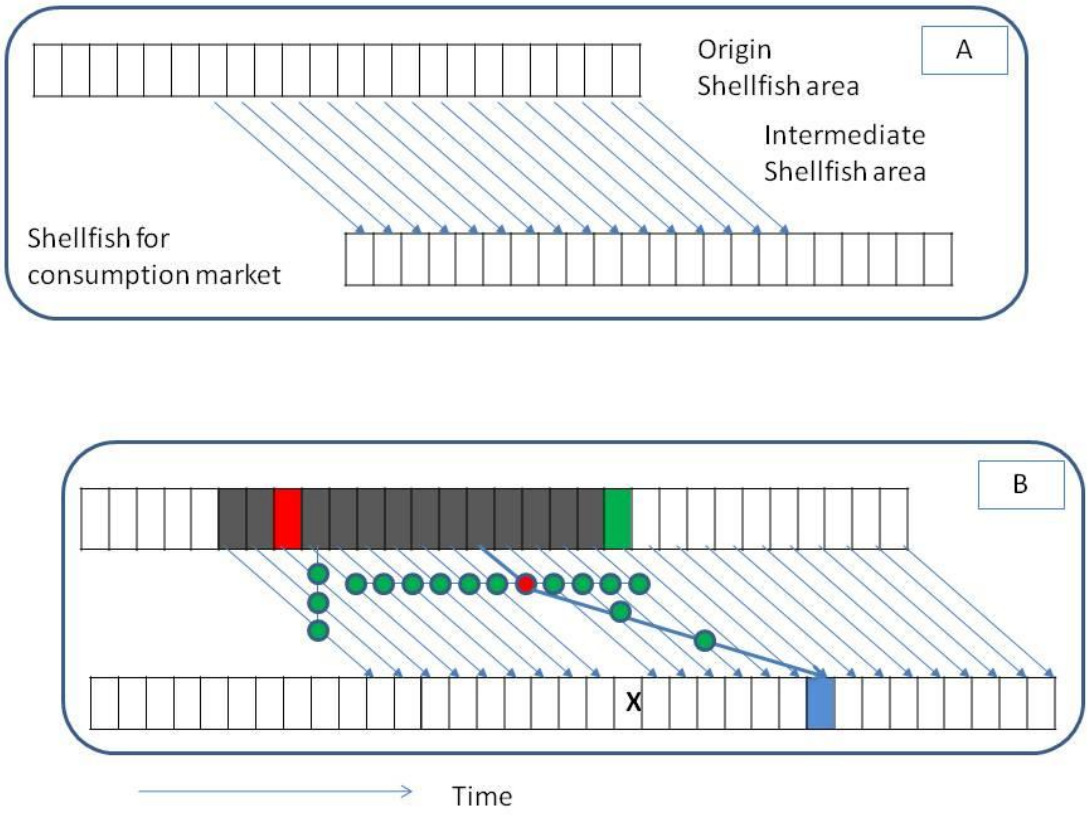

FIGURE 13: MANAGEMENT STRATEGY FOR TRANSFERRED SHELLFISH

Legend: A without management, relaying 15 days in "clean water",

B: red box: **day of the** closure of the area, green box: day of the re-opening of the shellfish area (and last day for checking transfers); red circle detection

of HAV; green circle no detection of HAV; X no selling; blue box: mean contamination between different arrivals at the same day.

The duration of shellfish production in the finishing area is set to 15 days, by definition free of HAV. If abnormal levels are detected in the area of origin, after confirmatory analysis, (such as “M2” monitoring in Table 5), controls should be performed on transferred shellfish (the day after the closure or 2 days after transfer). All samples transferred between the sampling date for confirmatory analysis until the re-opening of the area were checked. If positive results are detected at this check, (without confirmation), and whenever results are still positive (sampling every seven days), oysters cannot be sold. Two negative results and a period of five days were required between the last negative result and consumption. The effectiveness of this strategy, for people eating these transferred oysters is compared with a strategy of relaying in clean water 15 days, as new baseline (Figure 13).

II.4. PERSPECTIVES

The limitations of this work are described in the paper but we want to stress that could be done in future, to improve the quality and the impact of this work. Most problems evoked here for HAV can be extrapolated to Norovirus situation of contamination of shellfish. Accurate and recent data are accessible for norovirus, in particular in shellfish contamination (EFSA, 2012).

Data of monitoring of HAV are missing in shellfish, but also is missing for a precise modeling from the human contamination to contamination in the bay and in oysters. At the beginning of this chain data of excretion in human stools, are not numerous, not quantitatively done with a representative sample. Quantitative values in genomes for comparing excretion in symptomatic and not symptomatic cases are not available. The question can be expressed with quantitative RT-PCR results, knowing that cell culture is very difficult or not working in routine, infectivity of HAV genomes remains unknown. If those data gaps were better fulfilled, we could also investigate more precisely the epidemiological monitoring efficiency in human population in order to prevent contamination in the bay.

Discussion about infectivity is a general problem characteristic of QRA for Norovirus and HAV. For both cell culture is not available. Infectivity cannot be extrapolated from a situation of oysters contamination to treated drinking water by example. We assume here, as other authors, that we can make hypothesis about infectivity of genomes. Masago (2006) in drinking water, set the percentage of infectious genome at 100 % in norovirus, and Pinto et al set the limit at 1/60 for HAV. Extrapolation is regularly based on other viruses (AFSSA, 2007 page 302), however their resistance can be lower than for Nov and HAV (Lodder *et al*., 2005; Monpoeho *et al.,* 2004). No ways of quantification is perfect, and for instance using cell-culture it’s possible to miss non viable non cultivable micro-organisms. Then, assuming a percentage of infectivity or uncertainty distribution about this percentage, or assuming by clear formulated hypothesis that infectivity is assumed to be 100% of quantified genome, in particular situation can be different alternative for QRA.

The modeling from waste-water to shellfish is site specific dependent. Microbial particles (*E. coli*) are taken many often as surrogates of virus for spatiotemporal dispersion modeling in water in shellfish area. However size, weight and physico-chemical characteristics of virus are different. More over little is known about the size of aggregates of viruses in sea waters, and its impact on those spatiotemporal modeling. The accumulation and purification in shellfish, for HAV in particular is not well known. For Norovirus recent papers are now describing the situation (EFSA, 20111).

In this paper we investigated the temporal variability of contamination during the year. The main thing that could be made in future would be to investigate the spatial variability, and its impact of the relative efficiency of the monitoring system.

This variability can be described at different scales variation: between oysters, between batches of oysters and between different areas of the bay. Then knowing the spatial trend of contamination in the bay (by modeling it from a suspected source) and knowing the spatial position of points of monitoring, and the number of samples made at each time period, the relative efficiency of different monitoring-management could be better assessed (Schernewski *et al*., 2001; Bougeard *et al*., 2011). It could then be feasible to make recommendations about temporal and spatial frequencies of sampling to be make in shellfish (or other products) in particular situation of sources of suspected contamination by example (Sima Laura *et al.,* 2011; Da Silva *et al*., 2007; 2008).

The paper was also alluding to the monitoring of transfers. Real observed data about the transfers were missing. Also, we don't investigate, the use of monitoring sewage water before or after treatment, because we couldn't make, for this bay under study, inference of its relative impact in time (and space) (Bougeard *et al*., 2011).

Methodologically, it could be interesting what the parameters with main impact on results are. Further investigation could be to perform sensitivity analysis with Sobol's method (Ellouze *et al.*, 2011). However because this model is quite simple, and because 28 what-if scenarios were investigated, it was evidenced, that by example the time frequency of sampling is a critical parameter for HAV monitoring in particular for endemic situation. However such a descriptive analysis could be better and quantitatively assessed by further appropriate sensitivity analysis. Before doing that, an optimization of the program should be made in order to have the simulation runs faster.

In conclusion, we illustrated here that quantitative risk assessment is useful for investigating efficiency of management strategies in order to avoid human cases linked to viral shellfish contamination, even if data of contamination are expressed in genomes and not with known real infectivity.

We investigated the efficiency of management in the shellfish. Other management strategies were not explored further at this step, and could be done in future, such as different scenarios of transfer, or setting different quantitative limits of HAV genome contamination for management purpose, in shellfish, or weekly monitoring. Also it could be interesting in future to test the efficiency of the combination of different surveillance strategies, with monitoring waste-water, coastal water at critical point and shellfish.

Objective of classical QRA is to estimate the number of people infected, whenever the concentration in shellfish and human consumption is known, in order to estimate an order of magnitude of number of ill people that could be compare with epidemiological data. This is the idea of validation of the model. For HAV QRA we used dose-response of a surrogate, with low infectivity, Echovirus 12 as roughly validated in an outbreak associated with clams in Spain (Pinto *et al.,* 2009). For Norovirus the situation was different, but for those both viruses dose-response is a crucial step, for making realistic estimate of the number of cases linked to a particular level of contamination in food.

CHAPTER III: DOSE-RESPONSE, CRUCIAL STEP OF FOODBORNE TRANSMISSION

III.1. MAIN DOSE-RESPONSE MODELS FOR QRA PURPOSE

To establish the relationship between a dose and a risk of infection or illness, some different points of view made for quantitative microbial risk assessment can be re-assessed here.

Two ways of modeling are made for dose-response proposals:

(i) best-fitting or "empirical approach", (ii) mechanistic one (Teunis *et al*., 1999; Haas *et al*., 1999). The last approach is based on the idea of describing biological mechanisms by key parameters. We stress the description here of mechanistic point of view, because, in particular for Bayesian analysis, biological explanation of key parameters can give an idea to the prior to give to those parameters, and because a theoretical biological point of view can be refuted or improved by new scientific data.

We examine in a first step the theoretical and biological meaning of dose-response and then, briefly the way to estimate the key parameters of the data. Limitations of such approaches were examined in a third step of this part.

III.1.1. DOSE-RESPONSE MODELS: THEORETICAL AND BIOLOGICAL MEANINGS

In microbial risk assessment, the relationship between dose and response (infection or illness) (dose-response) are not assuming a threshold. A threshold should involve that an interaction occur between microbial pathogens and the human host, and because of discrete nature of a pathogen, more that one organism is required to survive to cause infection. Without threshold and without interaction, one pathogen can initiate infection (known as single-hit theory) (Teunis *et al*., 1999; Haas *et al*., 1999).

For infection two sequential process are assumed to occur (Haas *et al.,* 1999):

(1) "The human host must ingest one or more organisms that are capable of causing infection or disease". This is the exposure step, and the probability to ingest j organisms, knowing the mean dose of exposure, d. The notation is P1(j/d)

(2) "(…) only a fraction of the ingested organisms reach a site where infection can begin". The probability of k organisms to infect, knowing j are ingested is noted P2(k/j)

This two steps are leading to the equation

$$P\inf(\bar{d}) = \sum_{k=1}^{\infty} \sum_{j=k}^{\infty} P1(j / \bar{d}) P2(k / j)$$

The common definition for Infection state is defined by an immunological response (serological response) or excretion of viruses in feces. The common definition for disease state is defined by a typical human case, and is defined usually for epidemiological investigation by typical and characteristic clinical symptoms of the pathogen.

Different situations can be biologically plausible for establishing the relationship between dose and infection (and between dose and disease), linked to different assumptions of relationship between populations of pathogens and human populations.

DOSE-INFECTION RELATIONSHIP

For dose-infection relationship, two situations, with different biological assumptions, are regularly used for making inference with experimental data or used for QRA purpose (Haas *et al.,* 1999; Teunis *et al.,* 1999;2008;Pouillot *et al.,* 2012).

First situation: exponential model

(1)The pathogen is homogenously dispersed in dose (assumption 1) and there's no variability of the probability (p_m) for the pathogen to survive to m (real number unknown) barriers and infects, linked to the pathogen or to the host (assumption 2). We are in a general situation where the exact dose received by an individual is unknown, but the distribution of doses between individuals is known.

The first assumption (1) can be realistic, in experiment situation, or for a water outbreak, whenever an inoculum is homogenously dispersed and diluted in a watery reservoir, and if all doses are taken from this reservoir. By hypothesis, the pathogens shouldn't clump together (which is not very realistic for viruses, except in experiment situation).

For the second assumption (2), the situation can occur and be realistic, in general in experiment, such as human trial, where the human population can be assumed to be homogenous (for susceptibility to pathogen), and the pathogen can be also assumed to be pure (single strain by example) or homogenous for infectivity.

The structure of this dose-response model is given in Figure 11, corresponding to a situation where, an homogenous matrix with mean concentration λ are divided in doses. A group of N individuals are exposed to different doses, expressed in number of infectious viruse n. Each dose of each individual i is described by a Poisson distribution with mean λ (assumption 1). The event that an the individual i is infected, can be described by a Bernoulli distribution with a probability Pinf. Each virus has an identical and independent probability to infect, defined as p_m (assumption 2).

For an individual i:

$n[i,\lambda] \sim \text{Poisson}([\lambda])$

$Pinf[i, \lambda] = 1-(1-p_m)^{n[i,\lambda]}$

Knowing that p_m is a probability that one virus virus infect, 1-pm the probability that is this virus not infect, and for n virus ingested, Pinf is the probability that at least one virus succeeds in infecting the exposed individual.

If one individual is exposed, the event "be infected" is described by:

inf[i] ~ Bernoulli(Pinf[i,λ])

The DAG is given in Figure 14, for different dilutions of the same matrix. Each dilution is homogenously dispersed with mean λ[k], and with the change of notation Dose[i,λ_k]=n[i,λ_k]

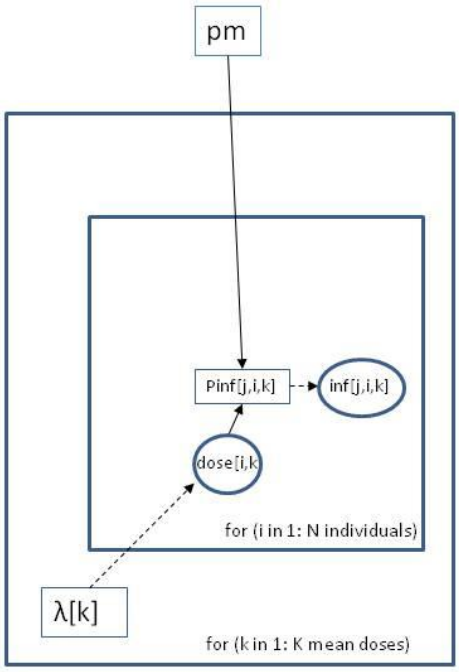

FIGURE 14: DIRECTED ACYCLIC GRAPH (DAG) OF EXPONENTIAL MODEL

legend: rectangle: data, random node circle, dashed arrow stochastic relationship, arrow deterministic relationship

The marginal risk of infection is a function of λ:

$$P\inf_m = 1 - \exp(-\lambda \times p_m)$$

This relationship is known as exponential dose-response. (Haas *et al.*, 1999)

Another parameter is also defined by this relationship: DMI_{50} which is the dose needed to achieve a 50% infection probability

$$DMI_{50} = \frac{\ln 2}{p_m}$$

Second situation: Beta-Poisson model

The two assumptions of this situation becomes (1) the pathogen is homogenously dispersed and (2) there's variability for the probability of a pathogen to infect, linked to the variability of both host and pathogen.

Again, each dose j of each individual i is described by a Poisson distribution with mean λ_k.

In this situation, p_m is not a constant and can be described by a Beta distribution corresponding to the variability of host and pathogen. This a more generalized situation, corresponding to outbreaks, where strains are mixed in the same exposure, and when human population response to a pathogen is not supposed identical (Teunis *et al*., 2004). Experimental trial can also includes variability of response between pathogen or host, and then Beta-Poisson model is often used, in particular when dose is homogenously dispersed. For outbreaks, the dose cannot, many often, be assumed to be homogenously dispersed. Then other relationship of dose-response are used (Haas *et al*., 1999; Teunis *et al*., 2008 b; Chen *et al*., 2006)

The relationship becomes, for an individual i:

n[i,λ] ~ Poisson ([λ])

$$P\inf[i,\lambda] = 1 - \prod_{j=1}^{n[i,\lambda]} (1 - p_m[j])$$

The probability is independent but not identical between pathogens, and for each combination of individuals and pathogen, p_m can be different.

The relationship raised difficulties to compute values for p_m, for each pathogen, whenever the dose of pathogens is high.

Whenever the exact dose is known, the marginal risk of infection is the Beta-Binomial model (Teunis *et al.,* 2008; Vose, 2008). The Beta-Binomial model is the integral of Pinf with pm, Beta distributed.

This characteristic is particularly useful for quantitative risk assessment, whenever distribution of doses, or exposure is not a Poisson distribution, is not homogenous and can describe by example the distribution of clumps of different size of viruses, that can be simulated. Inhomogenous distribution of virus in food contamination were already observed and fitted (Westrel *et al*., 2006; Teunis *et al*., 2008; Teunis *et a*l., 2010) .

The equation of Beta-Binomial model is

$$P\inf(d/\alpha,\beta) = 1 - \frac{\Gamma(\alpha+\beta)\Gamma(\beta+d)}{\Gamma(\beta)\Gamma(\alpha+\beta+d)}$$

Where Γ is a Euler gamma function.

The corresponding DAG is given in Figure 15:

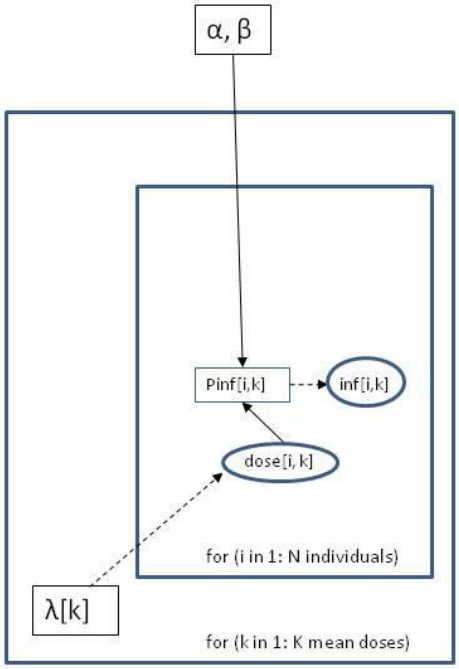

FIGURE 15: DAG OF THE BETA-BINOMIAL DOSE-RESPONSE

legend: rectangle: data, random node circle, dashed arrow stochastic relationship, arrow deterministic relationship

This DAG and the properties of Beta-Binomial model are used to fit a dose-response model for Norovirus in outbreak situation (submitted paper).

The marginal probability of infection linked to a Poisson distribution of doses (mean λ) :

$$P_{\mathrm{Im}}(\lambda\ ) = 1 - \int_0^1 \left[\frac{\Gamma(\alpha+\beta)\, {p_m}^{\alpha-1}(1-p_m)^{\beta-1}}{\Gamma(\alpha)\Gamma(\beta)} \right] \exp(-p_m\lambda\ )dp_m$$

$$P_{\mathrm{Im}}(\lambda\ ) = \int_0^1 g(p_m/\alpha,\beta)(1-\exp(-p_m\lambda\ ))dp_m$$

Where g is the Beta distribution, (parameters α and β), describing variability in $p_{m.}$

The integral is defined and can be solved by the function ${}_1F_1$ (Kummer confluent hypergeometric function) (Teunis *et al.*, 1999), therefore the marginal risk of infection for the Beta-Poisson model, as a function of a mean dose λ can be written as below:

- $P_I(\lambda_k) = 1 -_1 F_1(\alpha, \alpha+\beta, -\lambda_k)$

This relation, whenever β>>1 and α<β can be approximated by the equation (Furumoto, et Mickey, 1967; Teunis *et al*., 1999) known as approximate Beta Poisson model, and was used for the HAV QRA.

$$P_{\inf/\lambda} = 1 - \left(1 + \frac{\lambda}{\beta}\right)^{-\alpha}$$

$$DMI_{50} = \beta(2^{1/\alpha} - 1)$$

This last approximation is not valid for highly infectious pathogen such as Rotavirus and Norovirus. The mean of the Beta distribution, describing pm (probability of infection with one virus) is α/(α+β). If pm is suspected to be high (the case for Rotavirus and Norovirus), the condition α<β is not valid. This approximation (whenever conditions of approximation are fulfilled) is also overestimating the risk at low dose. With Poisson distribution of doses (mean λ), and with the maximum of possible value for pm (equal one), the maximum marginal risk of infection is:

$Pinf_{max}=1-e^{-\lambda}$.

It was shown that the approximate Beta-Poisson model exceeds this risk at low doses (Teunis and Havelaar, 2000).

- Other dose-response model exist, but they are not widely used for microbial risk assessment and in particular for foodborne viruses dose-response (Haas *et al*., 1999).

RISK OF DISEASE

Classically the probability of illness (mobidity ratio used in epidemiological studies) is conditional with probability of infection, and death probability (mortality rate) is conditional to probability of illness. Two approach of the probability of illness, knowing infection, are classically used (Haas *et al*., 1999; Teunis *et al*., 1999; Teunis *et al*., 2005;Teunis *et al*., 2010 a and b).

The first approach is setting that the probability of illness knowing infection is a constant, independent with the dose ingested. Usually ratio of morbidity is used for estimating uncertainty of this probability by a Beta distribution, and sometimes the hypothesis is checked by statistical approach, independent with dose (Haas *et al.,* 1999; Pouillot *et al*., 2004).

As illness is conditional on infection, the probability of becoming ill, can be written as

$$P(ill/dose) = P(ill/\inf) \times P(\inf/dose)$$

The second approach is setting that the probability of illness knowing infection is dependent with the dose ingested.

The second approach is valid when the probability of illness independent with dose is sometimes not confirmed by the data set and can be explained by biological mechanism: the probability of becoming ill may depend on the duration of the infection episode, linked to the dose ingested (Teunis *et al*., 1999). "The length of the infection period could reflect the balance between host defenses and pathogen growth, which may be dose dependent"(Teunis *et al*., 1999).

Given infection, the probability of becoming ill can then be written as:

$$P(ill/dose) = P(ill/\inf, dose) \times P(\inf/dose)$$

The duration of infection (τ)can be described with a Gamma distribution, with parameters r (shape) and θ (scale).

τ ~Gamma(r, θ)

$$\theta = \eta \times dose^{\lambda}$$

λ=1 if risk of illness increases with dose, λ=-1 if risk of illness decreases with dose.

Then, by example for Norovirus dose-response (Teunis *et al.,* 2008 a):

$$\tau \sim \text{Gamma}(r, \eta \times \text{dose})$$

The probability that an infected subject becomes ill due to this infection episode can then be written as:

$$P(ill/\inf, dose) = 1 - \exp(-\tau(dose))$$

The marginal risk of disease (knowing infection and dose) can be estimated with the above equations (Teunis *et al*., 1999; Teunis *et al.,* 2010 b?):

- If the risk increases with dose:

$$P(ill/\inf, dose) = 1 - (1 + \eta \times dose)^{-r}$$

- If the risk decreases with dose:

$$P(ill/\inf, dose) = 1 - (1 + \eta / dose)^{-r}$$

- If the risk is independent with dose:

$$P(ill/\inf) = 1 - (1 + \eta)^{-r}$$

III.1.2. KEY PARAMETERS ESTIMATE

Two methods are commonly used:

- The maximum likelihood approach is giving best values of dose-response parameters. The likelihood function l(X/θ) is the probability of observing X, the observed data, as a function of θ, dose-response parameters. A Best fitting value is obtained for each parameter of the dose-response which maximizes the likelihood. Credible intervals of uncertain key parameters are obtained by bootstrap (Haas *et al*., 1999; Pouillot *et al*., 2004). For HAV QRA, this approach

was used with Echovirus 12 data, for dose-response parameters estimates, with the approximate Beta-Poisson approach with good conditions of approximation (see Table below)($\beta >> 1$ and $\alpha < \beta$) (but overestimating risk at low dose).

- The Bayesian inference is based on the join distribution on the parameters and observations. The different steps are (1) define prior(s) for dose-response parameter(s), (2) calculate the likelihood conditional for the observed data (3) calculate the posterior as the product of likelihood and priors distribution, then normalizing the result. For multiparameter or hierarchical models, it is difficult to calculate the likelihood and posteriors. Technique based on Markov chain simulations (MCMC) where a transition distribution converges to the posterior distribution, after a sufficiently large number of simulations (Gilks *et al.,* 1996).

III.1.3. LIMITATIONS OF DOSE-RESPONSE MODELING

Limitations of dose-response studies were studied from different sources.
A summary of those considerations is expressed below:
Extrapolation at low doses, not observed in many cases, is reflecting a part of subjectivity in main hypothesis, accepting the idea of single hit or not. The fitting is made on limited data and information and the extrapolation to other host population can be subject of debates.
Extrapolation from experimental animal data to human population is not obvious because species susceptibility is often different (no animal model for Norovirus and HAV) (FAO/WHO, 2003; Armstrong and Haas, 2007; Tamrakar *et al.,* 2011; Hoelzer *et al.,* 2012; Teske *et al.,* 2011; Watanabe *et al.,* 2010). Extrapolation from trial study to general population is not obvious: characteristic of volunteers (young, healthy) are not representative in general of the population naturally exposed (FAO/WHO, 2003). Extrapolation from outbreak data to sporadic cases should take into account range of doses and again the representativeness of population in outbreaks for extrapolation to general population exposed. The state of acquire immunity before exposure is not taken into account in many cases, with some exception (Teunis *et al.,* 2002; Englehardt and Swartout, 2006). In outbreaks, because the study is retrospective to the event it's difficult to investigate. Extrapolation to more susceptible population such as immunosupressed population, pregnant women, older and children is difficult to justify (Haas *et al*., 1993; Gerba *et al*., 1996b; Crabtree *et al*., 1997; Balbus *et al*., 2004; Pouillot *et al*., 2004).
The characteristic of food matrix can be suspected to interfere with the response of the host, such as buffer effect or increasing the effect (acidity..) (FAO/WHO, 2003).

- Finally strains studied in dose-response can be different than those in general population we want to extrapolate (FAO/WHO, 2003).
- Mix of strains and more mix of pathogens and comorbidity impact on dose-response estimate is not well known (synergy, independence of action, inihibition) (FAO/WHO, 2003).
- The measurement quality of the technical method used for quantifying dose is rarely given in dose-response study. However the extrapolation of the dose-response with the measurement of doses

made with different methods, or with method with other performance of measurement than the dose-response can be criticized (FAO/WHO, 2003)..

- The definition of infection and cases in dose-response study should be carefully defined for its use in QRA. However dose–response are widely used for QRA purpose (FAO/WHO, 2003). Dose-response are used and recognized as valid for the risk management use, helping, by example, to set acceptable limit of contamination in food and water, or predict effectiveness of different management strategies (Regli *et al*., 1991; Schijven *et al*., 2011; Pouillot *et al.,* 2012).

III.2. LITERATURE REVIEW OF DOSE-RESPONSE FOR FOODBORNE VIRUSES

A summary of the state of knowledge for different viruses is given in the Table 8. Considering all these dose-response relationship for infection, parameters of dose-response were estimated by maximum likehood approach, except the last one, made both with bayesian inference and maximum likelihood approach . Confidence interval or credible intervals are not given, and results are focusing on best-fitting values. Many often this is because the classical method in quantitative risk assessment, for viruses, used point estimates and not probabilistic ones.

Most of these approaches were done with study of human volunteers, not perhaps representative of human population exposed, in an experimental context. The experimental context can be an advantage, because in particular, the dose can be homogenous dispersed in the inoculum, and then hypothesis of doses Poisson distributed is justified. The experimental context permits the use of a purified and well identified strain. In the other hand, purified strains, store in an experimental laboratory can change their infectivity, and in particular vaccine strains are perhaps not justified to be extrapolated to wild viruses.

The dose is given usually in a watery matrix. A matrix effect can be effective. For QRA purpose, with shellfish contaminated by viruses, it should be of particularly interesting to obtain dose-response parameters with data obtained with the same food matrix- oysters. Data could also of interest if the same method is used for measurement of virus contamination for dose-response or QRA (or known quality of method of measurement for each situation). It's sometimes difficult to extrapolate results of a dose-response obtained with a particular measurement of dose, in particular if relative performances are not well-known.

Extrapolation from one virus to another ("surrogate") or from a way of transmission to another, aerosol to oral ingestion should be also be used with caution. By example for Adenovirus type 4, two ways of aerial inoculation leads to different estimates of key parameters and infectivity (Couch *et al.,* 1966). Then direct extrapolation to oral way of transmission seems quite optimistic.

SARS analysis, is pooling data different studies of different animals and humans and also different viruses, close to SARS. For pooling all these different data the argument was statistical. However assuming no variability of host -response in this context, is perhaps an optimistic point of view, even if the few data available are not showing differences. The dose-response is assumed to be done in infectious unit (single hit theory). If the infectious agent is expressed in genome unit, the infective part is not known.

Virus	Type of model	mode values	DI_{50} $P_I(1)$	inoculum population infection definition	reference of the model/reference of the data	unit of measurement of dose	mean dose	exposed	Infected (disease cases in parenthesis)
Rotavirus isolated from disease children	Approximate Beta-Poisson	α=0.253, β=0.422		watery inoculum with buffer volunteers healthy adults rotavirus in stool or immune response	(Teunis and Havelaar, 2000)/data (Ward *et al.,* 1986)	unit of dose: FFU (Focus Forming Unit) 1 FFU=1,56.10^4 particles (see Ward)	0 9.10^{-3} 9.10^{-2} 9.10^{-1} 9 9.10^1 9.10^2 9.10^3 9.10^4	5 5 7 7 11 7 8 7 3	0 (0) 0 (0) 0 (0) 1 (2) 8 (6) 6 (2) 7 (5) 5 (3) 3 (2)
	Exact Beta-Poisson	α=0.253, β=0.422							
Poliovirus I (vaccine strain)	Exponential	p_m=0.0122	57	oral vaccine babies virus in stool	(Haas *et al.,* 1983)/data (Minor *et al.,* 1981)	$TCID_{50}$ Unit causing 50% of positive cell culture	7 16 27 42 50 55 65 80 90 160 210 280	1 2 2 1 6 3 6 1 4 3 2 1	0(0) 0(0) 0(0) 0(0) 3(0) 1(0) 0(0) 1(0) 3(0) 3(0) 2(0) 1(0)
	Approximate Beta-Poisson	α = 15 β = 1000	47						
Poliovirus I Sabin strain (LSc2ab)	Approximate Beta-Poisson	α = 0, 1097 β=1524	844 000	oral vaccine newborn infants virus in stool	Regli *et al.,* 1991/data Lepow *et al., 1*962	$TCID_{50}$	$10^{3.5}$ $10^{4.5}$ $10^{5.5}$	97 91 84	55(0) 52(0) 48(0)
Poliovirus III (vaccine strain-"Fox")	Approximate Beta-Poisson	α = 0.409 β = 0.788	3.5	oral intubation babies virus in stool	Regli *et al., 1*991/data(Katz *et al., 1*967)	$TCID_{50}$	1 2.5 10	10 9 3	3(0) 3(0) 2(0)
Echovirus 12 strain from disease children,	Approximate Beta-Poisson	α = 0.374 β = 186.7	1005	watery inoculum volunteers, non immune, male healthy adults	Regli *et al., 1*991/data Schiff *et al.,* 1984	PFU plaque forming unit 1PFU=41 particles	0 330 1 000 3 300	34 50 20 26	0(NA) 15(NA) 9(NA) 19(NA)

purified and cultivated				(18-45 years) infection is determined by seroconversion or intestinal shedding of virus			10 000 33 000 330 000	12 4 3	12(NA) 2(NA) 2(NA)
Adenovirus type 4	Exponential	pm=0.4172	0.72	aerosol inoculation volunteers free of serum antibody infection (serologic data)	Crabtree *et al., 1*997; Heerden et *al.*, 2005./data Couch *et al.*, 1966	$TCID_{50}$ 1 $TCID_{50}$=13.6 viral particulates (electron microscopy)	1 5 11 1000	3 3 3 6	1(1) 3(3) 3(3) 6(3)
Hepatitis A virus	not done but close to Echovirus 12			all infection are supposed to be symptomatic adult population outbreak data	Pinto *et al., 2*009/data	***	72 72 420 582	5 4 9 18	36** 33** 33** 33**
SARS and other coronaviruses (MHV,IBV,HCoV-229E)	exponential	0.002439	280[130-530] PFU 13 TCD_{50}	mice, rats, chickens, humans, intranasal or tracheal inoculation	Watanabe *et al.,* 2010; data from different bibliographic sources see Watanabe	PFU and TCD_{50} TCD_{50} 10 to 30 times less than PFU	see paper (not detailed here)	see paper (not detailed here)	see paper (not detailed here)
Norovirus Norwalk GI	Exact Beta-Poisson	$\alpha = 0.04$ $\beta = 0.055$	ID_{50}=18	For human volunteers, Se+ subjects (Se- were also studied, all negative results, see Teunis *et al.,* 2008)	Teunis *et al.,* 2008	genomes	*32.4 *324 *3, 24.10^3 *3, 24.10^4 *3, 24.10^5 *3, 24.10^6 *3, 24.10^7 *$3.24.10^8$ $6.92.10^5$ $6.92.10^6$ $2.08.10^7$	8 9 9 3 8 7 3 6 8 18 1	0 (0) 0(0) 3(1) 2(1) 7(6) 3(1) 2(2) 5(4) 3(2) 14(7) 1(NA)

TABLE 8: PUBLISHED FOOD-BORNE VIRUSES RELATIONSHIP BETWEEN DOSE AND INFECTION.

* for this experiment the inoculum is not homogenously dispersed. The inoculum is dispersed with aggregates.** the number of people exposed is not given explicitly in the published paper: the calculation of exposed is based on the number of cases and attack rates given in Pinto *et al.,* 2009.

***dose were re-estimated from concentration in genomes, corrected from extraction efficiency, infectivity (1/60), cooking loss (99.46%), and average consumption of 60g

For other pathogens than viruses, outbreaks data were, in recent publications, more commonly used (Teunis *et al*., 2004; 2005; 2008b; 2010b). For foodborne viruses, except for the particular case of HAV outbreak in Spain (Pinto *et al*., 2009), no data of human outbreak is used (Table) or available to estimate dose-response parameters, in particular for HAV and Norovirus).

	P(illness/infection)*100	P(death/illness)*100	P(illness/infection, dose)
Poliovirus	0, 1-1 % paralytic symptoms (Rose and Sobsey, 1993)	0,9% (Rose and Sobsey, 1993) 10% (Macler and Regli, 1993)	NA
Coxsackievirus A	50 % (aseptic meningitis aseptic or respiratory disorder) (Gerba *et al.,* 1996b; Rose and Sobsey, 1993)	5% (Rose and Sobsey, 1993) general population 86% immunosuppressed (Gerba *et al.,* 1996b)	NA
Coxsackievirus B	5-96% (Gerba *et al.,* 1996b)	0,59-0,94% (Rose and Sobsey, 1993) general population 13% newborne infants (Gerba *et al.,* 1996b)	NA
Echovirus	50 %(respiratory or cardiac disorders, diarrhea, meningitis)(Rose and Sobsey, 1993)	0.27-0.29% (Gerba *et al.,* 1996b) general population 3.4% newborne infants (Gerba *et al., 1*996b)	NA
HAV	75 %(hepatitis) adults (Gerba *et al.,* 1996b; Rose and Sobsey, 1993) 5 %(children) (Regli *et al., 1*991)	adult general population: 0,6 % (Rose and Sobsey, 1993) 1 % (Shuval, 2003) around 0.4% (Scallan *et al.,* 2011); between 0.3 to 2% adults>40 years of age	incubation linked with dose (Istre *et al*., 1985)
Rotavirus	56-60 % gastroenteritis for children (Rose and Sobsey, 1993) or 88 % (WHO, 2003)	0,01 % general population 1 % older population 63% immunosuppressed (Gerba *et al., 1*996b) 0,6 %for third world countries 0,015 % for developed countries (Havelaar and Melse, 2003)	NA
Adenovirus	0,5 (Crabtree, 1997))	0,01% (Crabtree, 1997) general population 53% (Gerba *et al.,* 1996) immunosupressed	NA
NoV	40-59 % gastroenteritis (Rose and Sobsey, 1993)	0.0001% (Rose and Sobsey, 1993) around 5.10^{-4}% (Scallan *et al.,* 2011)	$\eta=2.55*10^{-3}$ r=0.086 (Teunis *et al.,* 2008)
HEV	Hepatitis 50% (Worm *et al.,* 2002; Smith, 2001)	0.2 to 4% during outbreak (general population)(Worm *et al.,* 2002) 10 to 20% pregnant women (Worm *et al.,* 2002)	NA

TABLE 9: DISEASE AND LETHALITY RISK

Legend: NA not evaluated

The Table 9 shows that the probability of illness knowing infection with dose is, in most of cases, not estimated, and this is probably due to the lack of available data, in particular of dose ingested, whenever cases are observed.

III.3. NOROVIRUS DOSE-RESPONSE BASED ON SHELLFISH OUTBREAKS DATA

III.3.1. SUBMITTED PAPER

II.3.1.1. CONTEXT

Noroviruses (NoV) are the major cause of acute epidemic gastroenteritis in industrialized countries, leading to very high worldwide losses in years of healthy life. Very high infectivity estimates was found in human challenge studies for GI / Se+ (Teunis *et al*., 2008). Genogroup II, in particular the GII.4 cluster is predominant worldwide in human transmission, but genogroup I is regularly involved in foodborne outbreaks. Based on data from five oyster related outbreaks, detected in France infectivity of genogroup I and II NoV was estimated from the ingested dose, accounting for genetic determinants of susceptibility (histo-blood group antigens: secretors Se+/ vs. non secretors Se-).

The level of information is not the same between outbreaks. For some of them, individual consumption is known, for one of them the secretor status is known, and for all them, only cases and not infection are reported. Individual dose is unknown, and consumption can be different from one individual to another. Inside an outbreak, information can miss. Because of these missing data, Bayesian framework becomes useful. In all the outbreaks under study, each individual ate from the same common meal, then it was feasible to estimate a range for the numbers of oysters consumed. In case that information was missing, the dose ingested by each individual could be calculated by the prior as the product of a random sample from the Negative Binomial distribution of numbers of NoV per oyster, and a random sample from the numbers of oysters consumed, calculated separately for GI or GII NoV. And when secretor status is unknown, an informative prior about the probability to be secretor in general population, estimated in a separate and published analysis (Marionneau *et al*., 2005) can be used.

Our results confirm, for both genogroups in secretor positive individuals, the high infectivity and very low infectivity in non secretors. This is a significant advance in the field of microbial risk assessment, providing dose response relations based on outbreaks with natural diversity, in pathogen properties (infectivity, pathogenicity) and in host susceptibility. The results confirm NoV as (one of) the most infectious viruses known. Future regulations should take into account for this high level of infectivity in the prevention of outbreaks, for setting critical levels for NoVs in food. This is also useful for taking into account the foodborne part in outbreaks, associating different ways of transmission.

II.3.1.2. SUBMITTED PAPER

Article Summary Line:
The infectivity of noroviruses (GI and GII) for secretor individuals measured in foodborne outbreaks is confirmed to be high.

Running Title: Norovirus dose-response from outbreaks data

Keywords: norovirus, shellfish, Bayesian Analysis, Dose-Response Relationship, Fucosyltransferases

Title: Infectivity of GI and GII noroviruses established from oyster related outbreaks

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Abstract

Noroviruses (NoVs) are the major cause of acute epidemic gastroenteritis in industrialized countries. Outbreak strains are predominantly genogroup II (GII) NoV, but genogroup I (GI) strains are regularly found in oyster related outbreaks. The prototype Norwalk virus (GI), has been shown to have high infectivity in a human challenge study. Whether other NoVs are equally infectious via natural exposure remains to be established. Human susceptibility to NoV is partly determined by the secretor status (Se+/-). Data from five published oyster related outbreaks were analyzed in a Bayesian framework. Infectivity estimates where high and consistent with NV(GI) infectivity, for both GII and GI strains. The median and CI95 probability of infection and illness, in Se+ subjects, associated with exposure to a mean of one single NoV genome copy were around 0.29[0.015-0.61] for GI and 0.4[0.04-0.61] for GII, and for illness 0.13 [0.007-0.39] for GI and 0.18 [0.017-0.42] for GII. Se- subjects were strongly protected against infection. The high infectivity estimates for Norwalk virus GI and GII, makes NoVs critical target for food safety regulations.

Text:

Noroviruses (NoVs) contribute significantly to morbidity worldwide [1, 2]. NoV infection occurs primarily through person-to-person transmission but also through contaminated food or water, and in particular by exposure to fomites [3]. NoVs are the cause of approximately 90% of epidemic non-bacterial gastroenteritis outbreaks. The majority of outbreaks occurs during winter months but sporadic cases do occur throughout the year [4, 5].

Outbreaks occur in semi closed communities such as hospitals, schools, cruise ships, nursing homes and military settings [3]. Severity is higher for risk groups, such as immune-compromised individuals and the elderly [3]. NoVs are highly diverse genetically and antigenically, and among 5 genogroups, two are frequently associated with human outbreaks : genogroup I, and more frequently genogroup II [6]. Genogroup II (GII) NoVs, in particular the GII.4 cluster, have become predominant in human transmission of infection over the last two decades, but genogroup I (GI) strains co-circulate in the human population and are regularly involved in food and in particular oyster outbreaks [7, 8]. Like influenza, large outbreaks occur periodically with people of all ages infected [3]. One explanation is that immunity seems to be short-lived and incomplete [3], although continuing replacement of strains of the dominant GII.4 cluster suggests immune-driven selection to facilitate escape from protective (herd) immunity [9].

Differential genetic host susceptibility has also been identified. Since NoV strains bind to carbohydrates of the histo-blood group antigen family, pleiotropic interactions of alleles at three loci (*FUT3*, *FUT2* and *ABO*) determining the Lewis, Secretor and ABO phenotypes also contribute to explaining differences in occurrence of strains and genogroups in the human population [10,11].

The attributable fraction of NoV gastroenteritis linked to food consumption is estimated at around 26% for the US [2]. Nevertheless, this estimate varies a lot between studies and the data are sparse. Hygiene recommendations are required to limit the spread of outbreaks [12], in particular in closed settings. Limiting the contamination of food could be crucial, to prevent primary cases and curb outbreaks at the origin, as direct person-to-person transmission is likely and can be initiated by foodborne cases. Contaminated drinking water or food such as vegetables or molluscs has been shown to cause outbreaks [13-15]. To protect consumers and compare the effects of different management strategies, microbial risk assessment provides a comprehensive and reliable scientific prospective tool [16]. In order to evaluate the potential consequences of intervention measures in food contamination, the probability of primary cases of gastroenteritis should be predicted in an exposed population [16].

A human trial has been conducted to estimate infectivity and morbidity for a range of doses of Norwalk virus (GI.1) using a watery inoculum [17]. Genetic factors determining histo-blood group secretor status were taken into account and Se- status appeared to confer a complete protection against Norwalk virus (GI.1), in agreement with epidemiological studies [17]. The study highlighted the high infectivity of Norwalk virus with an average probability of infection of 0.5 for a single virus genome [17]. As the human trial was limited to a single virus isolate of a strain (G1.1), little is known yet on whether this may be generalized to other strains in food related outbreaks.

For such purpose, outbreaks are an important source of information, as a complement to human challenge studies [18]. In the present study we have used information on the genogroup of the virus and the secretor status of the human hosts in oyster outbreaks to investigate infectivity of NoV GI and GII in conditions of natural exposure.

Methods

Outbreak data

From a database of oyster related outbreaks in France, outbreaks were selected if the exposed population and the attack rate were known, as well as the numbers of oysters consumed, and if the concentration of NoVs was known from analysis of a sample of oysters from the same batch. Further conditions were that the same strain of NoV should have been detected in human stools and oysters linked with the outbreak, and symptoms had to be consistent with NoV gastroenteritis. In some of the outbreaks other enteric viruses (enterovirus, rotavirus) were detected in stool and oysters, but it was concluded retrospectively that the main cause was NoV [19]. A case was defined by the sudden onset of vomiting or diarrhoea or both with maximum incubation period of 48h [20], exposed subjects were included if they ate from the same contaminated meal. For the last outbreak, in 2008, the secretor phenotype for 33 individuals out of 34 was determined from saliva [21].

The outbreak data are summarized in **Table 10** and further details have been published separately [19, 21-23]. The numbers of exposed individuals ranged from 2 to 36. Individual data about consumption and host status (secretor phenotype and blood group), were not always present as summarized in **Table 10**. The contamination levels as numbers of RNA copies by oyster s and the genotype of strains found in the oyster samples for each outbreak are given in **Table 10**. In some outbreaks there was co-contamination by both GI and GII strains.

For all outbreaks, oyster analyses were performed by the same laboratory using the same method for NoV quantification [24]; thus all viral doses were measured on the same scale.

The dose was calculated as numbers of genome copies per oyster, without correction for extraction and amplification efficiency, by extrapolating from the weight of the digestive gland (where the contamination is 90 to 99% concentrated depending on the strain [25]) to the weight of the whole oyster (based on the weight of total meat). In all following analyses the dose is expressed in number of genome (RNA) copies.

Table 10 **Available information for each outbreak**.

Year of outbreak	Number exposed	Number ill	Individual status Secretor	Individual status ABO	Individual Consumption *	Range values **	Norovirus strain	contamination ***
2008	34	23	Yes	Yes	Yes	2-6	GII.4	18-955-37-0
2006 a	27	11	No	No	No	4-6	GII GI	1100 2300
2006 b	2	2	No	No	No	4-6	GI	275-6783
2002	36	21	No	No	No	1-6	GII.4+GII.8 GII.4+GII.9 GI.4	25 125 25
2000	4	4	No	No	Yes	7-18	GI.1	85-237

***Individual Consumption in number of oysters **Range values of number of oysters consumed**

***The level of contamination, (results of pool analysis of digestive gland of several oysters), are given by the number of genomes by oyster**

.

Dose-response model

The dose response models most commonly used for microbial pathogens are based on the conditional relation between exposure, infection and (acute) illness [26]. Exposure is equivalent to ingestion of one or more organisms (dose ingested). If p_m is the probability that any single ingested pathogen successfully passes all (m) defensive barriers in the host, this parameter summarizes the effects of host-pathogen interactions for infection [26]. Heterogeneity in this host-pathogen relationship can be modeled as a Beta distribution, with two parameters α and β [27]. Contamination in food products, in real world situations, can be described as a sample from a suspension with varying concentration. A Poisson-gamma mixture, equivalent to negative binomial distribution of number of genome copies, leads to a hypergeometric (2F1) dose response relationship [17]. Conditional on the ingested numbers of pathogens, this relationship can be described with a Beta-binomial distribution (**Table in appendix**).

The host (secretor status) and pathogen (genogroup) effects were incorporated as follows. The parameters of the infection dose response model were transformed as (Eq.1):

u = α /(α +β) and v = α + β (Eq.1):

The parameter u, the expectation of the Beta distributed probability p_m , depends on secretor status (Se) and genogroup (g) as (Eq. 2):

logit(u) = $\mu 0 + \lambda \times Se + \gamma \times g$. (Eq. 2)

Hence, for each combination of genogroup, and secretor status, parameters α and β can be defined, leading to 4 different dose response relationships.

For the probability of illness among infected subjects an existing dose response model based on the concept of illness hazard during infection was used, with key parameters r and η [26]. Under mild assumptions (gamma distributed duration of infection and linearly increasing illness hazard with dose) the conditional probability of illness (P_{ill}/ dose, inf) knowing dose (dose) and infection (inf) response can be described by the Eq3:

$$P(ill/dose,\eta,r,\inf) = 1-(1+\eta\times dose)^{-r} \quad \text{(Eq.3)}$$

For exposure to GI or GII the probability of infection becomes (Eq4.):

Pinf2= 1-(1-pinf(α_{GI}, β_{GI},dose $_{GI}$)) × (1-pinf(α_{GII}, β_{GII},dose $_{GII}$)) (Eq.4)

With pinf the probability to be infected by GI or GII, knowing specific parameters α, β (linked to GI, GII and secretor status) and ingested doses for GI or GII virus (see Source code in appendix)

For simplicity, as host and pathogen factors were assumed to act on early stages of infection (the virus entering host intestinal cells), they were assumed to only affect infection dose

response: The risk of illness is considering the dose as the sum of dose by GI and GII and the parameters of the illness dose response model, η and r were assumed independent of NoV genogroup (GI or GII) or secretor status.

Bayesian framework

A Bayesian framework was used to estimate parameters and predict the probabilities of primary interest. The directed acyclic graph outlining the parameters and their relationship in the model is shown in **Figure 16**. Details of chosen distributions for all parameters are given in the **appendix**.

We followed an approach similar to a published proposal [28]. In a first step, a core model describing the functional dose response relationship was defined. Prior distributions were allocated to all parameters in order to produce a very flexible prior dose response, in order to accommodate any possible variation in infectivity and morbidity. Then, in a second step, the core model was extended by incorporating all available data (**Figure 16**) to produce posterior estimates.

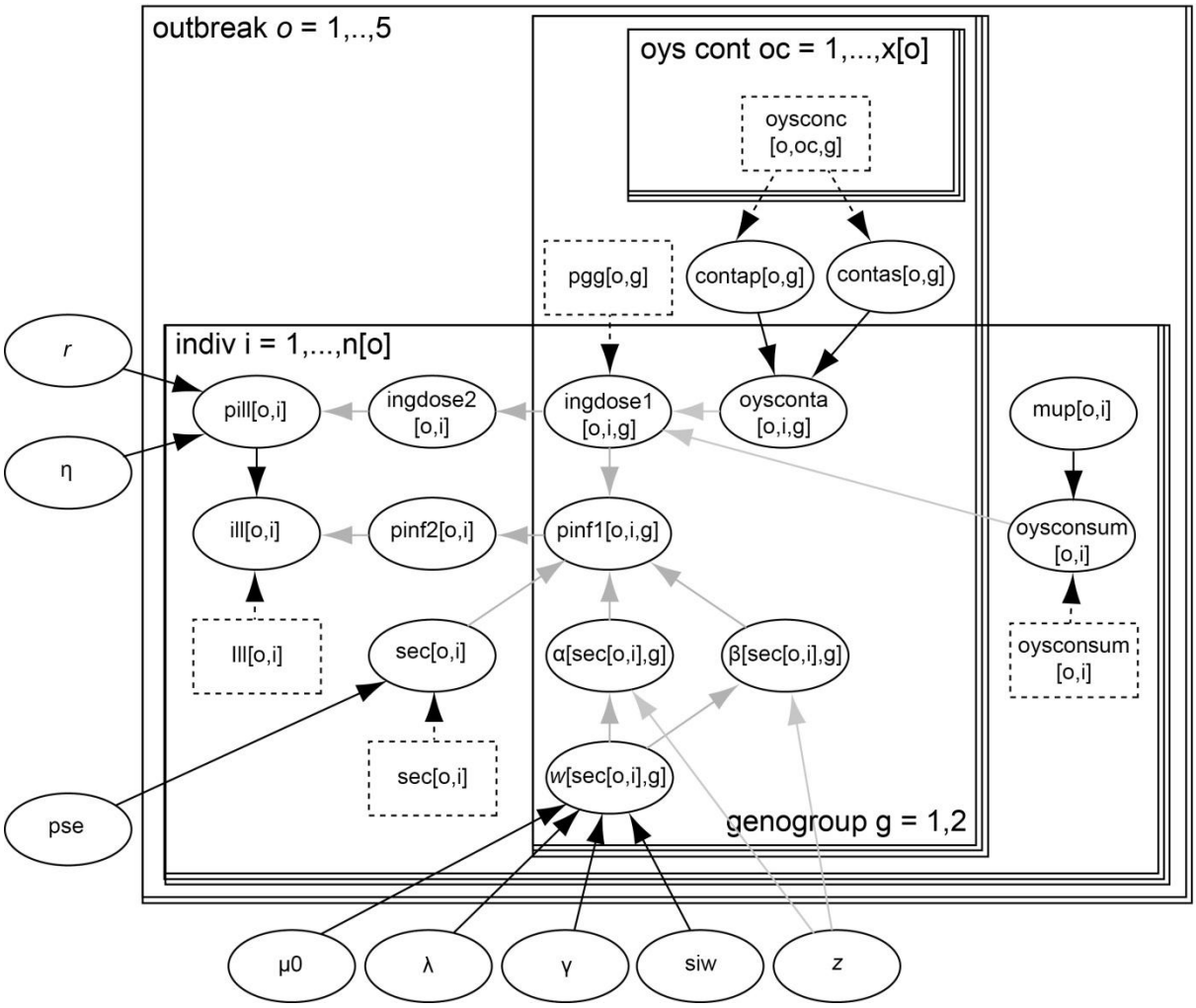

FIGURE 16: DIRECTED ACYCLIC GRAPH OF NOROVIRUS DOSE-RESPONSE MODEL.

Legend: All model quantities are presented as nodes. Data are denoted by dashed rectangle. Logical links are gray arrows and stochastic links black arrows. Solid rectangles describe the loops with reference to an index indicated in the corner of the rectangle (o for loop inside each outbreak, for example).

All model quantities are presented as nodes. Data are denoted by dashed rectangle. Logical links are gray arrows and stochastic links black arrows. Solid rectangles describe the loops with reference to an index indicated in the corner of therectangle (o for loop inside each outbreak, for example).
Oysconc in dashed rectangle: observed data of contamination in oysters; oc number of observation in oyster;Contap: parameter p (success probability) of Negative Binomial distribution;
contas: parameter s of Negative Binomial distribution;
oysconta: sampled value of oyster contamination from Negative Binomial distribution;
pgg in rectangle: data about the genotype involve in the outbreak
oysconsum in dashed rectangle individual consumption data;
oysconsum in oval sampled from truncated Poisson distribution with mean mup (prior);
ingdose1: Ingested dose for each individual and each genogroup;

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ingdose2: Sum of doses for GI and GII; pinf1: the probability of infection by one genogroup; pinf2 probability of infection by GI, GII or both;
pill: the probability of illness;
ill(in oval): illness knowing probability of infection and illness;
ill(in rectangle): illness data in outbreak
pse: probability to be Secretor(=1) in general population;
sec in rectangle : data of individual secretor status
r, η, μ0, λ, γ, siw, z: parameters of dose-response
See appendix Table 1 for other legend explanation.

Specification of prior distributions

The prior probability distribution ("prior") for the estimated fraction of Se+ among exposed individuals was defined by a Beta distribution with parameters estimated from published literature [11]. In case of co-contamination (both genogroups present) contamination with GI is assumed to be independent of contamination with GII.

Numbers of NoV in oysters may be clustered: numbers of NoV in oysters were modeled as a Poisson-gamma mixture (Negative-Binomial) distribution.

Because, in all the outbreaks we studied, each individual ate from the same common meal, it was feasible to estimate a range for the numbers of oysters consumed, in case that information was missing. The dose ingested by each individual could be calculated as the product of a random sample from the Negative Binomial distribution of numbers of NoV per oyster, and a random sample from the numbers of oysters consumed, calculated separately for GI or GII NoV. When individual secretory status is unknown**,** the information is kept at the level of the choosen (informative) prior, that is the probability of secretor positive status in the general population, estimated in a separate and published analysis [11].

Vague priors of all parameters of infection dose-response were chosen from a Normal (or Log Normal) (μ0, λ, γ, z) with mean zero. Priors of the log transformed parameters η and r are described by a non-informative Normal distribution. All prior distributions are given in **the appendix**. The priors of parameters for Se+ Se-/GI GII are set identical.

Model implementation

Models were run with Jags (Jags 3.2) [29] with R. 2.14.0 [30]. Parameter estimates were obtained with 3 chains of 15,000,000 iterations of the Gibbs sampler, thinning every 5000 iterations (to avoid autocorrelations), with a burn-in phase of 200,000 iterations. Source code of the extended core model is given in the **appendix**.

Model assessment

Convergence was assessed using the Gelman-Rubin diagnosis with three parallel chains [31]. A partial sensitivity analysis was performed, changing the standard deviation of key prior parameters (μ0, λ, γ) from 1 to 3. Posterior distributions from the eight resulting models

were graphically compared. Median values and 95% credibility intervals of posterior distribution of each key parameter (μ0, λ, γ, η, r, α, β) were evaluated with 9,000 posterior samples, with the model of increased flexibility (standard deviation of 3 for each parameter, μ0, λ, γ) . In order to characterize differences between dose-response relationships, the two parameters of the Beta (relation for infection) are given for each combination of covariates (Se+/Se-, G I and II). Other metrics include mean and variance in p_m characterizing the heterogeneity of the dose response [32, 33]. For a Poisson inoculum (fully dispersed virus, homogenously mixed), with mean dose (μdose) and heterogeneous of pm, represented by a beta distribution(α, β) the probability of infection can be integrated to yield the confluent hypergeometric function($_1F_1$). For each mean dose, median and 95th percentile of probability of infection are calculated and plotted, for sampled values of α and β, using the relation below (Eq.5):

$$p\inf(\mu_{dose}/\alpha,\beta) = 1 - {}_1F_1(\alpha, \alpha+\beta, -\mu_{\text{dose}}) \quad \text{(Eq.5)}$$

The (unconditional) dose-response relation curves for illness were also plotted. The dose-response probability for illness can be written as the product of the infection and illness dose response probabilities (Eq.5):

$$Pill(\mu_{dose}/\eta, r, p\inf) = (1-(1+\eta\times\mu_{\text{dose}})^{-r})\times p\inf \quad \text{(Eq.6)}$$

With Poisson distribution of doses (mean λ), and with the maximum of possible value for pm (equal one), the maximum marginal risk of infection is Eq.7 [27]:

$Pinf_{max}=1-e^{-\lambda}$ (Eq.7)

This curve is the maximum infectivity limitation curve, plotted with dose-response for infection and disease.

Further characteristics of those curves are the median infectious dose and the dose causing acute enteric illness with 50% probability (ID 50), and the quantiles 95% of the probability of infection and disease for a mean dose of one genome copy (as quantified by RT-PCR method). the difference between Pinf(1) and pm can be described by Eq. 8 and Eq. 9:

For Poisson exposure:

$Pinf(Dose) = 1 - \exp(-p_m \times Dose)$ (Eq.8)

(with pm beta distributed) therefore:

$Pinf(1) = 1 - \exp(-p_m)$ (Eq.9)

which is different from p_m (Pinf(1) approaches p_m only for $p_m<<1$).

Then, p_m is a conditional probability of infection (given ingested dose or exposure to 1 genome copy). Calculation of p_m separates the infection probability from the distribution of exposure. The distribution of probability of infection per virus (p_m) (exact single genome copy ingested), on a logistic scale [34], is plotted. Infectivity of the virus is characterized by p_m, knowing that it separates the infection probability from the distribution of exposure.

In order to investigate the adequacy of the model with the observed data, we calculated the posterior caracteristics (quantiles) of the expected numbers of cases in groups of individuals

with the same known exposure for the same outbreak. The contamination level was taken from posterior mean dose. Whenever the consumption and/or the secretor status were unknown, they were sampled from the model posterior distribution. Samples of size 9000 were simulated for this purpose.

Results

Boxplots of key parameters (μ0, λ, γ) for each model are given in **figure 17.**

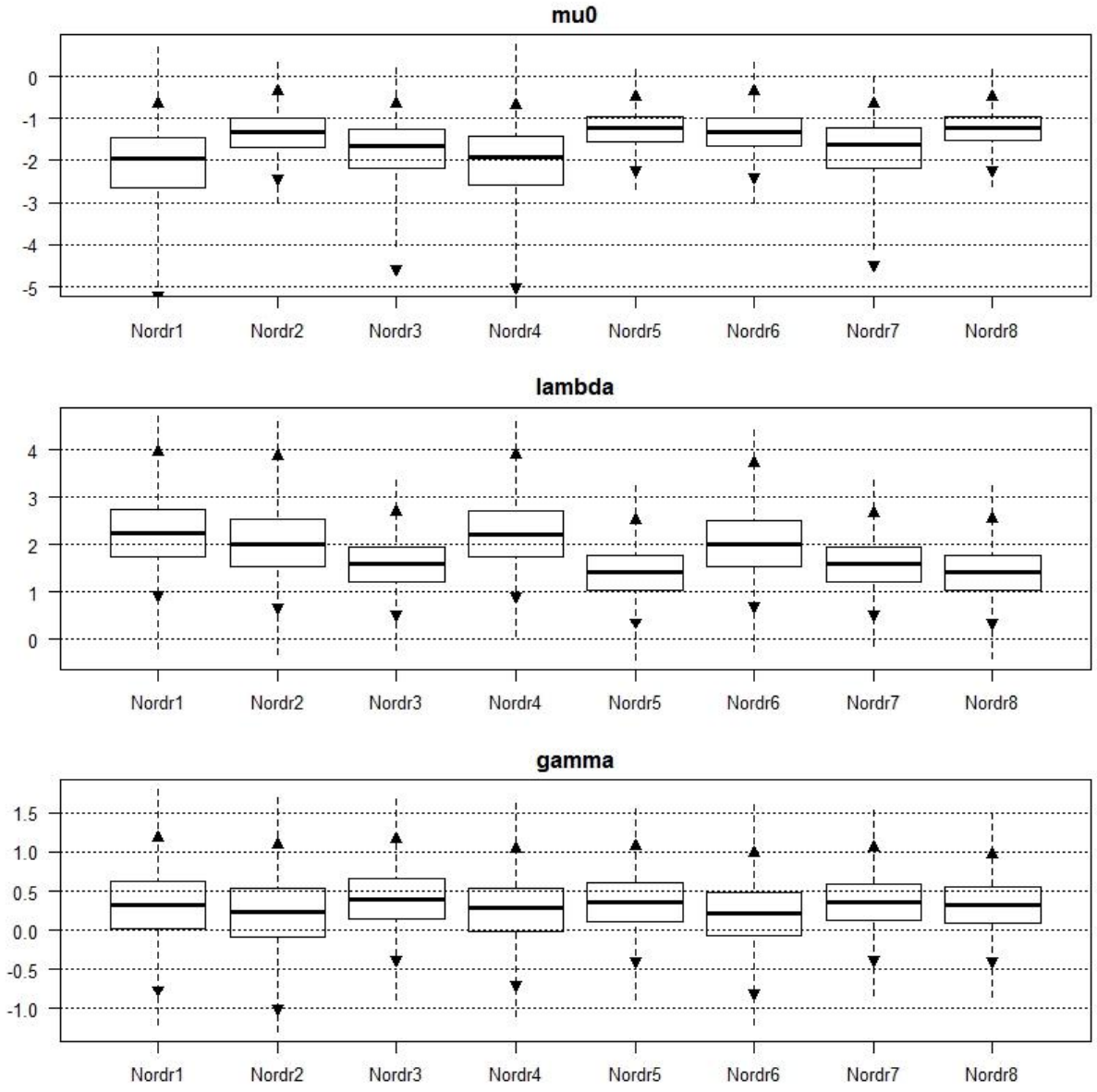

FIGURE 17: BOXPLOT OF POSTERIOR DISTRIBUTION.

Legend: median and 95% CI of the posterior distribution parameters μ0, λ, γ. Respective standard deviation of priors for each model: Nordr1($sd_{\mu 0}$=3,sd_{λ}=3,sd_{γ}=3), Nordr2($sd_{\mu 0}$=1,sd_{λ}=3,sd_{γ}=3), Nordr3($sd_{\mu 0}$=3,sd_{λ}=1,sd_{γ}=3), Nordr4($sd_{\mu 0}$=3,sd_{λ}=3,sd_{γ}=1),

Nordr5($sd_{\mu 0}$=1,sd_{λ}=1,sd_{γ}=3), Nordr6($sd_{\mu 0}$=1,sd_{λ}=3,sd_{γ}=1), Nordr7 ($sd_{\mu 0}$=3, sdλ=1, sdγ=1), Nordr8 ($sd_{\mu 0}$=1, sdλ=1, sdγ=1)

Category	Parameter	Posteriors		
		Median	2.5th percentile CI	97.5th percentile CI
All	μ0	-1.96	-5.22	-0.66
	λ	2.23	0.93	3.95
	γ	0.32	-0.76	1.18
	r	0.99	0.59	1.63
	η	0.99	0.37	2.67
for Se+/GI	α	$1.2*10^{-2}$	$3.13*10^{-5}$	0.59
	β	$1.13*10^{-2}$	$2.01*10^{-5}$	5.1
	mean(p_m)	0.45	0.025	0.96
	var(p_m)	0.17	0.004	0.25
for Se-/GI	α	$3.04*10^{-4}$	$5.3*10^{-7}$	$1.36*10^{-2}$
	β	$2.88*10^{-2}$	$8.14*10^{-5}$	5.57
	mean(p_m)	$9.4*10^{-3}$	$1.5*10^{-4}$	0.19
	var(p_m)	$8.49*10^{-3}$	$4.02*10^{-5}$	0.15
for Se+/GII	α	$1.72*10^{-2}$	$5.2*10^{-5}$	0.61
	β	$8.24*10^{-3}$	$1.6*10^{-5}$	5.19
	mean(p_m)	0.62	0.05	0.96
	var(p_m)	0.17	0.006	0.25
for Se-/GII	α	$5.5*10^{-4}$	$1.12*10^{-6}$	$2.16*10^{-2}$
	β	$2.79*10^{-2}$	$8.13*10^{-5}$	5.59
	mean(p_m)	0.018	$2*10^{-4}$	0.30
	var(p_m)	0.016	$5*10^{-5}$	0.20

TABLE 11: STATISTICS OF POSTERIOR DISTRIBUTIONS OF THE MAIN PARAMETERS

Priors for (μ0, λ, γ) were symmetric around 0 are given in appendix Table 2, by monte-carlo simulation to give an idea about the precision of the simulation. For all these models, posteriors show that for μ0, the posterior distribution is shifted to negative values. The positive posterior values of λ represent the strong protective effect of Se- status, as is also apparent for all these models. The effect of genogroup is described by γ. The fraction of posterior sample of gamma greater than zero, as shown in Figure 2, is not large or small (between 69.3 and 85%, for different variance of gamma distribution priors) indicating that we do not have strong evidence that GI and GII have different infectivities. Posterior 95% CI of key parameters (μ0, λ, γ, η, r, α, β), for the model with increased flexibility (standard deviation of μ0, 3, λ, 3, γ, 3 respectively), are given in **Table 11,** stratified by genogroup and secretor status. Priors of this model are detailed in the **appendix**. The estimated risk of infection per ingested virus particle p_m is high, with posterior median values for the mean around 0.5 for Se+ subjects, 2.5th percentile around 0.03, 97.5th percentile around 0.96. For non secretor, this value is much lower, around 1/40 for the mean of p_m (**Table 11**).

Dose-response graphs of predicted probabilities (median and 95% credible interval) of infection and illness as a function of doses are shown respectively in **Figures18** and 19.

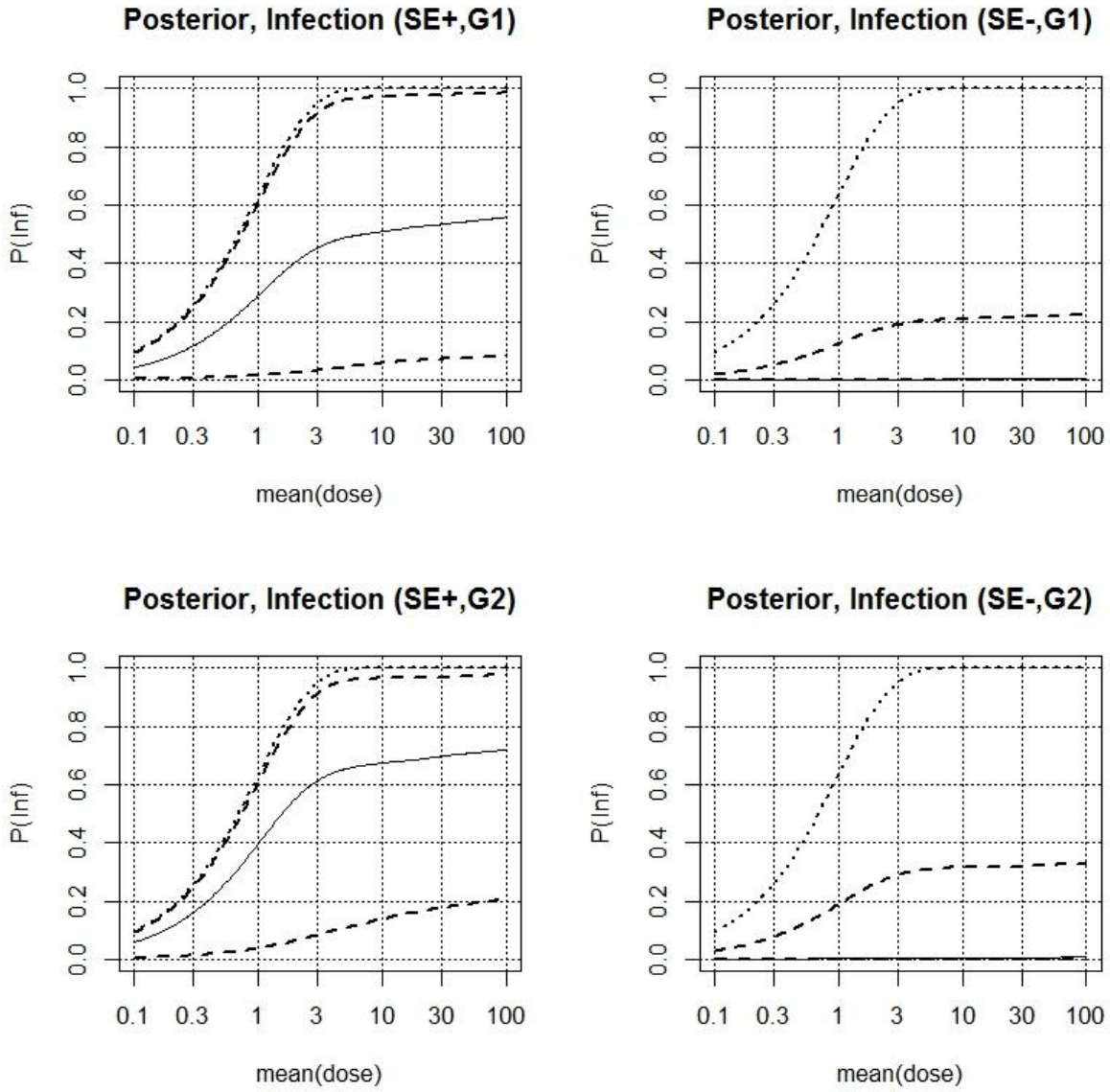

FIGURE 18: POSTERIOR DOSE-INFECTION RELATIONSHIPS.

Legend: Solid line: median of dose-response curves; dashed line: credible interval 95%; dot dash line: maximum infectivity limitation curve.

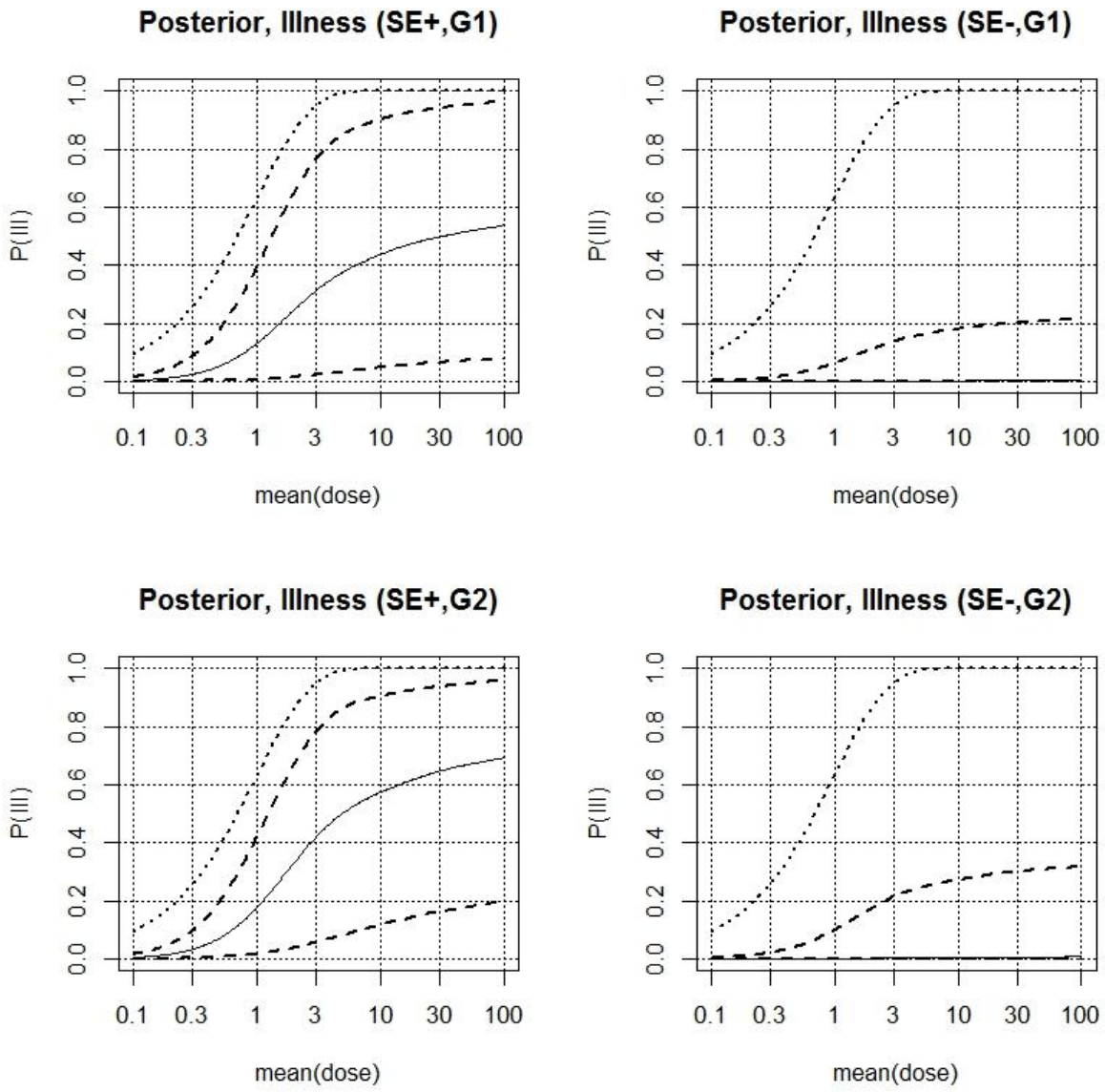

Figure 19: Posterior dose-illness relationships.

Legend: Solid line: median of dose-response curves; dashed line: credible interval 95%; dot dash line: maximum infectivity limitation curve.

Characteristics such as median infectious dose (ID_{50}) and probability of infection or illness at an average dose of a single virus are given in **Table 12**.

Category	Infection/disease	statistics	Median	2.5th percentile CI	97.5th percentile CI
Se+/GI	**Infection risk curve**	ID 50	7.1	0.73	$>10^6$
		prob inf with mean 1	0.29	0.015	0.61
	Disease risk curve	ID 50	32	1.32	$>10^6$
		prob dis with mean 1	0.13	0.007	0.39
Se-/GI	**Infection risk curve**	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob inf with mean 1	$9*10^{-4}$	4.4*10-6	0.12
	Disease risk curve	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob dis with mean 1	$4.25*10^{-4}$	$2.1*10^{-6}$	$6.19*10^{-2}$
Se+/GII	**Infection risk curve**	ID 50	1.6	0.74	$>10^6$
		prob inf with mean 1	0.4	0.04	0.61
	Disease risk curve	ID 50	4.86	1.24	$>10^6$
		prob dis with mean 1	0.18	0.017	0.42
Se-/GII	**Infection risk curve**	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob inf with mean 1	$2.12*10^{-3}$	$0.96*10^{-5}$	0.19
	Disease risk curve	ID 50	$>10^6$	$>10^6$	$>10^6$
		prob dis with mean 1	$1.03*10^{-3}$	$4.2*10^{-6}$	0.1

TABLE 12: STATISTICS OF POSTERIOR DOSE-INFECTION (AS PLOTTED IN FIGURE 1) AND DOSE-DISEASE CURVES (AS PLOTTED IN FIGURE 2)

Characteristics such as median infectious dose (ID_{50}) and probability of infection or illness at an average dose of a single virus are given in **Table 12**. Median ID_{50} estimates ranging between 1.6 and 7.1 genome copies per oyster consumed (**Table 12**), probability of infection with a mean dose of a single NoV genome (Poisson distribution) are 0.29 [0.015-0.61] for GI and 0.4 [0.04-0.61] for GII in Se+ subjects (**Table 12**). For Se- subjects the probability of infection and disease with a mean dose of a single NoV genome (Poisson distribution) are lower, 9.10^{-4}[$4.4.10^{-6}$-0.12] for GI and $2.12.10^{-3}$[$0.96.10^{-5}$-0.19]for GII.

In Se+ subjects the probability of acute enteric disease was also very high: with a mean dose of one genome copy, the median probability of illness is, for GI, 0.13 [0.007-0.39], for GII, 0.18 [0.017-0.42], and much lower for Se-individuals (**Table 12**)

A density graph of the probability density of p_m (transformed to logit scale) can be constructed using a posterior sample of the infectivity parameters (α, β) showing strong heterogeneity in infectivity for Se+ subjects and smaller heterogeneity in Se- subjects (**Figure 20**) for the 2.5th percentile.

Results of the prediction are given in the **Table 13**. The posterior predictive distributions look plausible with respect to the observed data, the observed numbers of cases are always within the predicted the 95% credible interval.

year of outbreak	group	SE+(1)/ SE-(0)	number of oysters eaten	observed contamination /oyster	number exposed	observed illness cases	posterior quantile estimate of number of illness cases				
							2.5%	25%	50%	75%	97.5%
2008	1	0	3	GII: 118-955-37-0	3	0	0	0	0	0	2
2008	2	0	4		2	0	0	0	0	0	1
2008	3	0	6		1	1	0	0	0	0	1
2008	4	1	2		3	3	1	2	3	3	3
2008	5	1	3		17	12	6	13	15	16	17
2008	6	1	4		2	2	0	1	2	2	2
2008	7	1	6		4	3	1	3	4	4	4
2008	8	NA	3		1	1	0	1	1	1	1
2008	9	1	2-6		1	1	0	1	1	1	1
2006a	10	NA	4-6	GII: 1100 GI: 2300	27	11	10	12	14	16	20
2006b	11	NA	4-6	GI: 275-683	2	2	0	1	2	2	2
2002	12	NA	1-6	GII: 25-125 GI: 25	36	21	17	21	22	24	27
2000	13	NA	7	GI: 85-237	1	1	0	1	1	1	1
2000	14	NA	9		2	1	0	1	2	2	2
2000	15	NA	18		1	1	0	1	1	1	1

Table 13. observed numbers of cases in some identically exposed individuals and related simulated predictions from the posterior distribution model

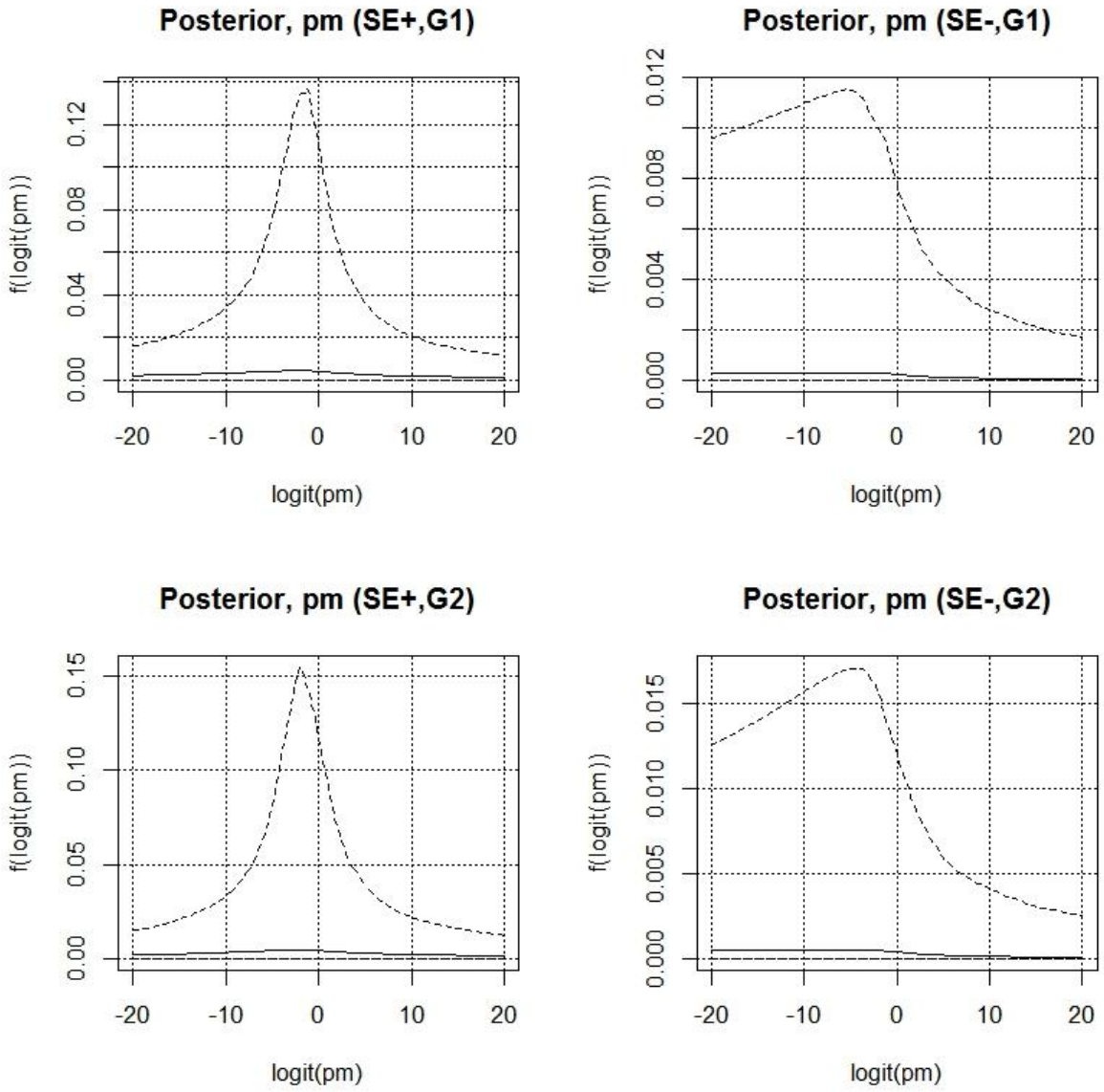

FIGURE 20. DENSITY GRAPH OF THE POSTERIOR SINGLE-HIT PROBABILITY OF INFECTION P_M, TRANSFORMED TO LOGIT SCALE.

Legend: Solid line: contour of median density of probability; dashed line: contour of a 95% credible interval.

Discussion

Strong differences were found between secretor and non secretor phenotypes (Tables 2 and 3). For secretor positives, infection probability and disease probability at low dose were high. In a human challenge study, the median infectious dose for Norwalk virus (GI.1) in Se+ subjects, was found to be around 18 genome copies [17], and probability of infection for a single Norwalk genome copy was close to 0.5. For Se+ subjects, our results are very similar to these clinical estimates, for both GI and GII NoV, with median ID_{50} estimates ranging

between 1.6 and 7.51 genome copies per oyster consumed (**Table 12**), and probability of infection(p_m) for a single NoV genome copy near 0.5(Table 11). In Se+ subjects the probability of acute enteric disease was high, tand this is in agreement with high attack rates reported in NoV outbreaks, keeping in mind that around 20% of the population is less susceptible (secretor negative)[10, 11]. This high apparent infectivity (low ID_{50}) in PCR units suggests that there cannot be large fraction of uninfectious (defective) genome copies.

However, credible intervals are wide and the lower limit of the credible interval should be examined carefully. The 2.5 th percentile represents the lowest plausible infectivity, and for secretor positives, those values are still high, with the 2.5^{th} percentile of E(p_m) (Expectation) around 0.05 (Table 11), compared to the lower limit of the prior near 10^{-5} (see Table in appendix).

At low doses, there are few symptomatic cases, so that the chance of reporting is low (endemic cases), while at high doses there are many illnesses among the infected cases and the cluster, or outbreak, is easily detected, as suggested recently in a study comparing shellfish implicated in outbreaks compared to background environmental levels [35]. However, exposure is different from place to place [40], and data of French outbreaks, show that there were identified outbreaks with values of contamination relatively low (see **Table 10**).

We did not detect any difference in infectivity between GI and GII strains among the five outbreaks analyzed here. However, the variances of estimated infectivities (p_m) are high, and inclusion of additional outbreaks might reduce the uncertainty and reveal a difference in infectivity.

It may be surprising to find GI NoV so frequently involved considering the large dominance of GII in human outbreaks [9]. Different factors such as distinct resistance to waste water treatment [36] or selective mechanisms in bio-accumulation of NoV strains have to be considered [25, 37].

The genogroup effect as two distinct classes of susceptibility is a simplification. Heterogeneity of responses can be found between strains within genogroups, possibly linked with the ABO blood group phenotype [21, 38, 39]. Because the ABO blood group was only known in one of the studied outbreaks and its effect could vary between strains within genogroups, this mechanism of genetic susceptibility was neglected as well as any pre-existing acquired immunity. However the use of a Beta-Poisson (Hypergeometric 2F1 in this case) dose-response takes into account any variability of response of the host, and we may assume that it is incorporated into the dose-response estimates reported here.

Analysis of the saliva of consumers in outbreaks suggested that the effect of secretor status may not always be all or none [21]. Susceptibility of secretor negative individuals requires the existence of other ligands with weaker binding or the occurrence of rare strains that can infect non-secretors.

The dose method used for oyster analysis includes quality control such as extraction efficiency and absence of RT-PCR inhibitors. Only samples complying with these controls (over 10% extraction efficiency and absence of inhibitors) were quantified [24] knowing that the extraction efficiency ranged between 13% and 38% in these shellfish analyses. As no method is currently available to evaluate NoV viability, we assumed that the fraction virus viable for infection was identical between the different oyster related outbreaks. All these factors have to be considered for future quantitative risk assessment studies.

For this first approach, we considered effects of genogroup or secretor status on infection and not on the illness dose-response relationship. It has been shown that secretor negative subjects are protected against infection, and thus their risk of becoming ill is also decreased [10]. For other enteric pathogens, variation in infectivity among strains has been demonstrated [18]. Since illness is conditional on infection, any effect acting on the probability of infection also modifies the marginal probability of illness. As in these outbreaks the data do not provide infection status, we have chosen the simplest way to take these covariates into account.

Contamination by multiple infectious agents is frequent in oyster-related outbreaks because of the fecal origin of contamination, by sewage contaminated water [19]. Since no information is available regarding mechanisms of cooperation or antagonism between infectivity or morbidity of NoV genogroups, we assumed there was no interaction. We consider also in this study only outbreaks with undetectable bacterial contamination and with identical NoV sequences in stool and shellfish samples of the same outbreak.

The actual scarcity of information is reflected by wide credible intervals. When additional outbreak data become available, with at least information on host secretor status, ABO blood type, size of exposed population, food intake and level of NoV in the contaminated food, the proposed dose response model may be improved, including ABO type as a covariate, and/or enable users to make more specific assumptions about effects on infection or illness. The generic model used here is described into the appendix, can also be used for the study of other outbreaks.

Conclusions

In conclusion, this study uses outbreaks to establish a human dose-response model for GI and GII, confirming that these viruses are highly infectious to humans with the secretor positive phenotype. Se- subjects have a strongly decreased susceptibility to NoV infection from either genogroup, as previously demonstrated with human challenge studies using G1.1. This is remarkable because the present results are based on outbreaks induced by consumption of contaminated oyster with a natural mix of strains and genogroups. For several years now, the increased recognition of the role of food, especially oysters, in gastroenteritis outbreaks has raised questions for safety regulations. Current processes (depuration, relaying, high pressure treatment or home cooking) as commonly performed are not effective to eliminate NoVs from oysters. Improving microbiological criteria for

shellfish or food items by including NoVs surveillance measures will help to improve the safety of food introduced on the market [40]. Oyster producers must avoid harvesting from fecally contaminated areas and food business operators need such information to consider their safety limits [40]. This work will be useful for risk assessors and risk managers to establish acceptable limit for NoV in oysters to be harvested and placed on the market, and may also be helpful for other risky food such as raspberries [41]. The present study provides new insights that will need to be considered for future regulation.

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Appendix

Parameter	status	Components	Value / Distribution	Rationale
o		Index of outbreak	1 to 5	
g		Index of Genogroup	1 or 2	
i, o		Index of individual i in outbreak o	1 to n [o],…see Table 1	see Table 1
pse	*marginal*	Probability to be Secretor(=1) in general population	Beta(79, 19)	[11]
Sec[i,o]	*conditional*	Secretor status of individual	Bernoulli(pse):(0, 1)	Informed by individual data or by prior pse
Contap[o,g]	*marginal*	Parameter p of Negative Binomiale distribution	$10^{\text{uniform}[-4,0]}$	Informed by observed data in oyster samples [o, g]
Contas[o,g]	*marginal*	Parameter s of Negative Binomiale	Round ($10^{\text{Uniform}(1, 1000)}$)	Informed by observed data in oyster samples [o, g]
oysconta [o, i, g]	*conditional*	Number of virus /oyster	Negbin (contap[o,g],contas[o,g])	

Ran[o, 1]	*fixed*	Minimum value of consumption (number of oyster)	Respective values		Data from outbreaks
Ran[o, 2]	*fixed*	Maximum value of consumption (number of oyster)	Respective values		Data from outbreaks
Mup[o]	fixed	Parameter of Poisson distribution	$\sqrt{ran[o,1] \times ran[o,2]}$		Geometric Mean value calculation
Oysconsum[o,i]	*marginal*	Oyster Consumption	Poisson (Mup[o]) Truncate (ran[o, 1]*ran[o, 2])		Informed by individual data or rank
Pgg[o,g]	*fixed*	Presence of genogroup in outbreak	0 or 1		Data from outbreak
ingdose1[o,i,g]	*intermediate*	Ingested dose for each individual and each genogroup	oysconsum[o,i]*Pgg[o,g]*oysconta[o,i,g]		
μ0	*marginal*	Intercept muw	Normal(mean=0,std=3)		Low informative
λ	*marginal*	Parameter of the Secretor effect	Normal(mean=0,std=3)		Low informative
γ	*marginal*	Parameter of the Genogroup effect	Normal(mean=0,std=3)		Low informative
Muw[sec[i,o],g]	*intermediate*	Expectation of beta distribution	μ0+λ*(sec[i,o]*2-1)+γ*(g*2-3)		
siw	*fixed*	Std of w	1		Low informative
w	conditional	Logit of the mean of beta distribution	Normal(mean=Muw,std=siw)		Low informative
z	marginal	Log (of quantity inversely related with variance)	Normal(mean=0,std=4)		Low informative
α[sec[i,o],g]	*intermediate*	First Parameter of dose-infection relationship	$\frac{\exp(w[i,g])}{1+\exp(w[i,g])} \times \exp(z)$		
β[sec[i,o],g]	*intermediate*	Second parameter	$\left(1-\frac{\exp(w[i,g])}{1+\exp(w[i,g])}\right) \times \exp(z)$		

pinf1[i,o,g]	*interm ediate*	Probability of infection knowing exact dose	$\left(1-\frac{\Gamma(a[i,g]+b[i,g])\times\Gamma(b[i,g]+Ingdose1[o,i,}{\Gamma(b[i,g])\times\Gamma(a[i,g]+b[i,g]+Ingdose1[o,i,}\right.$	Beta-Binomial
pinf2[i, o, g]	*interm ediate*	Probability of infection with both genogroups	1-(1-pinf1[i, 1])*(1-pinf1[i, 2])	independenc e of action
ingdose2[i, o]	*interm ediate*	Sum of doses for GI and GII	ingdose1[i,o, 1]+ingdose1[i,o, 2]	
η	*margin al*	1st parameter	$\exp(Normale(mean=0, std=0.5))$	Low informative
r	*margin al*	2nd parameter	$\exp(Normale(mean=0, std=0.25))$	Low informative
pill[o,i]	*interm ediate*	Probability of illness	$(1-(1+\eta\times \text{Ingdose2}[\text{o,i}])^{-r})\times pinf$	[38]
Ill[o,i]	conditi onal	Illness	$Bern(pill[o,i])$	

TABLE 13.DEFINITION, DISTRIBUTIONS AND VALUES OF PARAMETERS USED IN THE MODEL.

Legend: Status, gives the situation in Bayesian framework: marginal status for root random nodes, conditional for the other random nodes, fixed for constant values, and intermediate for the other nodes.

Category	Parameter	Priors		
		Median	**2.5th percentile CI**	**97.5th percentile CI**
	μ0	0.005	-5.83	5.84
	λ	0.01	-5.89	5.87
	γ	-0.02	-5.97	5.87
	r	1	0.6	1.63
	η	1.005	0.38	2.64
	pse	0.81	0.74	0.87
	z	-0.02	-7.86	7.74
Se+/GI	α	0.15	$2.06*10^{-6}$	969
	β	0.14	$1.86*10^{-6}$	955
	mean(p_m)	0.51	$3.2*10^{-5}$	0.9999
	var(p_m)	0.004	$2.5*10^{-7}$	0.23
Se-/GI	α	0.15	$2.1*10^{-6}$	986
	β	0.14	$2*10^{-6}$	997
	mean(p_m)	0.51	$3*10^{-5}$	0.9999
	var(p_m)	0.004	$4*10^{-5}$	0.15
Se+/GII	α	0.15	$2.36*10^{-6}$	943
	β	0.15	$2.2*10^{-6}$	943
	mean(p_m)	0.5	$3.2*10^{-5}$	0.9999
	var(p_m)	0.004	$2.1*10^{-7}$	0.22
Se-/GII	α	0.13	$2.12*10^{-6}$	941
	β	0.15	$2.16*10^{-6}$	928
	mean(p_m)	0.49	$3.22*10^{-5}$	0.9999
	var(p_m)	0.004	$2.4*10^{-7}$	0.224

TABLE 14: STATISTICS OF PRIORS DISTRIBUTIONS OF THE MAIN PARAMETERS

Appendix program:

R code preparing data file for BUGS code:

given on request

BUGS code describing the extended core model.

```
model {

  # ANCESTOR NODES = HYPERPARAMETERS
  #common between outbreaks (otb)
  # proportion of positive secretors people
  pse ~ dbeta(79, 19);

   #hyperparameters for dose-response (infection risk)
   # central dose response
  simu0<-3;
  mu0 ~ dnorm(0, 1/simu0^2);
  # effect due to the secretary status of the individual
  silambda<-3;
  lambda ~ dnorm(0, 1/silambda^2);
  # effect due to the genotype of the virus
  sigamma<-3;
  gamma  ~ dnorm(0, 1/sigamma^2);

  # common shape for the dose response
  # irrespective the attributes
  muz<-0;
  siz<-4;
  z ~ dnorm(muz, 1/siz^2);
  siw<-1;

  # hyperparameters for disease risk

  logetadis ~ dnorm(0, 1/0.5^2);
  logrdis ~ dnorm(0, 1/0.25^2);
  eta <- exp(logetadis);
  r <- exp(logrdis);
  #

  ###   looping on outbreaks with the help of indexes matrices
  ###       over all individuals from any outbreaks
  ###       over all sampled oysters

  for (otb in 1:nbotb) {
    #
    ###############
    # modelling the level of contamination of
    # the oysters for the two genotype
    for (ge in 1:2) {
      #   the parameters
      #       ancestor
      conts[otb,ge] ~ dunif(-4,0);
      contmu[otb,ge] ~ dunif(1, 1000);
      #       parameter to use
      contap[otb,ge] <- pow(10,conts[otb,ge]);
      contas[otb,ge] <- round (contmu[otb,ge]);
    }
    # modelling the contamination of sampled oysters
    for (oys in oyind[otb, 1]:oyind[otb, 2]) {
      oys.con1[oys] ~ dnegbin (contap[otb, 1],contas[otb, 1]);
      oys.con2[oys] ~ dnegbin (contap[otb, 2],contas[otb, 2]);
```

```
    }
    #
    ##############
    # modelling the illness of individuals
        for (ind in indind[otb, 1]:indind[otb, 2]) {
      #
      # consumption of oysters
      mup[ind] <- sqrt (ran[otb, 1]*ran[otb, 2]);
      oysconsum[ind] ~ dpois(mup[ind]) T (ran[otb, 1],ran[otb, 2]);
      # secretory status
      sec[ind] ~ dbern (pse);
      # loop onto the two genogroupes I an II
      for (g in 1:2) {

        #
       # oyster contamination
       oysconta[ind,g] ~ dnegbin (contap[otb,g],contas[otb,g]);
       # ingested dose
       ingdose1[ind,g] <- oysconta[ind,g]*oysconsum[ind]*pgg[otb,g];
        #
        # modelling the dose-response for infection
        #
       # expectation
       muw[ind,g] <- mu0 +
                     (sec[ind]*2-1)*lambda +
                     (g*2-3)*gamma
                   ;
       # variability around it
       w[ind,g] ~ dnorm(muw[ind,g], 1/siw^2);
       #
       u[ind,g] <- exp(w[ind,g]) / (1+exp(w[ind,g]));
       v[ind,g] <- exp(z);
       #
       a[ind,g] <- u[ind,g] * v[ind,g];
       b[ind,g] <- (1-u[ind,g]) * v[ind,g];
       #
       gammag1[ind,g] <- loggam(a[ind,g]+b[ind,g]) -
                         loggam(a[ind,g]+b[ind,g]+ingdose1[ind,g]) +
                         loggam(ingdose1[ind,g]+b[ind,g]) -
                         loggam(b[ind,g]);
       # proba of infection per genogroup
       pinf1[ind,g] <- (1-exp(gammag1[ind,g]));
      } # ending the loop over g
      #
      # probability of infection combining all genogroups
      pinf2[ind] <- 1-(1-pinf1[ind, 1])*(1-pinf1[ind, 2]);
      #
      # Looking for illness dose-response
      # common dose for conditional illness
      ingdose2[ind] <- sum(ingdose1[ind,])
      # probability to get ill for the assumed dose
      pill[ind] <- (1-pow(1+eta*ingdose2[ind],-r))*pinf2[ind];
      # modelling the illness
      ill[ind] ~ dbern (pill[ind]);
    } # ending loop over ind
    #
  } # ending loop over otb
  #
} # ending the model
```

II.3.2. COMPLEMENT OF THE PAPER

II.3.2.1. CHOICE OF THE MODEL

Other kind of modeling were possible, but were finally rejected, those possibilities and reasons for reject were developed here.

- The first kind of other modeling is concerning the effect of dose with the disease risk. The effect is supposed to be increasing with dose. This is justified by the previous work of dose-response with Norovirus, showing that the effect of dose was relevant for risk of diseases with GI in a trial experiment situation for Se+ individuals. By hypothesis, for the risk of infection and disease, an increasing effect of dose was also postulated for Se- individuals, in the model we have submitted. Our data were scarce, and our proposal was simple as possible, and can be changed in future with added information.

- In the same idea, the effect of Genotype and Secretor status is associated with risk of infection and not disease, or not on both risks. Our data cannot differentiate infection and ilnness, referencing only illness cases. Further studies on excretion on the exposed group, should help to refine and confirm hypothesis made on this model. Due to the scarcity of the data, and of combination presented, interactions between Genotype and Secretor status was not searched. Interaction between GI and GII, in case of co-contamination, was also neglected.

- Also the effect of genotype and Secretor status are treated as categorical variables, in linear relationship, with the mean of the Beta distribution describing variability of infectivity. Other parameterization can be investigated, again with more data.

  We didn't set a hierarchical model for dose-response parameters. Two level dose-response parameter, enabling analysis of the variation within and between outbreaks., were by example set for the dose-response of *E. Coli, Campylobacter* and *Salmonella* dose-response (Teunis *et al*., 2005; 2007; 2008). Variation among separate outbreak dose–response relations is described by the (joint) distribution of the infection parameters (α, β) and the illness parameters (ρ, η). Extra variability due to outbreaks was justified for *E. coli, Campylobacter* and *Salmonella*, because those outbreaks were identified in different countries, in different food matrix, and in different age group. Outbreaks data used for our modeling were not different in food matrix, exposed population (healthy adults), and country. However in future, outbreaks data from other situation could be used for better estimating dose-response parameters. The generalization of our work in the context of hierarchical approach, taking into account variability between outbreaks for dose-response parameters can be proposed. For infection risk, we should have then to consider if the effect of the outbreak is affecting the mean of the Beta distribution on all parameters describing u (muw in the Table 1 of the appendix of the paper), such as $\mu 0$,λ (Secretor effect) and γ (genotype effect) or only $\mu 0$ by example, and if the outbreak effect is also affecting v (inversely correlated with the variance of Beta distribution). We make the hypothesis that an outbreak effect is only affecting mean of the Beta distribution, with parameters $\mu 0$,λ (Secretor effect) and γ, and also parameters of disease risk, r and η. For each parameter we should have to define uncorrelated non-informative hyper-parameters, to describe variability between outbreaks, such as normal distribution, as suggested by previous studies (Teunis

et al., 2008). We imagine easily that the uncertainty of those hyperparameters should be high, unless we have enough outbreaks (and data) to investigate.

- In future, with more data, it can be plausible to investigate another model involving the strain effect of the virus, hierarchically with genotype effect or, not taking account a genotype effect (a strain is belonging to one genotype only). Same questions can be raised for A, B, O blood type and secretor status for the human host. Because genetically, the corresponding alleles are segregated independently, the question becomes about the effect of an interaction or not between those two characteristics of the host.

III.3.2.2. INFLUENCE OF THE CHOICE OF THE PRIORS ON THE PRIOR DOSE-RESPONSE RELATIONSHIPS

Even if this point is raised in the paper, the parameterization with u and v of alpha and beta parameters is done in order to avoid the correlationship between those highly correlated parameters (Teunis *et al*., 2005; 2007; 2008). The influence of the choice of priors can be checked by plotting the dose-response models with priors distribution of parameters. Figure 21 and 22, are giving respectively the marginal risk of infection and disease, (taking into account infection), conditional to a mean dose. Those figures showing that, the information given by those priors is so limited that credible interval of risk of infection or diseases, linked with mean dose is between 0 and the maximum limit of single hit dose –response ((1-exp(-λ)) (Teunis and Havelaar, 2000), or very close to this limit for risk of disease. The median of the credible interval is quite median for disease risk between 0 and maximum limit curve, which was what we expect, given as less as possible information by the priors, in order that estimates are driven more by the structure of the model and by the data.

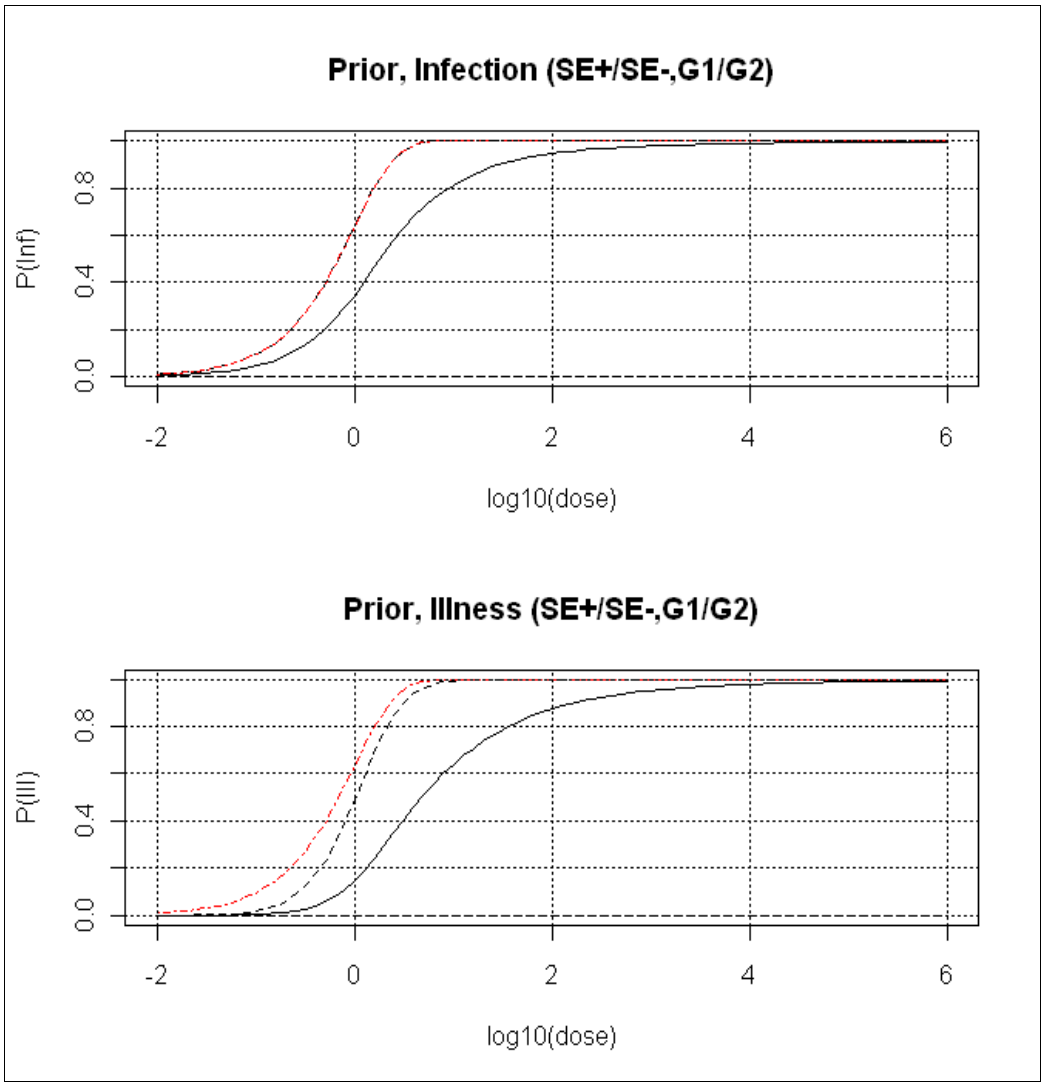


FIGURE 21: PRIOR DOSE-INFECTION AND PRIOR DOSE-ILLNESS RELATIONSHIP (SAME FIGURE FOR THE 4 SITUATIONS);

Legend: solid line: median of dose-response curves; dashed line: credible interval 95%; red dot dash line: maximum infectivity limitation curve (see text)

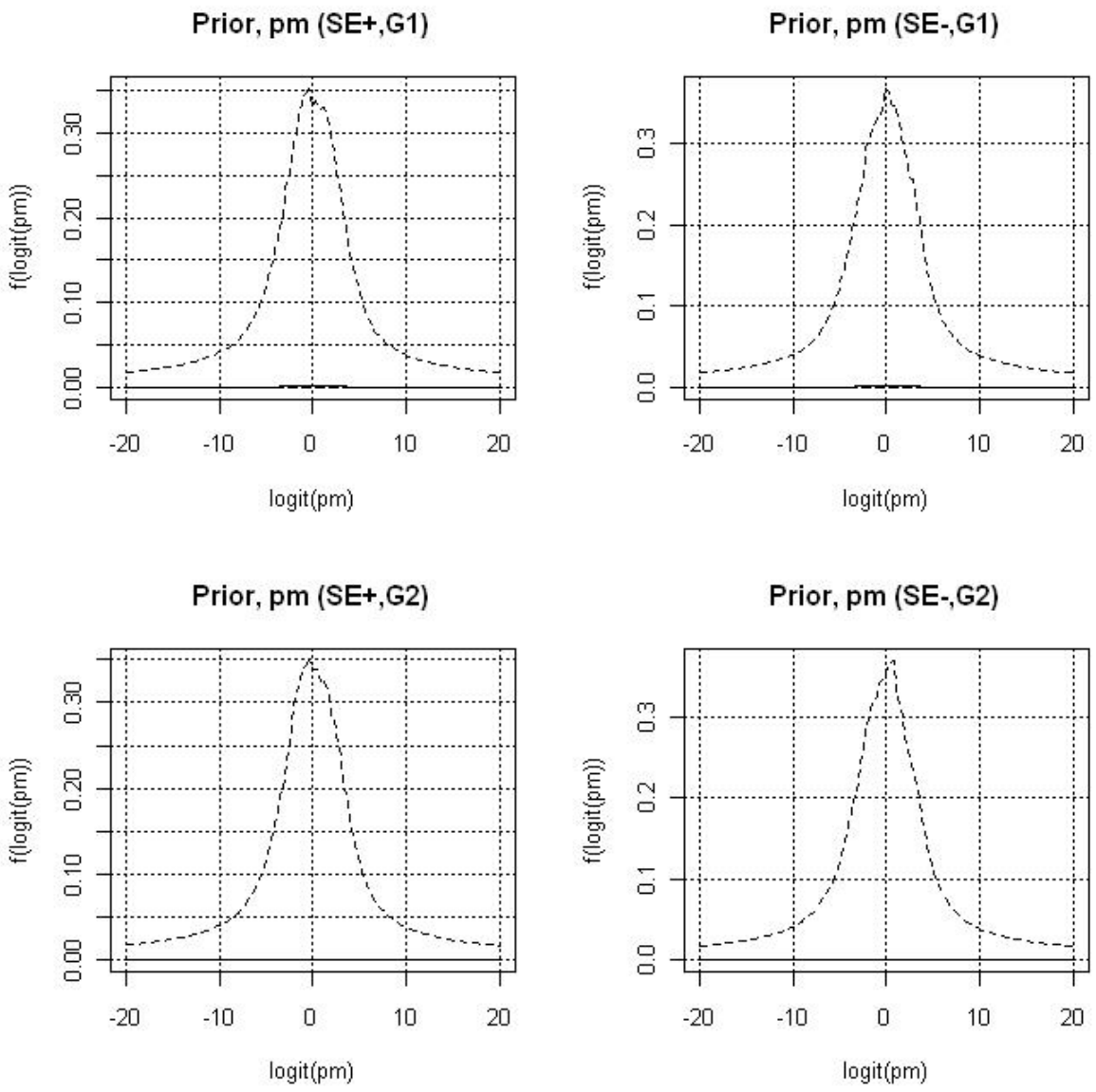


FIGURE 22: PRIOR FOR PM

Legend: solid line: median of dose-response curves; dashed line: credible interval 95%;

The Figure 22 is giving the prior of density of probability pm, in order to be compared with this obtained by the Bayesian inference. The prior of this density probability pm is symmetric around 0 and covering a large range of values, what we expected, in order to take into account all variability given by the data.

III.3.2.3. ADEQUACY OF THE MODEL TO THE DATA

With simplification, it is difficult to distinguish, in a bayesian inference, the validity of the model, and the validity of posterior distribution estimates for predictive aim.
Validity of prior is checked by sensitivity analysis, is done for some variance values (see submitted paper) and checked for consequences for prior of dose-response model (III.3.2.2).
Also the method used for establishing the model fitting criterion can applied to the same data set used for estimation, or to a new data set (which is currently described as "validation".
If we had a big data set we could have split the data into different subsets, to use part for establishing inference, and another part for validation purposes.

Because we only have limited data from few outbreaks, we used all the available data for Bayesian inference.
In this situation, fitting criterion applied to the same data set can be, theoretically, of use, such as or DIC (Deviance Information Criteria) or BIC (Bayesian Information Criteria)which add a penalty to the approximate error.

"The model with the smallest DIC is estimated to be the model that would best predict a replicate dataset which has the same structure as that currently observed. BIC attempts to identify the 'true' model, DIC is not based on any assumption of a 'true' model and is concerned with short-term predictive ability" (http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/dicpage.shtml).
However DIC, easily tractable (unlinke BIC) with rjags, is valid under the assumptions of asymptotic normality of posterior distributions of parameters, and not valid with missing data (neither hierarchical model). We have missing data in our study.

We compared our results to a completely different study, and show that infectivity values found for GI were close in both studies (Teunis et al., 2008) and discussion. (Reported attack rate in Norovirus outbreaks are also known to be strong)

Then, a practical and simple point of view is a posterior predictive checking with data used for Bayesian inference (Gelman *et al*., 2004). The observed data should look plausible under the posterior predictive distributions (Table 13).

The results given in Table 13 are showing adequacy between posterior estimates and observed data, used for Bayesian inference,with a little over-estimation in median, but showing observations in 95% CI for each situation. A more complex model, with interaction process between two genogroups, was not used, taking into account the few number of data available (scarcity principle). Validation of the model was not feasible without the use of new data. We prefer to take all the data for the inference estimates.

III.4. PERSPECTIVES

If the aim of dose-response modeling is to estimate a mean number of cases linked to a particular mean dose ingested, the approach developed here, with the different known dose-response model have been shown to be enough. This is the classical of dose-response, known as hazard characterization and is widely used for QRA.

For Norovirus or HAV, because inter-human transmission is feasible after foodborne cases, incorporating time to excretion, duration and level of excretion, or time to incubation can be of interest. Other published models are incorporating incubation time dependency (duration between exposure and illness) (Huang *et al.,* 2009 a and b). The prediction is concerning the number of cases, as a function of mean dose but also as a function of time post-inoculum (Huang *et al.,* 2009). However, this kind of data is not available for Norovirus, and the duration of incubation is usually short (1-2 days). For HAV, the incubation is long and more variable–(15 to 50 days), then the study of a function depending of dose, and time post-inoculum, and interaction between those two factors could be interesting to investigate in future. The link with the duration and importance of excretion or/and severity of symptoms with the dose and strain ingested can be also a matter of consideration in dose-response modeling. Data of outbreaks with immuno depressed situation in human population, linked to previous of illness or age effect should be better documented in order to be taken into account in dose-response estimates.

The effect of co-infection is not, also well established, between strains, genogroups or even different pathogens. The previous immune status of susceptible individuals exposed to Norovirus is not so – well known, and is not known in retrospective outbreak studies. The effect of airborne transmission, with exposure of aerosolized viral particles, where exposure to vomiting is high could also be explored in future (Marks *et al*., 2003; Teunis *et al*., 2010).

The dose-response we established here can be easily used in future for QRA purpose, establishing limitations of contamination in food in order to avoid sporadic cases and foodborne outbreaks. We propose here to use NoV dose-response model to better understand the potential part of oyster consumption when an epidemic occurs in human population.

CHAPTER IV: DYNAMIC MODEL FOR COMPREHENSIVE ANALYSIS OF THE FOODBORNE AND NOT FOODBORNE TRANSMISSION PATHWAY

IV.1. DYNAMIC MODELS USED IN EPIDEMIOLOGY: BIOLOGICAL AND THEORETICAL MEANINGS

IV.1.1. INTRODUCTION

Quantitative modeling may have different purposes. In epidemiology, different management strategies can be rarely tested in the real world, and if so it is always in a particular context. Reproducibility of results is therefore a critical question that should guide policy decisions (Wallinga and Teunis, 2004; Vynnycky and White, 2010) .

Epidemiological models provide theoretical results of management decisions, avoiding insofar as possible situations that are non observable in the real world. For making quantitative predictions that can guide policy decisions, modeling results must be used with caution. Real-world data are required for fitting the main parameters of the model, particularly those identified as critical by sensitivity analyses. Testing the model in other situations, not used for estimation, makes the validity of the model more accurate (validation step). Our purpose here is not to provide quantitative estimates for risk managers. The models were designed to provide a comprehensive analysis, explaining how the infection can spread, and how some control scenarios can interfere with results, under various assumptions. This comprehensive analysis sets out to explain the links between the main factors, with an idea of causality and plausible link pathway. Finally, our model also seek to highlight some gaps in our knowledge and help identify what kind of field data is required for fitting the model to real-data situations.

We examine the spread of infection and not just the spread of disease cases. For the dynamic of epidemics, the spread of infection is considered separately from the spread of symptoms (Figure 13). The disease period, when symptoms are manifest, is not necessarily completely correlated with the period of infectivity (Anderson and May, 1992; Keeling and Rohanni, 2008). More over asymptomatic individuals can be infectious and can contribute to the spread of infection, as in HAV and NoV cases (Sukhrie *et al.,* 2012).

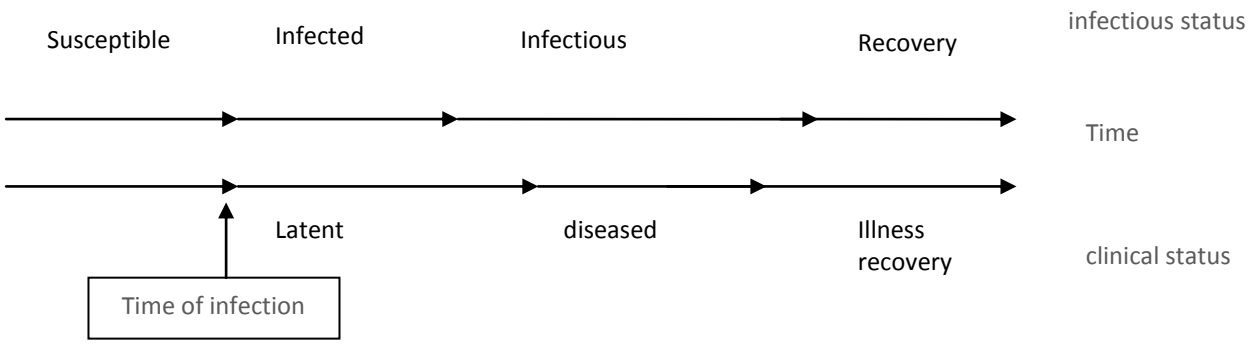


FIGURE 23: THE DYNAMICS OF INFECTION AND DISEASE PROGRESSION (FROM KEELING AND ROHANNI, 2008)

A basic concept of dynamic epidemic models is R_0 "the Basic Reproduction Number that is the average number of secondary infectious persons resulting from one infectious person following their introduction into a totally susceptible population" (Vynnycky and White, 2010). If R0 less than 1, epidemics go to extinct, if R_0 is greater than 1, epidemics can remain endemic (or go extinct with some probability). R_0 characterizes, the infectiousness and rapidity of a pathogen in a specified population of all susceptible individuals. R_0 is related to the concept of the herd immunity threshold, or critical immunization threshold, defined in a SIR (Susceptible-infectious-Removed) model as $1-1/R_0$. To eradicate an infection, in a SIR process the proportion of the population that is immunized must exceed this threshold value (Vynnycky and White, 2010). Whenever the epidemic continues, the average number of secondary cases produced by a single individual during its entire infectious period is characterized by the effective reproductive number R_N. This reproduction number can be changed by efficient management strategies, or can be evaluated to monitor the efficiency of control measures (Lipsitch *et al*., 2003; Wallinga and Teunis, 2004; Heesterbeek and Roberts, 2007).

The type of model used depends on the epidemiological purpose. The first step is to identify the question that can be treated by modeling (Vynnycky and White, 2010). Deterministic formulations are used for describing the spread of infection in large populations, whenever randomness cannot interfere with the main results. Stochastic formulations are necessary whenever randomness can play an important role, such as in small populations, or when rare events are under study. From one initial situation, with fixed parameters and a deterministic framework, only one type of epidemic situation can be predicted. This may be unrealistic in some cases, e.g. when a small number of individuals can play an important role in an epidemic, when studying disease invasion or disease extinction (in which rare chronic carriers or super-spreader events can play a significant role (Cori *et al.,* 2009). We will focus here on probabilistic models.

Probabilistic models can be described by the structure of the population considered, by the time analysis and by their formal mathematical framework. Individual-based models (Ajelli *et al*., 2008), in comparison with compartmental models, can take into consideration individual variation, e.g. in contact rates (superspreader individuals for example). Heterogeneity in the population (age, contact rate, vital dynamics) can be analyzed as a number of discrete compartments in the population or in an individual-based model (Ajelli *et al.,* 2008). With additional dimensions, meta-population models can explore relationship between distinct populations, in space (Ajelli *et al.,* 2011) and/or between different hosts populations (Keeling and Gilligan, 2000; Durand *et al*., 2010).

A model can be based on continuous or discrete time processes. In discrete time processes, the choice of the interval is crucial: two possible transition states should not be possible to occur during a single time step. The time step duration should be less than the mean duration of a given health state. Computation is faster, and less subjective for the choice of the time step, with short time steps in a long period of study, in a quite large population, with continuous-time compartmental models (Gillespie algorithm)(Vynnycky and White, 2010).

The diversity of mathematical formalisms in stochastic modeling can be illustrated by extensions of the Reed-Frost equation (O'Neill and Marks, 2005), generation-time processes (Wallinga and Teunis, 2004; Cauchemez *et al*., 2006; Heijne and Teunis, 2009), jumping processes with discrete time, Galton Watson branching processes (Bruss *et al*., 1984), jumping processes with continuous time,

Crump-mode-Jagger branching process (Antia *et al*., 2003) and the Gillespie algorithm (Breban *et al*., 2009; Wang *et al*., 2012). The generation time is the mean interval between infection of the primary case and its secondary cases.

For simulating jumping processes with continuous time, we used the Gillespie algorithm. We chose to develop the principle of this algorithm here, due to its particular use in our work (see section IV.3).

IV.1.2. Example of a stochastic process with continuous time: Gillespie algorithm

The principle of the Gillespie algorithm is given below, in a simple example:

A population of size N (closed population) can be divided into three compartments, with a particular number of individuals in each, (S) for susceptibles, (I) for infectious, and (R) for removed.

Possible transitions or events, include the following:

1 susceptible individual can be infected (migration of 1 from compartment S to I): event (SI)

1 infectious individual can be removed (migration of 1 from compartment I to R):event (IR)

The resulting simplistic compartmental model is given in Figure 14:

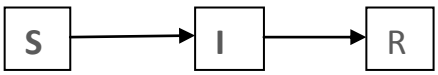


Figure 24: compartmental model illustrated with a flow diagram

Legend: Arrows indicate migration between the compartments. (S) for susceptible, (I) for infectious, and (R) for removed.

The algorithm proceeds as follows:

1. All possible events are labeled; here there are two events, ESI and EIR.
2. For each event the transition rate at which events occur is calculated
   for event ESI, the transition rate is given by the following equation:
   F1=β S I/ N (where β is the contact rate between S and I, I the number of Infected, S the number of susceptible).
   β is the exact per capita rate at which specific individuals come into effective contact per unit time.
   The number of individuals newly infected by inter-human transmission is related to the number of susceptible individuals, infectious individuals, and the contact rates between them. The force of infection is the rate at which susceptible become infected per unit time " (Vynnycky and White, 2010). It can be expressed by βI or βI/N.
   Under a frequency based assumption, the risk of infection remains unchanged as the population size increases. There is no crowding, nor change of individual behavior with the

population size. This assumption is deemed to be true, for large epidemics in humans such as the Flu. The transmission term for infection is thus βI/N. The alternative assumption is the density dependence assumption, used for example in animal diseases (Roche *et al.,* 2009). The force of infection is then βI.

for the event EIR, the transition rate (F2) from state I to R is given by:

F2=γ I (where γ is the removal rate or 1/average duration of infectiousness)

3. For short time periods, the number of events that occur is Poisson-distributed. The time to the next event is given by an exponential distribution and is memoryless. It describes the time between two events, i.e. a process in which events occur continuously and independently at a constant average rate:
    - for one event, this time for ESI is tSI and for EIR is tRI:
        - $tSI \sim exp(F_1)$, where $F_1 = \beta S I / N$
        - $tRI \sim exp(F_2)$, where $F_2 = \gamma I$
    - the minimum time to the next event (ESI or EIR), considering independent exponentially distributed random variables (tSI and tRI) with transition rate parameters F_1 and F_2, is a random variable, exponentially distributed, with a rate parameter equal to the sum of parameters of each separate exponential distribution (using the property of exponential distribution):
        - $t_{min} \sim exp(M_T)$
        - where $M_t = F_1 + F_2$
4. After sampling the time to the next event, the kind of event is being sampled.

    The probability of each competing event is

    $pESI = F_1 / M_t$

    $pEIR = F_2 / M_t$

    The next event to be ESI can be described by a Bernoulli distribution (pESI). (1 is an ESI event, 0 is an EIR event)

    The number of individuals in each compartment changes.
5. Return to the step 2.
6. Stop when the time of simulation is reached.

The Bernoulli distribution is a special case of Binomial distribution, with only one trial sample. Generalization to N events can be then easily obtained by changing the Binomial distribution to a multinomial distribution. The future state of the system depends only upon the current state (Markov chain property).

IV. 1.3. Parameter estimates and sensitivity analysis

The parameter estimation for dynamic modeling is classically performed in one of three ways: a maximum likelihood approach (Wallinga and Teunis, 2004), Bayesian inference (Cauchemez *et al*., 2004; O'Neill and Marks, 2005; Cauchemez *et al.,* 2006; Courcoul *et al*., 2010; Sukhrie *et al*., 2012), or Approximate Bayesian Computation based on sequential Monte Carlo simulations(ABC-SMC) (Liepe *et al*., 2010; Toni *et al*., 2009). This last method, given a parameter θ, its prior distribution π(θ) and a data set x, estimate the posterior distribution π(θ/x), as in classical bayesian inference. The ABC

rejection sampler is more flexible than the Gibbs sampling-Metropolis-Hasting algorithm (Gilks *et al*., 1996), allowing a tolerance parameter, that can be set by the user, for rejection criteria (Liepe *et al*., 2010).

The sensitivity analysis is usually done using different sampling methods (Monte-Carlo, Latin hyper cube, Full Factorial) and using different rank order estimate. Sensitivity analysis orders the input estimate by the magnitude of the uncertainty that it causes in the outcome variable. Here, the sensitivity analysis focuses on parameters with uncertain estimates rather than on parameters with variability estimates, already taken into account in the probabilistic framework. To do so, different metrics are used such as the Spearman partial rank correlation coefficient, the Pearson correlation coefficient, and the partial rank correlation coefficient (with different assumptions for the use of each) (Le Menach *et al*., 2006; Hoare *et al*., 2008). Global approaches, suitable for non-linear or non-additive models, and measuring the effect of an input factor when all other factors are varying, use Sobol's method and the Fourier Amplitude Test (FAST) (Saltelli *et al*., 2002; Reusser *et al*., 2011). For stochastic modeling, which takes into account each time point of each dynamic output, there are specific approaches based on principal component analysis and on analysis of variance (Lamboni *et al*., 2009; Lurette *et al*., 2009; Courcoul *et al*., 2011).

IV.2. LITERATURE REVIEW OF PUBLISHED DYNAMIC MODELS WITH FOOD-BORNE TRANSMISSION

Two categories of papers were selected: (1) examples of published dynamic models on foodborne transmission, with pathogens other than viruses (not exhaustive) and (2) published dynamic models of foodborne viruses, with or without foodborne transmission.

The information is provided in **Table 15** for foodborne pathogens other than viruses, taking into consideration foodborne transmission (in particular for humans) and in **Table 16** for foodborne viruses. For HAV some papers on dynamic modeling were omitted (Gay, 1996; Armtrong *et al*., 2002; Jacobsen *et al.,* 2004, Bauch *et al*., 2007) to focus on those that explicitely describe foodborne transmission (Ajelli *et al*., 2008; 2009; 2011).

Several papers on indirect transmission of Vibrio cholerae via water use a logistic dose-response curve (Codeço *et al.,* 2001; Longini *et al.,* 2007; Righetto *et al.,* 2012). This dose-response model does not assume a minimum threshold as do exponential or beta-poisson dose-responses. The resulting estimate can take any value as an input ranging from minus infinity to plus infinity, whereas the output is confined to values between 0 and 1, as a probability. The logistic function is a sigmoid curve, and is widely used, to introduce more or less complexity to models in ecology (Lotka-Volterra equations) (Brown and Rothery, 1993; Dennis *et al*., 1989;Breban *et al.,* 2010) and bacterial growth (Rosso *et al*., 1995).

The initial stage of growth is approximately exponential; then, as saturation begins, growth slows, and at maturity, growth stops, with the limitation of available resources. Studies of avian influenza viruses in southern France, (i.e. the Camargue area) also use logistic dose-response curve. This dose-response is not used in classical microbiological QRA, and does not have the same biological

assumptions, in particular, (i) exposure and (ii)survival and infectivity of the pathogen (see section III.1). However recent papers have used the well-known exponential dose-response (section II) (Breban *et al*., 2009) to describe avian influenza epidemics in ducks populations (Breban *et al*., 2009; Wang *et al*., 2012). Consumption is described by a constant rate.

Two papers relating to *Cryptosporidum parvum* and an enterovirus consider an exponential dose-response in a dynamic framework (Eisenberg *et al*., 1998; 2004; 2005) see **Tables 15 and 16**. Main dynamic models on viruses generally do not consider indirect foodborne transmission, given their objectives and context, and consider that foodborne transmission is negligible (**Table 16)**. HAV is an exception, in endemic situations and coastal populations (Ajelli *et al*., 2008; 2009; 2011). The kinetics of virus excretion and depletion has been considered for polioviruses and HAV (Ranta *et al*., 2001; Ajelli *et al*., 2008; 2009; 2011). For HAV, the foodborne transmission rate is a function of viral load for an infectious individual and the rate of depletion in the environment, and a periodic factor β_2, that can be observed and is fitted to describe individual consumption (Ajelli *et al.*, 2008; 2009; 2011) (**Table 16**). The rate of exposure (**Table 15**) describes the consumption by unit time. Consumption is not based on observations, but is fitted indirectly, based on seasonal fluctuations.

In the work described here, introducing stochastic QRA as a part of a dynamic modeling framework, with human consumption data, and dose-response variabilty described by a Beta-Distribution (Beta-Binomial), for foodborne viruses, is therefore a methodological improvement for modeling the foodborne pathogen dynamics (with several transmission routes).

Foodborne pathogen	reference	context	objective/mitigation strategies tested	Type of modeling/type of dose response	type and rate of foodborne transmission considered
Vibrio cholerae	Righetto *et al*., 2012	Human cholera insurgence with seasonality	Role of aquatic reservoir fluctuations in long term cholera pattern-	SIR /logistic dose-response curve (Codeco, 2001)	$\beta \times \frac{dose}{K_{50} * V + dose}$ (1) β=rate of exposure to contaminated water (day^{-1}) (water consumption/unit of time Hartley et al.2006) dose=total number of cholera bacteriae in the water reservoir of volume V dose is a function of Infectious individuals K_{50} concentration in bacteria that grants 50% probability for a susceptible of contracting the disease V volume of reservoir (with its own dynamic) logistic dose-response curve (Codeco, 2001)also used in Hartley and al, 2006 and in Shuai and Van den Driessche, 2011.
Vibrio cholerae	Longini *et al.*, 2007	Human cholera	effect of oral vaccine for controlling endemic cholera	SEIR with infectious asymptomatic individuals /two transmission routes: (1)person-person (2)person-environment-person	The probability, *P(t)*, that a susceptible person is infected with cholera in sub-region *i* on day *t* is then given by: $P(t) = \left[1 - (1 - \theta^x br\pi)^{u_i(t)} (1 - \theta^x \phi br\pi)^{v_i(t)}\right]$ Where π = probability that an unvaccinated susceptible is infected either from the environment or from direct contact due to the presence of a single unvaccinated infective in the sub-region (transmission probability), *r* = relative susceptibility multiplier. *x* = 1 if susceptible is vaccinated, 0 if unvaccinated, θ = 1 – vaccine efficacy against susceptibility, ϕ = 1 – vaccine efficacy against infectiousness, $u_i(t)$ = number of unvaccinated infectious people in sub-region i on day t, $v_i(t)$ = number of vaccinated infectious people in sub-region i, on day t; and *b* = seasonal boost factor for first 30 days of each run. Indirect transmission (via water is proportional to Infectious)

Cryptosporidium	Eisenberg *et al*., 2005	Humans massive outbreak of Milwaukee, 1993	Relative impact of 3 transmission pathways	SEIR: 3 transmissions pathways: (1)environnent-person (2) person – environment-person (3) person-person	proportional to Infectious (taking into account shedding, depletion rate, transport time, and failure of water plant treatment) in previous studies (Eisenberg *et al.,* 1998;1996) β(d)=1-exp(-rd)
Avian Influenza Virus (AIV)	Roche *et al.*, 2009	wild birds	Effect of ecology of the host (density, migration) and AIV persistence in water environment linked to epidemiological cycles	SIRS/logistic dose-response curve	$\omega \times \frac{dose}{\theta + dose}$ same (1) ω contact rate with water or drinking volume year^{-1} θ minimal viral load to initiate an (50%) infection (Roche *et al.,* 2009)
Avian Influenza Virus (AIV)	Breban *et al.,* 2009	Ducks	reservoir of virions that have long persisted in the environment. explain the pattern of periodicity and persistence of AIV; major results: environmental transmission provides a persistence mechanism in small community size, may explain 2-4	SIR/exponential dose-response	environmental transmission: ρS(1-exp(-αD) with α=log(2)/ID_{50} S number of susceptible individuals ρ per capita fraction of v virions ingested per unit of time and exposure rate, per capita consumption rate scaled by lake volume. V viral exposure.

			year periodicity, long term persistence by reservoir		
Avian Influenza	Wang *et al.,* 2012	Wild waterfowl	Show environmental transmission role in the periodicity of influenza outbreaks (2-4 years) and persistence of the virus	SIR/exponential dose - response	as Roche *et al.,* 2009
Avian Influenza multistrain)	Breban *et al.,* 2010	wild waterfowl	Results suggest that the endemic strain with non mixed transmission is more resistant to invasion	SIR multistrain/exponential doose -response perfect cross immunity between strains two strains with same Ro, one with one transmission route, one with two transmission routes.	As Roche *et al.,* 2009

TABLE 15: EXAMPLES OF RATES OF FOODBORNE TRANSMISSION IN DYNAMIC MODELS OF FOODBORNE PATHOGENS

(Potential) Foodborne pathogen	reference	context	objective/mitigation strategies tested	Type of modeling	Foodborne transmission investigated
Enterovirus (poliovirus)	Eisenberg *et al.,* 2004	Consequences of exposure to biosolid for human exposed population	To provide a method for assessing the consequences of pathogen exposure, example of exposure through consumption of biosolid-amended soil	SEIRS with person-environment person pathway	yes, approximation of a Beta-Poisson dose-response with ingestion rate of soil 0.01g/day dose is a function of Infectious individuals and external contamination
Poliovirus	Ranta *et al.,* 2001	Large unstructured (metropolitan) population, with endemic or epidemic transmission of Poliovirus	theoretical assessment of the likely efficiency of environmental surveillance (sewage water) compared to surveillance of human cases	I(t) for different situations	no
Poliovirus	Tebbens *et al.,* 2006	human outbreaks	consequences of reintroduction of poliovirus	SEIR	no
Poliovirus	Rahmandad *et al*., 2010;	human outbreak	consequences of network structure on the poliovirus transmission process understanding the dynamics of outbreaks	SEIR	no
HAV	Ajelli *et al*., 2008	coastal population of seafood consumers unit of time: month length of time: years	Role played by risk factors (mussel consumption) on equilibria, stability and the period of HAV oscillations, with and without vaccination program.	Deterministic SIR with a constant homogenously mixed population. 3 sources of infection: (i) direct transmission between S and I (ii) indirect transmission by locally contaminated raw	$dS=\mu N-\mu S-\lambda(t)S$ (μ mortality and fertility rate, N size of population, S susceptible, λ force of infection) $\lambda(t)=\beta_1 I/N+\beta_2(t)U+\beta_3$ (I infectious, U HAV contamination, β_2 periodic

				seafood (iii)traveling to higher endemic areas	transmission rate) dU=d (I-U) where d is the rate of depletion (virus)d=0.33 month^{-1}
HAV	Ajelli *et al.,* 2009	endemic areas of Italy (Campania and Puglia) unit of time:week length of time: 50 years	effect of target vaccination, social distancing measures, improvements in standards of living and hygiene in endemic areas of Italy 50% of cases in those areas due to ingestion of infected seafood (Mele *et al.,* 2006) combination of vaccination coverage and hygiene improvement decreases the number of notified cases by half in 50 years, social distancing alone can be counterproductive, hygiene improvement alone not very efficient (in the range under study), most efficient mitigation strategy: targeted vaccination program.	stochastic individual-based model of HAV	same consideration with Ajelli *et al.,* 2008. β is linked with age hygiene mitigation strategy decrease U(t) by a reduction factor (20 to80%) accounting for improved conditions(other fishing areas, or better hygiene in fish market) d=0.033week^{-1}
HAV	Ajelli *et al.,* 2011	Italy unit of time=month length of time 50 years	Effect of the vaccination program in Puglia on the decline of HAV incidence in the country as a whole Effect of the continuation of vaccination program in the endemic areas from Puglia to Campania. Differences in consumption during the year is not considered, between areas considered but not detailed.	spatiotemporal dynamics, metapopulation model, SIR based model with two main sources of infection direct and indirect (via food)	The decay of virus δ in the environment may vary from region to region (length of the pipe of sewage system or harbor). With the same notation as in Ajelli *et al*., 2008 G(U)=δe$^{-\delta U}$ (exponentially decay) dU=δ[I-U)]
NoV	O'Neill and Marks, 2005	human outbreak data =school (primary school and nursery)	Effectiveness of vomiting episodes in enhancing the spread of the virus via aerosol transmission	individual stochastic model (Reed –Frost) S Susceptible, I Infective or	no

		unit of time =day length of time=22 days		Vomiter and R.	
NoV	Heijne, Teunis *et al*., 2009	human outbreak unit of time=days length of time =16 days data on outbreaks in 11-14, 15-17 and 1 camp for staff >18 years old; disease attack rate 2.3% to 10.7%	Effectiveness of hygiene measures in reducing the number of cases in an outbreak (=the spread of norovirus infection). Effect on the Effective Reproduction Number R from 14 to 2; (number of secondary cases per primary case) mean generation time 3.6 days	generation time modeling and individual based stochastic model SI	no
NoV	Vanderpas *et al*., 2009	hospital outbreaks unit of time day length of time 1000 days	effectiveness of patient turnover on the endemic prevalence of norovirus acute-care setting is at –risk of endemic situation attack rate 41%-R0=3.74	deterministic SEIR (differential equations	no
NoV	Sukhrie *et al.,* 2012	hospital/health care facilities outbreak (human) unit of time: days length of time 38 to 77 days	Contribution of symptomatic and asymptomatic individuals (patient and healthcare workers HCW) to the spread of norovirus in health care facilities. symptomatic individuals are main contributors	generation time to construct plausible pathway of transmission and reproduction number for symptomatic and asymptomatic individuals (patient and HCW)	no
NoV	Heijne *et al*., 2012	hospital/ outbreak in 4 wards of psychiatric institution unit of time days length of time 33 days	contribution of transmission route between HCW to patient, patient to patient, patient to HCW, main was patient to patient, second patient to HCW	generation time modeling-transmission tree	no

TABLE 16: DYNAMIC MODELS OF VIRAL FOODBORNE PATHOGENS

IV.3. Dynamic model of norovirus cases in a coastal area

4.3.1. Context

In 2011, the French ministry of Agriculture asked ANSES, about the efficiency for public health, of preventive measures such as the duration of closure of a norovirus contaminated shellfish area, in the context of a Norovirus epidemic in the human coastal population (ANSES, 2011). The shellfish by this public health issue was the Thau Lagoon.

The risk associated with consumption of contaminated shellfish in coastal areas can be studied, using QRA to estimate primary cases (linked to food consumption). It is known that selling contaminated oysters, outside the coastal production area can generate human foodborne outbreaks (ANSES, 2011). However for the coastal area population itself, it was not obvious, in a context of occurrence of a human epidemic, that the total number of cases would be affected significantly by foodborne transmission. It was not easy to define the end of the foodborne risk for the human population, linked to the duration of the epidemics and environmental parameters. The winter 2002-2003, 2005-2006, 2009, 2010-2011 outbreaks showed the same sequence of events, with the beginning of a winter gastroenteritis outbreak in the human coastal population, heavy rainfall some weeks after the beginning of the human outbreaks (associated with malfunctions in waste water treatment), the subsequent sale of shellfish, some days (or weeks) thereafter, creating foodborne outbreaks in the human population. The contamination of shellfish by Norovirus was confirmed later. The human foodborne outbreaks involved coastal and non coastal population, because oysters can be transferred for sale elsewhere in France. Microbial monitoring was shown to be inefficient in preventing these foodborne outbreaks.

In this context the report (ANSES, 2011), based on a qualitative analysis of the situation in Thau Lagoon, recommended that one month after the last negative results in the shellfish production area, without any further human epidemic, and without any new episode of heavy rainfall, are suitable criteria to re-open the shellfish area to sale for human consumption, and prevents new foodborne outbreaks (ANSES, 2011). Other preventive measures were proposed (ANSES, 2011; Vaillant *et al.,* 2012b), as for HAV contamination episodes (ANSES, 2010), associating improvement in early reporting of the warning information on potential contamination of shellfish in light of certain environmental factors, targeting in particular shellfish producers and local authorities, and improvement in shellfish traceability. Improvement in the exhaustivity of mandatory declaration of foodborne outbreaks is also suggested (ANSES, 2011; Vaillant *et al.,* 2012b).

The duration of a norovirus epidemic in the general population is from 1 to 19 weeks, with an average of 7 weeks (sentiweb, 2012). The average number of (incidence) cases is 450 cases per 100,000 population, reported by physicians (sentiweb, 2012). In the Languedoc Roussillon region (including Thau Lagoon), the average peak is from 500 to 800 cases per 100, 000 (from 2004 to 2009), with two other observed peaks around 1100 to 1268 cases /100, 000 in 2010-2011 (sentiweb, 2012). Based on data from 7 winter outbreaks of this period the peak is, roughly, at the median of the

period of the epidemic. This number of reported cases can be corrected, by the number of people with Acute Diarrhea (AD) who consult general practitioners, around 33% (27-40%) (Van Cauteren *et al*., 2012). This reporting rate should also be corrected by the asymptomatic rate, which can be estimated at around 30%: 30% of exposed persons had positive collected stool specimens (Gallimore *et al*., 2006, Heijne *et al*., 2009). However not all gastroenteritis cases are linked to noroviruses (see part I), only 19.2% of AD stools are found to be NoV positive (Chiki-Brachet *et al.,* 2002).

The estimate of the total number of cases in France, seeking medical assistance with AD during the winter epidemic is approximately 2-9.5% (9.4% in 2010) of the French population (sentiweb, 2012). A more precise number of cases for fitting an epidemic model on coastal population should be used for more precise statistical analysis.

We investigated a situation close to that described above using dynamic stochastic modeling, to examine the different sources of variability, such as the epidemic pattern and shellfish contamination, with a QRA approach in a dynamic model framework. Our aim was to identify biological factors involved in a causative pathway, and also to explain some qualitative trends of the results with some specific parameter calibration, based on recently published studies (Chan *et al*., 2006; Atmar *et al.,* 2006; Teunis *et al*., 2008; Maalouf *et al.*, 2011) . Our work was designed to identify the data gaps in the available data for a modeling purpose. The model was also designed to investigate the public health effect of the closure of the shellfish area in a theoretical situation.

When fitted with available data, and validated, in other context, this model can help risk managers to make valuable decisions about the closing the area (and the duration of closure), in the case of shellfish contamination.

For norovirus, shellfish outbreaks raise another question. The relative contribution of Genotype GI (and other strain such as GII.3) in shellfish outbreaks is surprisingly higher in comparison to other outbreaks, in particular compared to inter-human main transmission outbreaks, which are more associated with GII and in particular with the GII.4 genotype (Glass *et al*., 2000; FAO/WHO, 2008; Verhoef *et al*., 2010; Matthews *et al*., 2012; EFSA, 2012). Biological differences in bio-accumulation in oysters, have recently been detected, in addition to other parameters (survival in the environment, infectivity, excretion) that may explain the epidemiological difference between the GI and GII NoV (Lindesmith *et al*., 2008; FAO/WHO;2008; Maalouf *et al*., 2011).

Our dynamic model aimed to investigate, whether some parameters, particularly excretion level, infectivity (contact rate and dose-response parameters), and bio-accumulation in shellfish, can explain (or affect) the spread of infection and the number of cases attributable to each genotype in an theoretical exposed coastal human population (with shellfish consumers and non shellfish consumers) with two transmission routes, interhuman and foodborne. Specific Dose-response results on GI and GII were used (Teunis *et al*., 2008; Thebault *et al*., submitted).

Se- subject appeared to be better protected from infection with GI (Teunis *et al.,* 2008) or GII (see section II), although perhaps less homogenously for GII (Lindesmith *et al.,* 2003; Marionneau *et al.,* 2005; Le Pendu *et a*l., 2006; Tan *et al*., 2008; Le Guyader *et al*., 2010). Genetic factors determining histo-blood group secretor status were taken into account in our dynamic model.

Co-contamination by GI and GII in Thau Lagoon and in human outbreaks has been observed several times: in 2002-2003; 2005-2006 in oysters only; 2009; but not observed in 2010-2011 (GII only) (ANSES, 2011). We explored co contamination, with assumptions of cross-immunity between norovirus GI and GII.

Although previous dynamic models of Norovirus are available, describing the inter-human dynamics of infection in a semi-closed environment (school, hospitals, scout camp) but do not explicitly consider the foodborne pathway (O'Neill and Marks, 2005; Heijne and Teunis, 2009; Vanderpas *et al*., 2009; Sukhrie *et al*., 2012, Heijne *et al*., 2012).

4.3.2. MODEL STRUCTURE

4.3.2.1. DEFINITION OF SPATIAL AND TIME SCALE, DEFINITION OF HUMAN POPULATION UNDER STUDY

The duration under study is similar to that observed during the epidemics in Thau Lagoon: around 100 days, occurring in winter, with an epidemic lasts around 7 weeks (6-8 weeks).

During this period, the human population was modeled as closed, (no migration), the demographic parameters (birth, death rates) are considered negligible, the population size is assumed to be constant. The mixing of individuals is perfect and the efficient contact rates control inter-individual transmission (direct and indirect). Indirect contact rates exclude oyster foodborne transmission.

The population was considered homogenous and randomly mixed, the heterogeneity of contact with age, in particular, was not considered in the first simplistic model. Nevertheless there are no available data for describing such heterogeneity in the coastal population.

Half of the population is considered to consume oysters, at least once during the period (including Christmas and New Year's Day), the other half does not eat oysters. The mixing rate is assumed to be the same between and within both populations. Representative data estimate of the percentage of oyster consumers in coastal population is assumed to be higher than in the general population (i.e. below 10% in INCA1)(ANSES, 1999; 2009). The coastal population, close to a seafood production area eats more fish and shellfish products than the general population (Calipso study) (see part II),however the actual proportion of shellfish eaters in coastal populations is unknown (Leblanc *et al.,* 2006; Sirot, 2010).

Genetic factors determining histo-blood group secretor status were taken into account, but simply. Se- (secretor negative) subjects seem to be better protected from infection with GI (Teunis et al;2008, or GII (section II), although less homogenously so for GII (Lindesmith *et al.,* 2003; Marionneau *et al*., 2005; Le Pendu *et al*., 2006; Tan *et al*., 2008; Le Guyader *et al.,* 2010). A percentage of the population, lacking the Se+ receptor is considered to be resistant to infection. This assumption slightly overestimates the resistance, but is introducing too much complexity in the model. The proportion of Se- subjects in the population, based on published data (Marionneau *et al*., 2005) is estimated at around 20% (**Table 18**), and is assumed, for an individual, to be independent oysters consumption status. Therefore, 20% of this population is then considered to be Se-, and with

simplification, not sensitive to GI or GII (resistant to infection), either via direct transmission between individuals, or indirect and foodborne transmission.

Chronic carriers (Sukhrie *et al*., 2010), hyper infectious individuals (or super-spreaders) are ignored. Asymptomatic individuals are not considered.

The size of the theoretical population is 2,500 individuals, with 1, 250 oyster consumers, 1, 250 non oyster consumers. For each population 250 individuals are expected to be Se-, and thus resistant to infection. For the remaining 1,000 population consumers and non consumers, no protective immunity was assumed before the epidemic: all individuals of these populations are susceptible. Protective immunity may be absent or short lived (in term of weeks or months) (Karst *et al*., 2010).

The beginning date of epidemic is declared the 1st December, which is plausible, given the data on the observed beginning date of the gastroenteritis epidemic in the Languedoc –Roussillon region in previous years (Doyle *et al.,* 2004; sentiweb, 2012; ANSES, 2011; Vaillant *et al.,* 2012). This is important for human oyster consumption which is taken into consideration in the model, (see section "environmental transmission"). The data of consumption where daily based. The winter period is important to consider, because norovirus and gastroenteritis epidemic occurring during the winter (Lopman *et al*., 2009). The specific winter bioaccumulation rates in shellfish were used (Maalouf *et al*., 2011).

4.3.2.2. Model formalism

A stochastic approach was chosen to include the variability of some parameters in the model, and to examine its potential impact on results. The length of time considered is around 100 days. The unit of time can be one day or less, because the norovirus transmission pathway can be very short, and mean length of time in one compartment can be close to one day (duration of incubation). The heterogeneity in human populations is treated with a compartmental model, with few homogenous categories. We therefore chose a hybrid model (Breban *et al.*, 2010), in continuous time, for describing the inter-human epidemic with a simple stochastic model, using the Gillespie algorithm, and in discrete time for describing virus environmental dynamics, and foodborne transmission risk dynamics. Human food consumption has daily time basis, and it is quite plausible that environmental foodborne risk assessment changes daily. Oysters are generally harvested once a day. The dynamics of foodborne transmission was evaluated in discrete time, with a single day as the unit of time. Fifty Monte Carlo simulations were performed for each situation, and quantiles (median, 95%CI) were plotted in results with R.2.14.1. The number of simulations can be improved upon publication request for more precise estimates of quantiles (one to several thousand simulations) .

4.3.2.3. Human epidemic model structure

The different health states in the human population, and the possible transitions between them, in the general situation of co-infection with GI and GII are given in Figure 25. The list of events associated with the transitions rates for the Gillespie algorithm is given in Table 19. The definition of parameters, calibration values and references for setting calibration values are given in Table 20.

The latent period is of the same order of magnitude as the infectious period (**Table 18**) . The basic model assumes that hosts can be divided into four health classes, susceptible (S), exposed or infected (E), infectious (I) and immunes R (Vanderpas *et al*., 2009). No protective immunity before the

epidemic is considered. The genetic diversity of norovirus can play a role in this system. Infection with a strain of one genotype may not confer immunity against strains of other genotypes or even variants within a genotype (Lindesmith *et al.,* 2008). However, in the period of time considered, each individual can only become infected once by one genotype, and then enters the removed state (R).

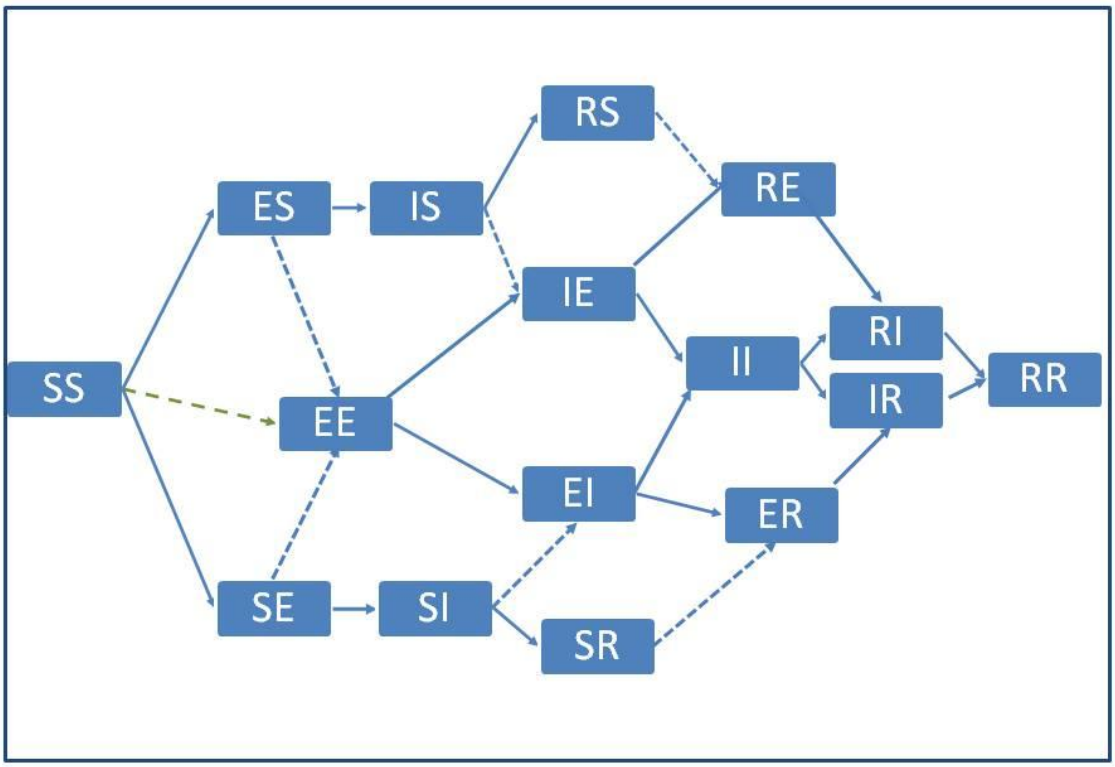


FIGURE 25: STRUCTURE OF THE TWO STRAIN MODEL IN THE INTER-HUMAN TRANSMISSION FOR GI AND GII.

Legend: Green dashed arrow: indirect foodborne (via oysters) transmission, only possible for the population of oyster consumers; dashed blue arrow transition with an infectious state; other blue arrows, other transition between health states.

In the Table 19, the two letters reflect a status-based formulation, with GI and GII respectively, but also a history-based formulation (Keeling and Rohanni, 2008) (Figure 25). Each transition between result is the departure of one individual from the source compartment (or origin) and the arrival of one individual in the target compartment (of destination) (**Table 17**). Parameters and their definition are given in **Table 18.**

Event (reference number)	Health status(from)-1	Health status (to)+1	Event Transition rate
1	SS	ES	B1*SS*(I1/N)
2	SS	SE	B2*SS*(I2/N)
3	ES	IS	L1*ES
4	ES	EE	δ1*B2*ES*I2/N
5	IS	RS	G1*IS
6	IS	IE	δ1*B2*IS*I2/N
7	RS	RE	δ1*B2*RS*I2/N
8	SE	SI	L2*SE
9	SE	EE	δ2*B1*SE*I1/N
10	SI	SR	G2*SI
11	SI	EI	δ2*B1*SI*I1/N
12	SR	ER	δ2*B1*SR*I1/N
13	EE	IE	L1*EE
14	EE	EI	L2*EE
15	IE	II	L2*IE
16	IE	RE	G1*IE
17	EI	II	L1*EI
18	EI	ER	G2*EI
19	ER	IR	L1*ER
20	IR	RR	G1*IR
21	II	RI	G1*II
22	II	IR	G2*II
23	RE	RI	L2*RE
24	RI	RR	G2*RI
25 (food transmission)	SS	ES	ε*pmal1*SS
26 (food transmission)	SS	SE	ε*pmal2*SS
27 (food transmission)	SS	EE	ε*pmal3*SS
28 (food transmission)	SE	EE	ε*pmal1*SE
29 (food transmission)	ES	EE	ε*pmal2*ES

TABLE 17: LIST OF EVENTS WITH TRANSITION RATE FOR HUMAN EPIDEMIC DYNAMIC

Legend: for I1 and I2: with index c for consumers and nc for non consumers:

$I1=sum(IS,IE,IR,II)_c+ sum(IS,IE,IR,II)_{nc}$

$I2=sum(SI,EI,RI,II)_c + sum(SI,EI,RI,II)_{nc}$

Parameter	Definition	Value for GI	Value for GII (GII.4)	Remark and Reference
B1, B2	effective contact rate parameter	B1=0.8	B2=1	calibrated for an outbreak $B1_{cc}=B1_{NCNC}=B1_{NCC}=B1_{CNC}=B1$ $B2_{cc}=B2_{NCNC}=B2_{NCC}=B2_{CNC}=B2$ $_{C}$ consumers $_{NC}$ non consumers e.g. Bncc contact rate between non consumers and consumers etc…
1/L1 for GI 1/L2 for GII.4	duration of latent period	1.5 days	as for GI	first virus shedding in stool (Atmar *et al*., 2008) 2 days (Heijne *et al*., 2009)
1/G1 for GI 1/G2 for GII.4	duration of infectious period for inter-human transmission	2 days	as for GI	symptomatic duration Karst *et al.,* 2010;, and with the assumption that the symptomatic phase is the main contributor to inter human transmission (Karst *et al*., 2010) in agreement with 1-3 days postchallenge peak(Heijne and Teunis, 2009); 49% enter the symptomatic phase within 1-2 days (Van derpas *et al*., 2009)
δ1 δ2	cross-immunity parameters	0.8 or 0.1	as for GI	Assumption (see text)
durexcret ($_{g1\ or\ g2}$)	duration of excretion of genomes in stool by an infectious individual	x ~ Betapert (5, 10,30) (min 5, mode 10, max 30)	as for GI	excretion last a median of 28 days after inoculation (Atmar *et al*., 2008) extrapolated from GI (Norwalk) to GII 10 days with ELISA (Atmar *et a*l., 2008)
$c_{max(g1\ or\ g2)}$ $c_{min\ (g1\ or\ g2)}$	concentration in genome /g of stool	1 to 4 days: c_{max}~$10^{Norm(7, 1)}$ 4 to x days < 24 days: c_{min}~ $10^{Unif(0,4)}$	c_{max} ~$10^{Norm(9, 1)}$ c_{min}~ $10^{Unif(0,6)}$	Median peak at 3 days after begins of symptom (4 days after inoculation) (Atmar *et al*., 2008) extrapolated from GI (Norwalk) to GII difference between GI and GII, in excretion (Chan *et al*., 2006) 100-fold higher for GII compared to GI estimate conc/g ~10^BetaPert (2,6,8) (Mokhtari *et al*., 2009)
weightstoolday$_{min}$ weightstoolday$_{max}$	quantity of stool excreted per individual per day	250 g during first 2 days 130 g during x-2 days	as for GI	criteria 200 g for symptoms (Atmar *et al.,* 2008) severity can be higher for GII (Atmar *et al*., 2008)
ε	consumption authorized or not	0: closure- 1: open-authorized		assumed identical to open/closure of shellfish area efficiency of closure (=0)assumed to be perfect
percse	% of Se- in general population	20%	as for GI	Marionneau *et al.,* 2005
N	total population size	2500		

TABLE 18: HUMAN PARAMETERS DEFINITION, CALIBRATION VALUES, REFERENCES

Inter-individual transmission is described with a frequency dependent transition rate. However the size of the population is constant and demographic parameters are not considered here.

The model is similar in structure to other two-strain models (Keeling and Rohanni, 2008; Roche and Rohanni, 2010). Simultaneity of change in health state for both genotypes is considered negligible, except for simultaneity of co-infection, which was possible for indirect transmission via food. Finally, after infection by both genotypes, the individual is completely removed (resistant to infection). If the modeling had been built for more seasons or several years, immunity should decrease and individuals should become susceptible individuals, with the assumption of no long term immunity (in a SEIRS system).

The effective contact rates per unit time (B1 for genotype I and B2 for genotype II) are estimated to be the same within the population of consumers and non consumers and between these two populations (**Table 18**). The total number of Infectious I1 and I2 that can infect consumers and non consumers, is the total sum of infectious in both populations (**Tables 17 and 18**). B1 and B2 are calibrated to generate a small epidemic in the human population. B2 is assumed to be more important for GII.4 than for GI, to take into account the fact that GII.4 is dominant in inter-human epidemics (Lindesmith *et al.,* 2008).

The duration of latent phase (1/L1, 1/L2) is estimated to be similar to the incubation period (Karst, 2010), and similar to the time to the first excretion of viruses in stools defined for Norovirus, in a median time of 36 h, (range 18-110 h)(Atmar *et al.,* 2008) (**Table 18**). The duration of the infectious period (1/G1, 1/GII) is estimated as the usual range of the symptomatic phase, given that the high excretion rate in stools can be found during the first days of clinical symptoms (24-48 hours) (Karst, 2010; Atmar *et al*., 2008). The symptomatic phase for norovirus, which sometimes includes vomiting, can result in a higher transmissibility phase (Sukhrie and Teunis 2012).

The interaction between strains over time is described by parameters of partial cross-immunity. In the model, the probability of being infected by a genotype I(II.4) was lower after being removed from one infection with genotype (II.4)(I). In our model competition also occurs before being removed from one infection (**Table 17**), during the latent and infectious period. The interaction between strains is represented by δ1 and δ2 (**Table 17**). Between 0 and 1, these factors decrease the probability to be infected by the other genotype. Co-infection in stools occurs but is not common, making unlikely any enhanced susceptibility, but can be confirmed by further data analysis. A mild immune response, short term, is detected (Karst, 2010). The combination of no long-term protective immunity and the huge diversity of strains is a mechanism that underlies the regular repeated epidemics of norovirus. Therefore the factors δ1, δ2 of 0.1 and 0.8 (**Table 18**) should be interpreted as an exploratory ways, for investigating their influence on co-contamination. These factors can be set differently for GI and GII.4, however, for this analysis, we chose to keep the same values for both.

Finally symptomatic individuals, and chronic carriers were ignored, although they may play a role in direct and indirect transmission (Sukhrie *et al*., 2010; 2012; Partridge *et al*., 2012).

Indirect transmission is considered by following transition probabilities per unit time, regarding the consumer population (equation 25 to 29 in **Table 17**).

The mean probabilities of daily illness in the population of consumers (pmal1, pmal2 and pmal3) are functions of I1, I2, I1 and I2 respectively, environmental parameters, consumption data and dose-response parameters as described in section III. For NoV, we assume that each genome corresponds to one infectious virus. Details of calculations of the indirect transmission rate (pmal1, 2,3) are given in the next section.

4.3.2.4. INDIRECT TRANSMISSION RATE

All parameters of indirect transmission and their distribution are given in **Tables 18 and 19.**

Parameter	Definition	Value for GI	Value for GII (GII.4)	Remark and Reference
waterconsump	waste water per individual, daily	180 liters	as for GI	(ADEME, 2011)
$conc_{wi}$	concentration in water influent	$(\sum(weightstoolday_i * C_m i))/(N*waterconsump)$	as for GI	
pabatstep	probability of surviving water treatment and loss in the system's network	10-4	as for GI	can be documented in the future (site-treatment dependent); can be different between GI and GII no strong survival difference (Flannery *et al*., 2012); survival difference (Da Silva *et al*., 2007;2008)
delaystep	residence time in treatment plan	2 days	as for GI	data obtained from Brittany (aNSES, 2010) (can be documented in the future, in particular for maturation pond)
T_{90}	time to a 90% decrease of initial quantity of GI (in treatment plant or in oysters)	12 days	as for GI	T_{90} between 8 and 12 days, estimated from data (Le Guyader *et al.,* 2008; Dore *et al.,* 2010)
$conc_{we}$	concentration in wastewater effluent/liter	n~Binom($conc_{wi,,}$ pabatstep) $conc_{we}=n10^{-(delaystep/T90)}$	as for GI	
dilustep	treated waste water dilution and loss in the sea	10^4	as for GI	indirectly calibrated in order to obtain realistic value of concentration in genomes in sea water
$conc_{mwT}$	conc in sea water in contact with shellfish/liter with wastewater treatment plant	$conc_{mwT}= conc_{we}$/dilustep	as for GI	
rawdilu	conc in sea water in contact with shellfish/liter without wastewater treatment plant (raw reject)	none	as for GI	assumption
Residence time in sea	1 day			assumption
γ_g	bio concentration between concentration	$10^{-Unif(-2;0)}$ mean: $10^{-1.5}$	$10^{-unif(-8;-6)}$	November-January-march data of bioaccumulation in 24 h, (Maalouf *et*

	of digestive tissues (DT) in oysters from concentration in sea water		mean: 10^{-7}	*al.,* 2011)
r_g	Biodilution factor between DT and edible tissues	0.01	1	January-march data of bioaccumulation in 24 hours, (Maalouf *et al.,* 2011)
pro_{DT}	Average weight of digestive tissues (W_{DT})/ average weight of edible oyster tissues W_O	pro_{DT} =0.08 W_{DT}/W_O Wo~Unif(10;20) $W_{DT=}$ $pro_{DT.}$ W_O	as for GI	data from Le Guyader, published in Thebault et al.2012
Vge	mean quantity of genome per gram of oyster edible tissue	Vge=(($W_{DT.}Vg_{DT}$)+(W_{o-} W_{DT}) r_g Vg_{DT})/Wo Vg_{DT}: mean quantity of genome (g for GI or GII) by gram of digestive tissue	as for GI	
Q_g	quantity of genome/g of oyster	Q_g~Poisson (Gamma(Vge, 1))	as for GI	heterogeneity of contamination
$t_{postharvest}$	time between harvest and consumption	0	as for GI	no delay, because coastal consumption
consum(i)	consumption data in gram/indiv/day	empirical distribution with 1000 consumers data set	as for GI	from Calipso, SECODIP and INCA data (Thebault *et al.,* 2012)
α,β,η,r	Dose-response parameters	posteriors distribution of dose-response outbreaks data for GI	data for GII	posteriors distribution of dose-response outbreaks (Thebault, A., Teunis, P., Le Pendu, J., Le Guyader, S., Denis JB. submitted

TABLE 19: ENVIRONMENTAL PARAMETERS INVOLVED IN INDIRECT TRANSMISSION

Legend : Unif: Uniform distribution, Poisson: Poisson distribution; Gamma: Gamma distribution

For each new Infectious (I1 or I2) individual, two period of infectivity in stools were considered:

- A first period, corresponding to the maximum symptomatic phase, and a hyperinfectious state of viral excretion in stools, corresponding to high concentration of virus (genomes)/g stool (c_{max}) . The first period was set to 4 days **(Table 18).**

  A second period, corresponding to asymptomatic phase, and less viral excretion in stool, c_{min}. The duration of the second period is sampled from a Beta-Pert distribution (**Table 18**). Calibration of parameters was considered plausible based on published studies (Atmar *et al.,* 2008; Chan *et al*., 2006; Lee *et al*., 2007; Mokhtari and Jaykus, 2009).

  The excretion rate (concentration in stools/g) were set differently for GI and GII (**Table 18**), at 100-fold higher for GII than for GI (Chan *et al*., 2006).

- For each infectious individual, daily excretion was estimated for the first and second period. For each day and each infectious individual, the quantity of virus excreted with the product of the concentration in genomes and the average weight of stools produced daily by an individual, diluted by the quantity of water consumed each day (**Table 18**).

- The duration of each period was set identical for GI and GII, but could be different (GII.4 is suspected to have longer excretion periods (Atmar *et al*., 2008; Chan *et al*., 2006).

- At the sewage treatment plant, each day the influent contains the sum of all daily quantities associated with each infectious individual for GI and GII.4, diluted by the sum of sewage water produced by the population of size N. This approach was used for estimating Poliovirus concentration in a city influent (Ranta *et al*., 2001). The concentration in influent is noted $conc_{wi}$.

- The effectiveness of sewage treatment at the plant is defined by the probability that one virus (or genome) survives the treatment and the aggregation in the network sewage system or sewage sludge (**Table 19**).

- The residence time in the sewage plant is taken into consideration before the effluent is discharged into the environment. The concentration in the effluent, noted $conc_{we}$ is a function of the survival of the virus in the environment (in the plant reservoir) and residence time (**Table 19**). Using an exponential decay, the time to 90% loss was defined based on published data (Le Guyader *et al*., 2008; Dore *et al*., 2010).

- The quantity of virus in the effluent is diluted, to obtain plausible values of concentration of viruses per liters in sea water, in the neighboring shellfish area. Sea water are set daily memoryless. This assumption should be estimated for a specific coastal site, because the residence time is site dependent (effect of tide, hydrological characteristics, depth) and period (i.e.season) dependent (tide, tempest, rainfall) (Pommepuy *et al*., 2005;Rueda *et al.,* 2006; ANSES, 2010). This assumption probably underestimates the virus concentration in sea water. The residence time is not the only factor to consider: shellfish activity in production areas (cultivated or wild) can deplete the concentration in sea water, and sedimentation with aggregates of organic matter. Therefore, our assumption mildly underestimates the concentration of NoV in sea water.

- In case of heavy rainfall, and during periods of heavy rainfall, a by-pass system is considered in replacement of the sewage treatment plant. The dilution in sea water is considered to be null in comparison, to the regular system, because points of accidental discharge were not elaborated to minimize sea water contamination. During the period of heavy rainfall, sea water are again daily memoryless. The quantity of virus in sea water is result of the concentration of viruses in the influent, taking into account the dilution of wastewater population consumption (**Table 19**).

The mean concentration in oysters is a function of concentration in sea waters, taking into consideration the bioaccumulation factor γ_g (with g specific to GI or GII) (Maalouf *et al*., 2011). The

mean quantity of virus per gram digestive tissue (DT) in shellfish V_{gDT} was obtained using the following dynamic for GI or GII.4 (day j)

$V_{gDT}(j)=\gamma_g . Cmw_{gj}+Vg(j-1)10^{(-1/T_{90g})}$

where t is the time in days, T_{90}, the duration for 90% decay of virus, γ_g the bioaccumulation in oysters, taken as a random variable (time of oyster variability during winter period) (**Table 19**).

- The mean quantity per gram edible oyster tissue was different for GI and GII (g=1 or g=2), and is different between days j. This mean quantity was noted as $V_{gE}(j)$. The relationship between the quantity of viruses in the digestive and edible tissues is given by:

- $Vge=((W_{DT.}Vg_{DT})+(W_{o-}W_{DT})\ r_g\ Vg_{DT})/W_o$

with r_g the biodilution factor as the relative percentage of virus in tissues other than in digestive gland, compared to the digestive tissue (different for GI and GII.4) (Maalouf *et al*., 2011), W_{DT} mean weight of digestive tissues, Wo, mean weight of oyster (**Table 19**).

Heterogeneity of contamination in oysters was introduced (in the idea of small scale spatial heterogeneity), assuming that the contamination per gram oyster is Gamma Poisson distributed ($Q_g(j)$)
The parameter of the Poisson distribution is Gamma distributed with shape= $Vg_{E,}$ and scale=1. (The mean of the Gamma distribution is the product of shape and scale).

The oysters were assumed to come from a large homogenous population, with some heterogeneity of contamination. Their demography (linked to human practices) is not considered. During the considered period, transfers for sale for human consumption and other transfers of shellfish were assumed to have no impact on the distribution of contamination in oysters.
Knowing the quantity of genome per gram oyster per day, daily shellfish consumption data was used. Shellfish consumption is highly seasonal, and higher in coastal population. We took the consumption data from the 1st December for the beginning period of the simulation to focus on the period of interest in Thau Lagoon (ANSES, 2011).The way these consumption data were compiled from three different data bases has been already described (Thebault *et al.,* 2012 and section II) . We obtained for each day j the consumption of oysters in gram for each individual i in a population of 1000 consumers, noted consum(ij).

In this population, we evaluated the daily mean risk of illness (Pill) due to oyster consumption from each day of the period. For a population of 1000 consumers, SE+ subjects, individual daily risk was evaluated using the following equations:

$Doseing_{gij}=consum(I,j)*Q_g(j)$

$P(inf_{gij})=1-Gamma(\alpha_g+\beta_g)Gamma(\beta+Doseing_{gij})/((Gamma(\beta_g)Gamma(\beta+\alpha+Doseing_{gij}))$

where Gamma is the Gamma function, α and β parameters specific to GI and GII and were taken from previous estimates (see section III)

- The probability of illness, conditional to infection and dose for each genogroup is given by

$P(ill/inf_{gij})=1-(1+\eta\ Doseing_{gij})^{-r}$

The probability of illness, taking into account infection risk is given by

$Pill_{g,i,j}= P(inf_{gij})P(ill/inf_{gij})$

For individual i, day j, the event of illness with GI (or transition from S to E for one individual) is Bernoulli distributed with parameter ($Pill_{g,i,j}$) and is noted ill(1,i,j) for illness with GI, for illness with GII ill(2,I,j).
The infection by GI and GII is given by the result of the Bernoulli distribution for GI and GII via Pinf, with both events=1; the sum of doses for risk of illness; it is noted ill(1and2,i,j).
The mean risk of illness, on day j in the population, of size S=1000, by GI only, is given by:

$$ill_{1,j}=\frac{\sum_{1}^{S}\left[ill(1,i,j)-ill(1and2,i,j)\right]}{S}$$

The mean risk of illness, on day j in the population, by GII only, is given by the same equation, replacing (1) with (2)

$$ill_{2,j}=\frac{\sum_{1}^{S}\left[ill(2,i,j)-ill(1and2,i,j)\right]}{S}$$

$$ill_{1and2,j}=\frac{\sum_{1}^{S}\left[ill(1and2,i,j)\right]}{S}$$

Pmal1(j), is the mean of the daily risk of illness on day j by GI, Pmal2(j) is the mean risk of illness on day j by GI, Pmal3(j) is mean of risk of illness on day j by GI and GII. One set of dose-response parameters was taken from the posterior distribution of dose-response estimates (see section III).

This calculation allows for comparison with observational studies, on the influent of the sewage treatment system, on the effluent of the sewage treatment system, on sea water and on shellfish for both the digestive and edible tissues.

The corresponding concentration are plotted in the **Figure 26.**

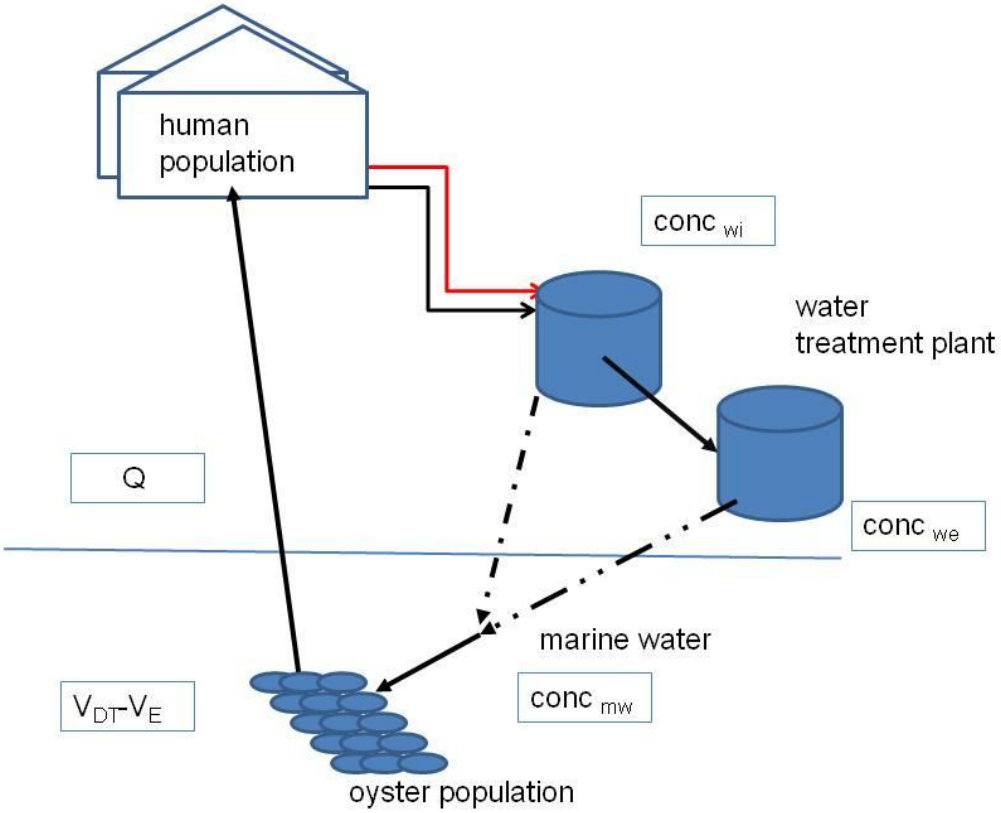


FIGURE 26: CORRESPONDENCE BETWEEN INDIRECT TRANSMISSION PARAMETERS AND DIFFERENT STEP OF CONTAMINATION (DEFINITION TABLE 10).

Legend: Black arrows virus transmission to each compartment (red arrow initial contaminated). One point dashed arrow is raw reject (no sewage treatment or further dilution); two points dashed arrows step reject (sewage treatment and dilution in sea water

From **Tables 18 and 19**, it can be seen that only four categories of parameters differentiate GI from GII: human excretion, transmissibility rate (B1,B2), genotype-specific oyster accumulation (γ_g, r_g) and genotype-specific dose-response parameters. Differential survival estimates should be further documented, but were not taken into account here, whenever suspected (Da Silva *et al*., 2007; 2008; Sima *et al.,* 2011; Flannery *et al*., 2012).

In the QRA part of the model, the calculation of daily risk comprises the variability in contamination parameters and consumption data.

4.3.2.5. MITIGATION STRATEGIES AND INITIAL PARAMETERS.

The **Table 20** gives the initial states and the period parametrization

Scenario (1 to 5) describes the mitigation strategy implemented.

definition of period date of beginning: 1 December	days since the beginning date	Environmental source of contamination	Initial state
1st period	1 to 20	WTP	• 5 infectious GI or GII • 995 S consumers • 1000 S non consumers • 500 RR other initial state Null N= 2500 • Environmental contamination null
2nd period	20 to 23	CCR	from 1st period
3rd period	23 to 30	WTP	from 2nd period
4th period	30 to 51	WTP	from 3rd period
5th period	51 to 120	WTP	from 4th period

TABLE 20: DEFINITION OF PERIOD AND INITIAL STATES

Legend: WTP contamination of sea water by sewage water treatment plant

CCR contamination of sea water by raw reject only

The dynamics of the system and the definition of period were based on those observed in the Thau Lagoon, with epidemics at winter time, associated with heavy rainfall for some days and failure of the water treatment system (by-pass of waste water influent into sea waters), and some days (weeks) later, food-borne outbreaks detected within and outside the shellfish production area (**Table 20**). Other strategies, such as relaying, purification, and effectiveness of monitoring were not tested here. The initial states were 5 infectious individuals of GI, or GII according to the genotype under investigation. In case of co-contamination, 5 infectious individuals of G and GII were involved in the initial states. We were not interested in explaining how these five individuals became infectious, or in the beginning of epidemic itself. We can make the assumption they became infected after travelling to other areas, where epidemics occur in the winter time. These infectious individuals contribute to initial environmental contamination.

Five different mitigation strategies were investigated for contamination by only GI GII or by both GI and GII (**Tables 19 and 20**), and then for co-contamination of GI and GII.

The scenarios (1 to 5) describe the mitigation strategy implemented. The mitigation strategy is called closure of the area or local shellfish consumption ban, both of which produce same effect, i.e. they stop the foodborne transmission.

The mitigation strategies investigated were “scenario 1”, no foodborne transmission; “scenario 2”, oyster consumption during the whole period; "scenario 3", 3 weeks consumption ban; "scenario 4" long term consumption ban, "scenario 5" early implemented consumption ban (**Table 21**).

	scenario1	scenario2	scenario3	scenario4	scenario5
period 1	forbidden	authorized	authorized	authorized	authorized
period 2	forbidden	authorized	authorized	authorized	forbidden
period 3	forbidden	authorized	authorized	forbidden	forbidden
period 4	forbidden	authorized	forbidden	forbidden	forbidden
period 5	forbidden	authorized	authorized	forbidden	authorized

TABLE 21: SUMMARY OF MITIGATION STRATEGY INVESTIGATED BY THE MODEL

Legend: mitigation is different with local shellfish consumption authorized or not for particular period

4.3.6. RESULTS

The **Figure 27** shows median results for scenario 3 for GI. **Figure 27 A** gives the changes in median health states in time, and environmental results are given in **Figure 27 B.** The red vertical dashed lines indicate the mitigation periods. A strong effect of consumption (foodborne cases) is shown (more than half the cases in consumer population). The period of consumption is quite long enough, but shown some cases after 51 days. Consumption of shellfish is high (during Christmas holiday period).

The **Figure 27 B** shows median concentration in sea(marine) waters, and concentration of virus per g (with 95% IC) edible oyster tissues. This figure illustrates the high bioconcentration effect in GI, even if biodilution is lower (in comparison with GII; **Figure 29**). The contamination of shellfish is governed by the episode of raw contamination in the environment.

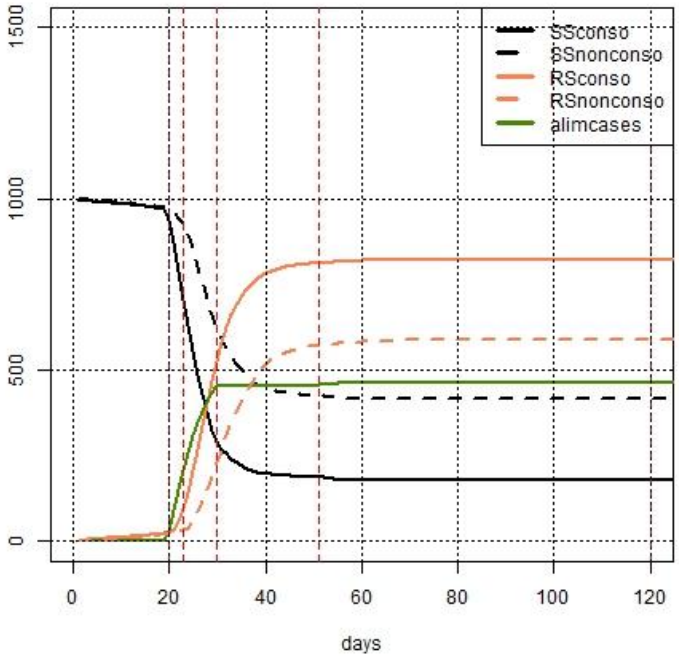


FIGURE 27 A. HUMAN EPIDEMIC SITUATION WITH GI CONTAMINATION, FOODBORNE TRANSMISSION, AND PARTIAL TIME FORBIDDING CONSUMPTION (SCENARIO 3).

Legend: SSconso susceptible oysters consumers, SSnonconso susceptible oysters non consumers, RSconso, removed oyster consumers (GI cases in consumer population of 1000), RSnonconso, removed non oyster consumer (GI cases in non consumer population of 1000), alimcases, foodborne cases in consumer population.

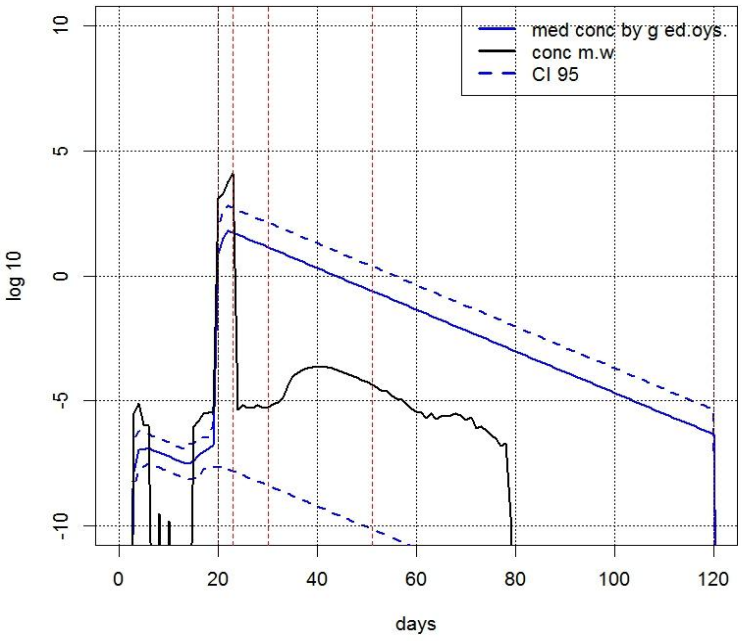


FIGURE 27.B. CONCENTRATION IN SEA WATER AND IN SHELLFISH WITH TIME (IN LOG10)

Legend of Figure 27. B:

Med.conc. by g ed.oys: median concentration of virus /g (with IC95 in hatched lines) in the edible part of oyster

conc m.w : median concentration in sea waters

The **Figure 28** shows the results for the different scenario of duration of closure of the area with GI contamination only.

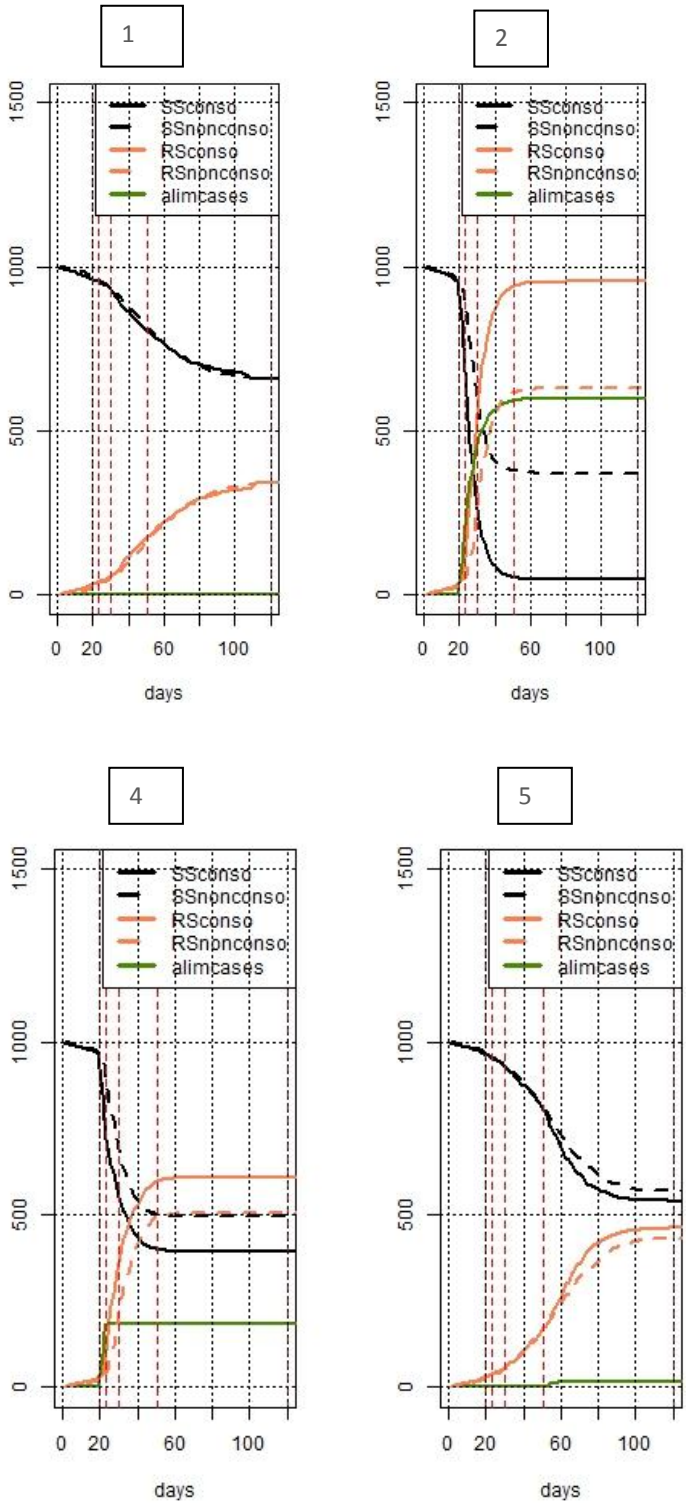


FIGURE 28. HUMAN EPIDEMIC SITUATION WITH GI CONTAMINATION, FOODBORNE TRANSMISSION, FOR SCENARIO 1, 2,4,5

Legend: upperleft scenario 1; upperright scenario 2; bottom left scenario 4; bottom right scenario 5.

The figure 28 shows strong effect of foodborne transmission for GI cases.

The Figure 29 shows median results for scenario 3 for GI. **Figure 29 A** gives the changes in median health states in time, and environmental results are given in **Figure 29 B.** The red vertical dashed lines indicate the mitigation periods. An effect of consumption (foodborne cases) is shown but less than before (comparison 27 A). The period of consumption is quite long enough. The **Figure 29 B** is showing median concentration in sea(marine) waters, and concentration of virus per g (with 95% IC) edible oyster tissues. This figure illustrates the less bioconcentration effect in GII, even if biodilution is higher (in comparison with GII; **Figure 29**). The contamination of shellfish is governed by the episode of raw contamination in the environment.

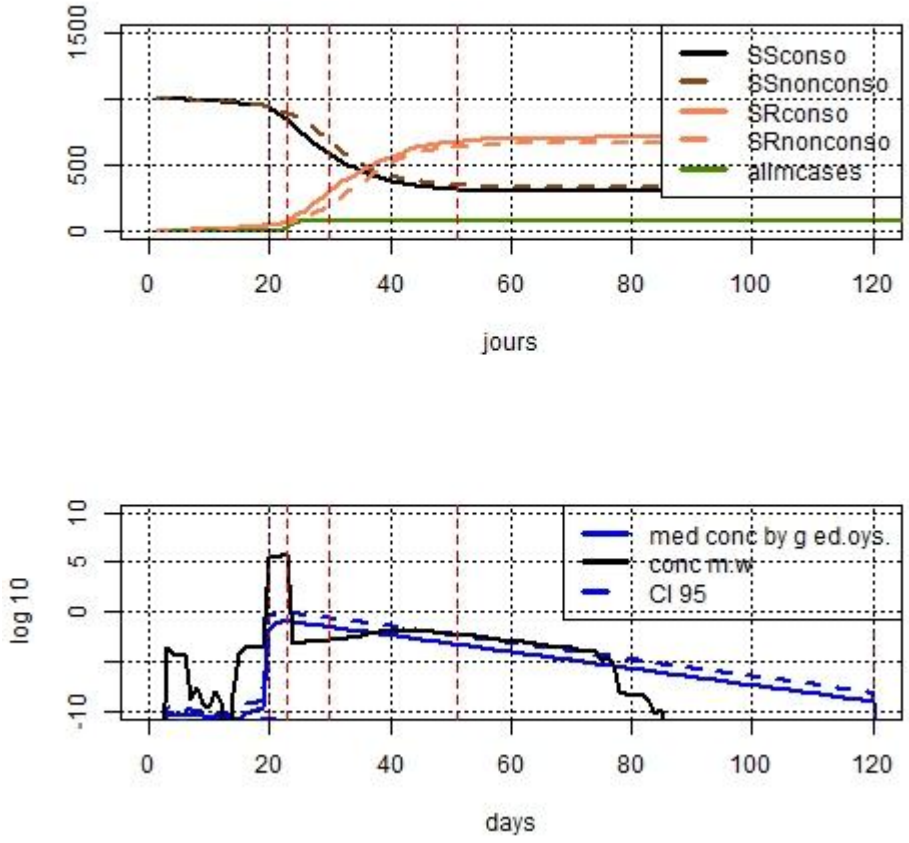


FIGURE 29 : A: HUMAN EPIDEMIC SITUATION WITH GI CONTAMINATION, FOODBORNE TRANSMISSION, AND PARTIAL TIME FORBIDDING CONSUMPTION (SCENARIO 3). B CONCENTRATION IN SEA WATER AND IN SHELLFISH WITH TIME (IN LOG10

Legend : 29 A upper Figure, 29 B Bottom Figure

Legend 29 A: SSconso susceptible oysters consumers, SSnonconso susceptible oysters non consumers, RSconso, removed oyster consumers (GI cases in consumer population of 1000), RSnonconso, removed non oyster consumer (GI cases in non consumer population of 1000), alimcases, foodborne cases in consumer population
Legend of Figure 29 B:
Med.conc. by g ed.oys: median concentration of virus /g (with IC95 in hatched lines) in the edible part of oyster
conc m.w : median concentration in sea waters
The Figure 30 shows the results for the different scenario of duration of closure of the area with GII contamination only.

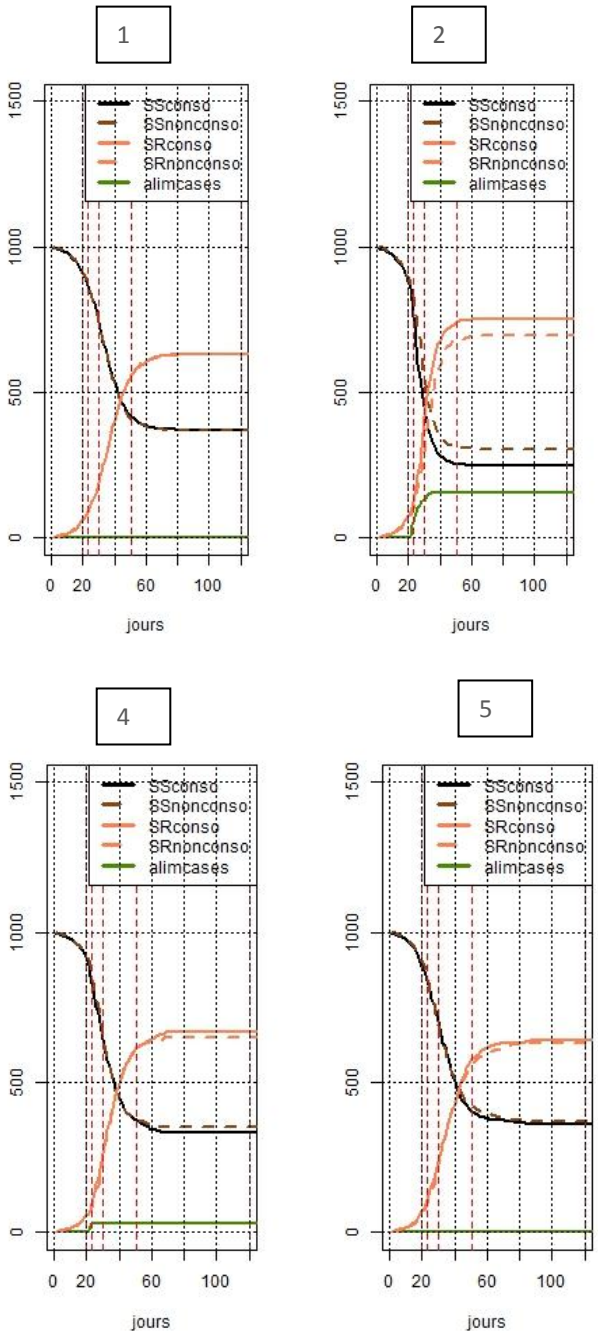
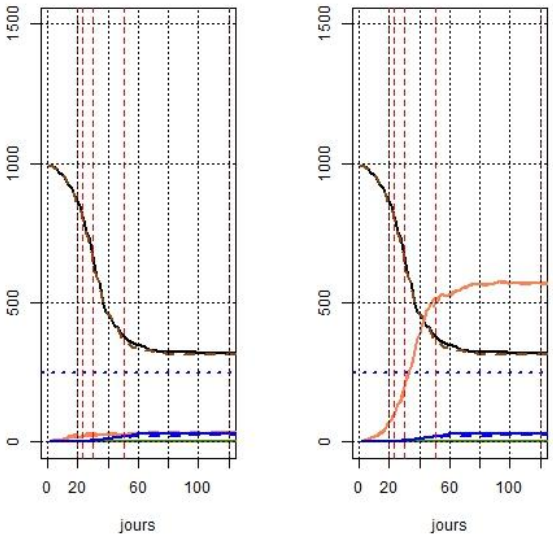


FIGURE 30: HUMAN EPIDEMIC SITUATION WITH GII CONTAMINATION, FOODBORNE TRANSMISSION, FOR SCENARIO 1, 2,4,5

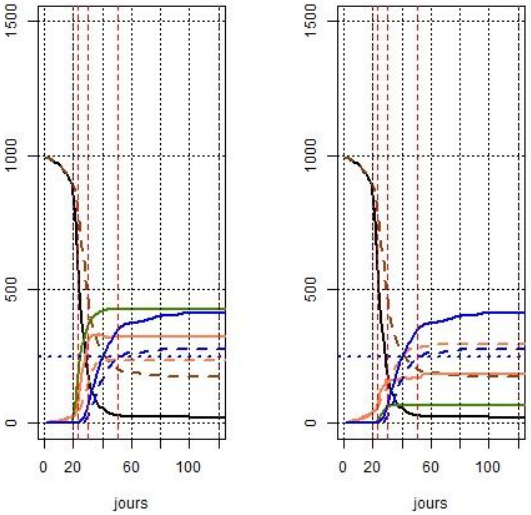
Legend: upperleft scenario 1; upperright scenario 2; bottom left scenario 4; bottom right scenario 5.

The figure 30 shows potential effect of foodborne transmission for GII cases in scenario 2.

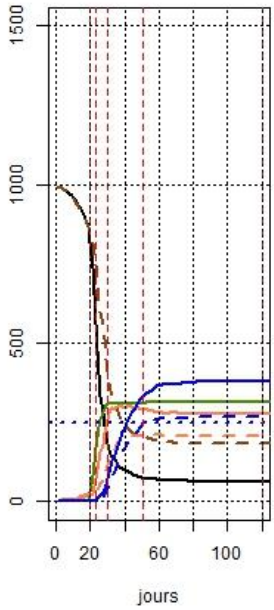
Figure 31 show the results for GI (left) and GII (right) cases with GI and GII coinfection with cross immunity parameter at 0.8 (low level of cross immunity), for scenario 1, 2,3,4,5. At the end of epidemic an SSconso can become RSconso, RRconso, or SRconso, or can remain SSconso.

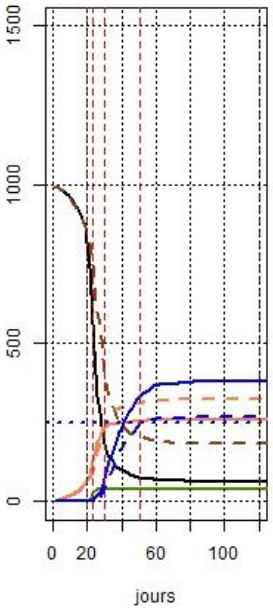


Scenario 1

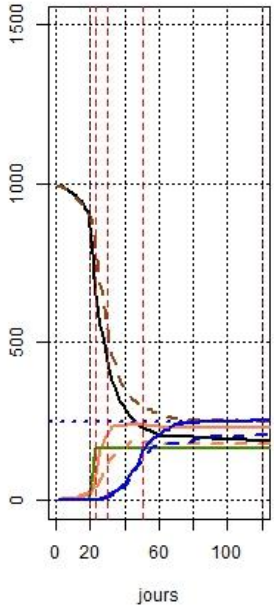


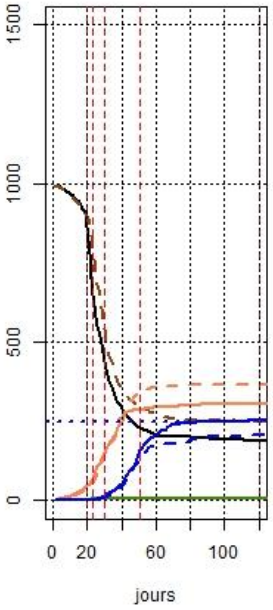
Scenario 2



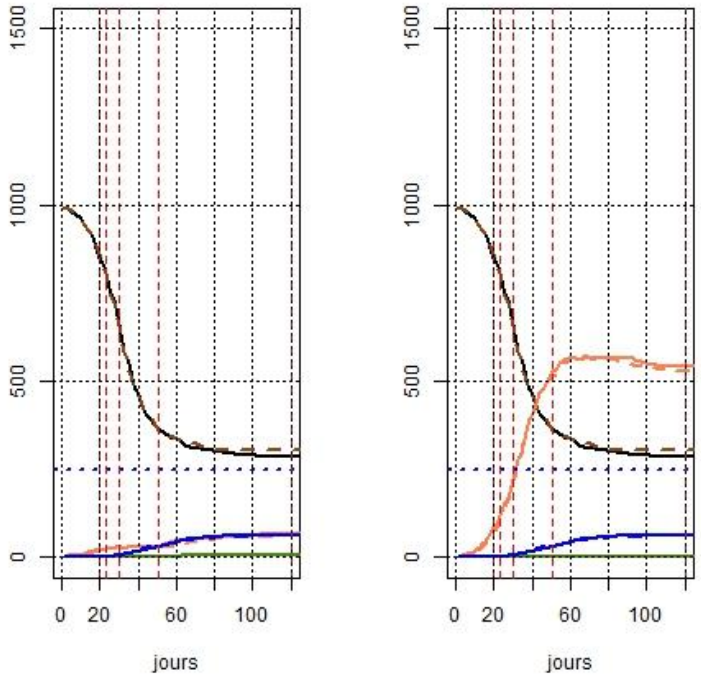


Scenario 3





Scenario 4



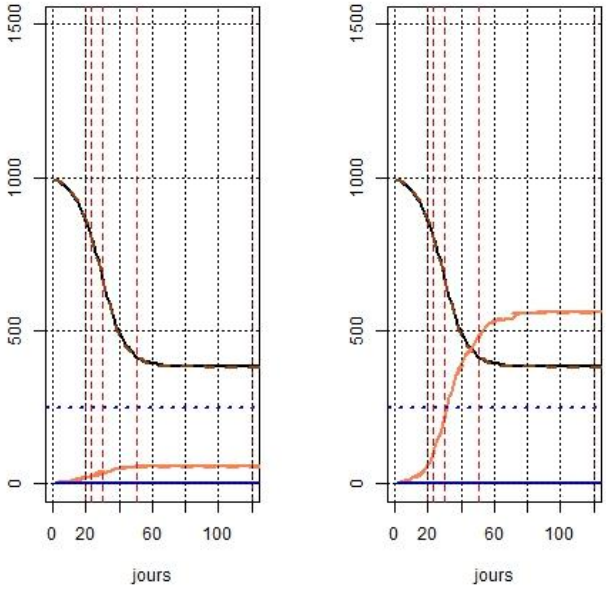
scenario 5

FIGURE 31: GI AND GII CASES WITH GI AND GII COINFECTION WITH LOW CROSS IMMUNITY FOR SCENARIO 1 TO 5

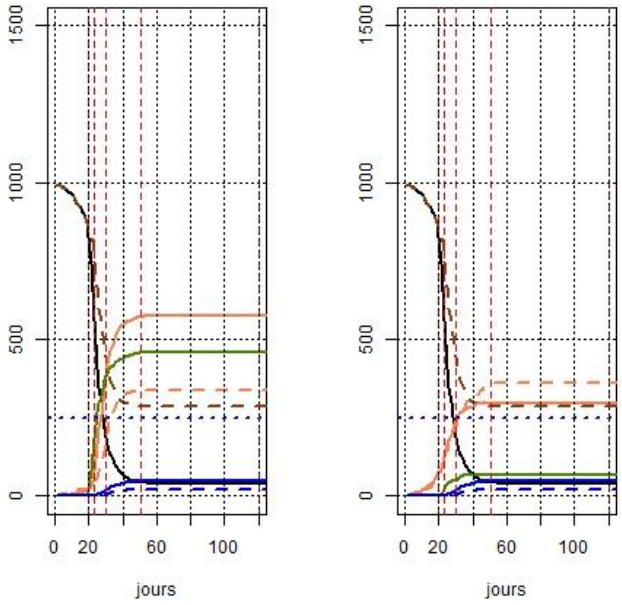
legend: show the results for GI (left) and GII (right) cases with GI and GII coinfection with cross immunity paramter at 0.8 (low level of cross immunity), for scenario 1, 2,3,4,5. At the end of epidemic an SSconso can become RSconso, RRconso, or SRconso, or can remain SSconso. Red curve left GI cases only (dashed non oyster consumers), Red curve right GII cases only (dashed non oyster consumers), blue curve RR (dashed non oyster consumers), black curve SS (dashed non oyster consumers), green curve foodborne cases, right GI, left GII.

The effect of foodborne transmission is shown by GI cases and RR cases in scenario 2, by example, in comparison with scenario 1 (Figure 31). Oyster consumers are more likely to be RR or GI cases in scenario 2. The total number of cases (SS curve) is more important with foodborne transmission.

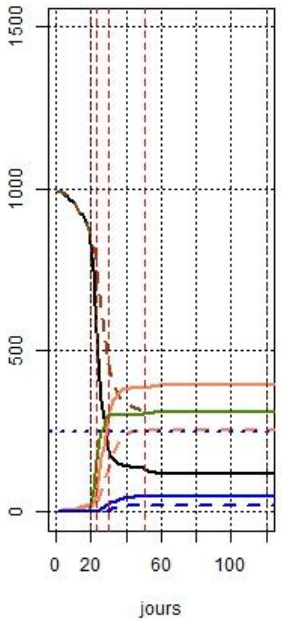
Figure 32 show the results for GI (left) and GII (right) cases with GI and GII coinfection with strong cross immunity parameter at 0.1 strong level of cross immunity), for scenario 1, 2,3,4,5. At the end of epidemic an SSconso can become RSconso, RRconso, or SRconso, or can remain SSconso.

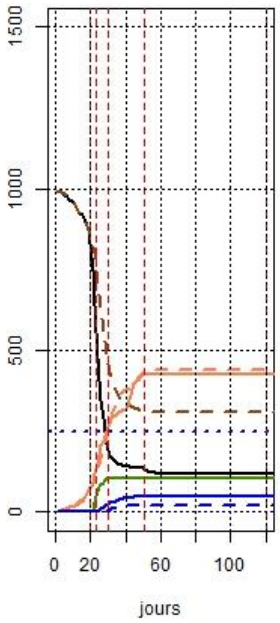


Scenario 1

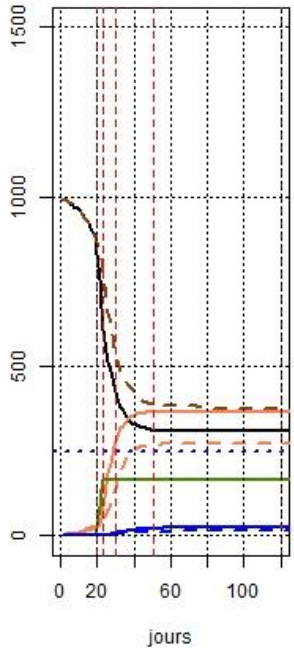


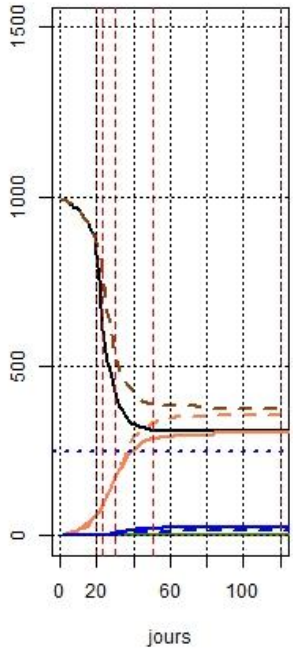
Scenario 2





Scenario 3





Scenario 4

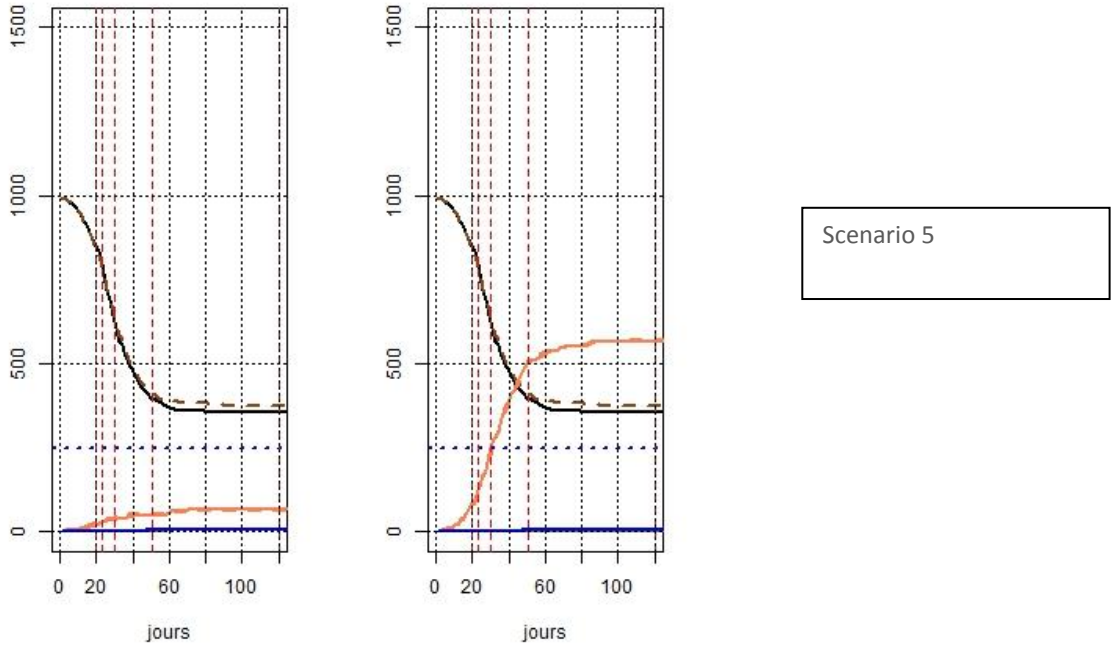


FIGURE 32: GI AND GII CASES WITH GI AND GII COINFECTION WITH STRONG CROSS IMMUNITY FOR SCENARIO 1 TO 5

Legend: show the results for GI (left) and GII (right) cases with GI and GII coinfection with cross immunity paramter at 0.8 (low level of cross immunity), for scenario 1, 2,3,4,5. At the end of epidemic an SSconso can become RSconso, RRconso, or SRconso, or can remain SSconso. Red curve left GI cases only (dashed non oyster consumers), Red curve right GII cases only (dashed non oyster consumers), blue curve RR (dashed non oyster consumers), black curve SS (dashed non oyster consumers), green curve foodborne cases, right GI, left GII.

The effect of foodborne transmission is shown by GI cases and RR cases in scenario 2, by example, in comparison with scenario 1 (Figure 31). Oyster consumers are more likely to be RR or GI cases in scenario 2. The total number of cases (SS curve) is more important with foodborne transmission. The number of RR is less important in Figure 32 than in Figure 31, with the strong effect of cross immunity.

4.3.7. DISCUSSION

The mitigation strategy of closing shellfish areas to protect human shellfish consumers seems effective, even for a population exposed to a winter Norovirus epidemic, whatever the genotype, but (more for GI), for shellfish consumers and non consumers. Comparing results in scenario 1 (without foodborne transmission) and scenario 2 (with food borne transmission) (Figure 28 for GI and 30 for GII) show an increase in the number of cases, for consumers and non consumers, with a high number of cases attributed, for consumers, to foodborne transmission for GI (Figure 28).

This effect is not surprising, already intuitively explained by the fact that the reproduction number and the size of an epidemic are linked to transmissibility parameters. Introducing environmental shellfish transmission adds another component to transmissibility, and, as a consequence to the reproduction number and the size of the epidemic (Rohanni *et al.,* 2009). Estimates of the changes in reproduction number over time and according to the implemented mitigation strategy are potential future avenues of investigation.

How should the mitigation strategy be implemented ? Long closure periods (scenario 5) or early closure measures (scenario 4) seemed to be, on average effective (Figure 28 for GI and 30 for GII; 31; 32). Whenever abnormal level of contamination is suspected, NoV contamination should be confirmed quite rapidly with real-time RT-PCR technique, and the decision for closure can be made rapidly and implemented for a long enough period to avoid foodborne contamination cases (scenario 5). Or, if suspicion of contamination is strong enough (epidemic in population, failure in treatment) , decision to declare closure can be made under the precautionary principle, while waiting for results from the PCR analysis (scenario 4). Waiting for one month after the end of epidemic, and until enough negative results to re-open could be explored in future developments of the model. However scenario 3 for GI shows that some foodborne cases could happen if the closure of the area (forbidding consumption period) is not long enough. The decreasing of the virus concentration in shellfish is a long process, and in the case of raw sewage discharge, the initial contamination can be high (Figure 27B and 29 B), whenever the infectivity of GI and GII by food transmission are high. Alimentary cases continue to happen with concentration close to 1/g in edible tissues (in DT the concentration should be 100 X more). During this period of the year, consumption of oysters reaches its yearly maximum increasing foodborne risk (France AgriMer, 2008)

The sporadic or accidental contamination effect has already been described for HAV (Thebault *et al.,* 2012 ; section II), and microbial monitoring was found to be inefficient in this situation to prevent cases, paving the way to other management strategies, such as improvement of sewage water treatment, virus monitoring, and an alert system for shellfish producers and local public authorities (Thebault *et al.,* 2012; ANSES, 2011).

Some parameters were set different for GI and GII. Bioconcentration effect is shown in Figure 27B and 29B.The median concentration in sea waters was more elevated for GII than for GI, and that can be explained by higher rate of excretion, and higher number of infectious individuals at the beginning of the epidemic, linked to the higher transmissibility rate assigned for GII. Without environmental transmission, the number of cases is more elevated for GII than for GI (scenario 1, Figure 28 and 29). In the end, however, the concentration in edible tissues is higher for GI than for GII, due to the higher bioaccumulation factor for GI than for GII in winter period (Maalouf *et al*., 2011). The relative contribution of foodborne cases to epidemic cases is stronger for GI than for GII (as shown in Figure in scenario 2 of Figure 28 and 29. The mechanism and calibration of this model could explain, along with the differences in the biological characteristics of each genotype, the relatively higher incidence of GI in shellfish-relatedfoodborne outbreaks compared to GII.4

The last point was to explain the effect co-contamination. The results are linked to value given to cross-immunity factor (Figure 31, 32). If cross immunity is high (Figure 32), GII given its higher

transmissibility rate reaches a higher number of cases, and co-infection (RR) is rare. If cross immunity is low (0.8), RR cases became more frequent (Figure 31). Food related cases can be the cause of RR cases (co-infection or successive infection GI GII), SR (GII cases) or RS cases (GI cases). The effect of foodborne transmission is shown by comparison with scenario 1 and 2, with an increase number of GI and RR cases with foodborne transmission (Figure 31-32). This effect (for RR) was limited in the case of strong cross-immunity.

The long term effect of environmental transmission was not studied here, but is suspected to be different from previous studies. Environmental reservoirs are known to increase the inter-epidemic period for scrapie in sheep (Woolhouse *et al*., 1998,), cholera (Codeço, 2001), bubonic plague (Keeling and Gilligan, 2000), avian influenza (Roche *et al*., 2009; Wang *et al*., 2012) and HAV (Ajelli *et al.*, 2008). However, the duration of immunity and demographic conditions should have an impact on these results, which could be explored in the future for Norovirus dynamics.

Another human reservoir impact was not explored here: chronic shedders. The estimate of the proportion of chronic shedders and their impact on the dynamics in the general population is another challenging question. Other environmental reservoirs such as other shellfish species, or sediment can be explored, in particular those involved in viral contamination after storms or tempest (e.g. Xynthia, Grodzki *et al.,* 2010). Survival rates in water (cool and marine) and in sediments (and soil) are factors to consider for long term exploration of the reservoir effect with higher survival rate (Breban *et al.,* 2010)(Figure 33).

Some waste water treatment plants have higher residence times (1 or 2 months), including reservoir ponds, and these can have a different effect on virus survival.

We ignored here the problem of infectivity, because there is no kind of culture system for Norovirus. Alternative assumptions should be done by comparing with closely related viruses, but there is no global agreement on the percentage of genomes that can be infectious. We therefore assumed that all genomes correspond to infectious genomes, which is partly justified by the high infectivity found in dose-response estimates (Teunis *et al*., 2008; Thebault *et al*., submitted). We also ignored other sources of contamination, such as irrigated crops, water consumption and winter bathing in the sea. Contaminated fruit and vegetables could be investigated from contamination data on these products, and their involvement checked by case-control studies from data on outbreaks. For consumed water, the source and treatment of water can be known, and classified, roughly according to different level of viral risk for consumers, involving superficial water sources, and treatment efficiency (AFSSA, 2007). Before trying to analyze coastal epidemic data, the origin of water consumption must be checked. However, as in all sources of indirect transmission other than oysters, we can assume that these factors are included in and therefore overestimated, the inter –individual transmission parameter in our model.

The effect of demography and zootechnical practices, heterogeneity of shellfish in space and time, the effect of environmental conditions on shellfish activity (filtration) all probably affect the contamination level of shellfish on the market level (post harvest). This should be investigated to better estimate the associated parameters and examine their effect on results. For example results

of NoV bioaccumulation in shellfish in the summer time are different than those reported for the winter(Maalouf *et al*., 2011).

Differences between the biological characteristic of each genotype can explain, under all assumptions made for the model, the relatively higher incidence of GI in shellfish outbreaks compared to GII.4. Another genotype GII.3 with some characteristics similar to those to GI should have more or less same effect as GI (Maalouf *et al*., 2011). The survival rate, in different kinds of situations and treatment, is not well defined for the different strains and genotypes of norovirus and should be better investigated, even if some papers have already been published on the subject (Le Guyader *et al.,* 2008; Flannery *et al., 2*012). Although not surprising, but the effect of cross immunity could be better explored with other hypotheses, with full cross immunity and lower values of cross immunity (Roche *et al.,* 2010), after or before removal of symptoms, in particular for longer time scales, to see their effect on dominance or coexistence patterns in cases in coastal areas . Understanding and making estimates of immunity and cross-immunity parameters for Norovirus, is a fundamental question in the understanding of the persistence of strains and genotypes in the human population.

The long term effect of role shellfish in the maintenance of some genotypes, such as GI or GII.3 rather than GII.4,is an interesting point to explore in the future. First the structure of the model for inter-individual transmission should be changed from a SEIR model to a SEIRS model, to take into account the short immunity generally associated with Norovirus infection (Karst, 2010). Then a metapopulation model could explore, including demography (and migration effect) in light of previous work done on HAV (Ajelli *et al*., 2011). However more precise data on shellfish consumption in different kinds of areas (coastal, non coastal) is needed. Furthermore our model could be generalized using more genotypes or strains, each having its own dynamic, depending on data availability (Figure 33). We chose to apply the stronger contact rate for GII than for GI. The excretion rate is higher for GII than for GI, and perhaps survival and infectivity of the GII virus justifies this assumption. The dominance of GII.4 in inter-human epidemics is a complex phenomenon, and strain diversity dynamics need to be included in the model (Lindesmith *et al.,* 2008). "The rapid evolution of RNA viruses, means that their evolution and ecology occur on the same timescale, and therefore must be studied jointly to be fully understood"(Pybus and Rambaut, 2009). A general multistrain model should then be developed, perhaps based on genotype cluster characteristics (Lipsitch *et al*., 2009; Breban *et al*., 2010) (Figure 33). However the dominance of a particular genotype is illustrated here by a higher transmissibility rate.

The calibration choice should be investigated using other parameter values. Uncertainty analysis should be carried out to confirm results on a wider range of values for uncertain parameters, for example for $\delta 1$ and $\delta 2$, describing cross immunity or dose-response parameters (α, β, r, η). We could imagine, as for QRA, a second level of the dynamic model, taking into account the uncertainty of parameters, and focusing on median of the mean of variability estimates (see section II).

A sensitivity analysis would indicate which parameters are the most influent to results. It would also highlight the gaps in data.

The effective contact rate parameter β should be better informed with specific data, localized at small scale in shellfish production areas and estimated with real-data (Melegaro *et al.,* 2011). Heterogeneity in the population was oversimplified here. Dealing specifically with the norovirus infection, specific effective contact rates of chronic shedders (Sukhrie *et al*., 2010), asymptomatic carriers (Sukhrie *et al*., 2012), and secretor-negative populations could be better explored (Marionneau *et al*., 2005). However, data from the general population on which to base estimates are lacking. At a lower scale, strain effect and ABO blood type can also be involved. Even if infection sensitivity does not seem to be age-specific, age and social habits interfere with the contact rate and transmission risk (transmission at primary school for example) and even duration of excretion (Partridge *et al.,* 2012). Therefore introducing an age factor could be interesting also because shellfish consumption in France can be associated with age, sex, season, location.

The proportion of shellfish consumers with representative data in coastal areas in France is not known. The proportion of negative secretors is based on few available data. Contact rates between consumers and non consumers were assumed to be equal even if age can differ between those groups, because again, informed data were missing. The knowledge of risk factors associated with gastroenteritis can provide more information on heterogeneity in the population that the model could take into account.

Finally estimating the latency and infectious periods for Norovirus is a challenging question. Excretion data were used to determine the latency period, knowing that incubation and excretion data are give close estimates (Karst, 2010, Atmar *et al*., 2008). We chose to keep excretion data and infectious period separate. Short generation time (Heijne *et al.,* 2009) and short infectious period are reported (1.8 days) (Van Der Pas, 2009)whenever excretion in stool seem long and elevated. The symptomatic phase seems to be linked to transmission rather than asymptomatic, even with high rates of excretion (Sukhrie *et al.,* 2012). Other approaches can be explored using viral excretion data (Cori *et al.,* 2012), and also exploring alternatives such as modifying the structure of the model with a hyperinfectivity state (Hartley *et al*., 2006) or different infectivity states.

In any case, this work is the first attempt to explain the effects of the management strategy that involves closure of the shellfish area in a context of a Norovirus epidemic in human population, and is the first attempt to understand what kind of role shellfish selectivity plays in the incidence of a particular genotype in a human norovirus epidemic.

Acknowledgement: financial support ANR COQENPATH

4.4. PERSPECTIVES

We chose to focus the dynamic model on Norovirus due to the particular context in shellfish areas, where winter epidemics occur yearly in human coastal populations, in comparison with HAV, where epidemics are becoming rarer (ANSES, 2010).

The dynamic model of Norovirus provides insights into situations, where two (or more) transmission infection routes are possible, in order to attribute cases to specific pathways. Results seem to justify closing shellfish production areas, during contamination period, to prevent foodborne cases in coastal population, even during epidemics in the human population, which is not possible to evaluate with QRA. On other hand, introducing QRA in a dynamic model made the estimate of foodborne cases more realistic. Moreover, this point was defined as a challenging point (EFSA, 2011) for understanding what the consequences of virus foodborne contamination are in the whole process.

However we do not estimate the risk of an emerging epidemic due to food (oyster) contamination. QRA gives an estimate of primary cases, linked to foodborne transmission only. For this assessment, precise contact rate estimates for inter-human transmission, and consumption data, are needed. For Norovirus secondary cases are known to occur after or along with foodborne cases. However the winter epidemic of gastroenteritis is not driven by foodborne cases, but by inter-human transmission, and the seasonal pattern is not explained by any particular food contamination or consumption. However it may be useful in closed environments to prevent primary and secondary cases of food origin, by setting a limit for contamination in food, even if there are other ways of introducing infection in these environments (asymptomatic carrier by example)(Alfano-Sobsey, 2012). In hospitals, cases can be more severe for older and immunosuppressed individuals (Gustavsson *et al.*, 2011; Partridge *et al.*, 2012; Greig and Lee, 2012).

The epidemic following foodborne cases is known for HAV and the size of the epidemic can be tremendous (Halliday *et al.*, 1991). A long term dynamic model of HAV for coastal populations has already been constructed in Italy (Ajelli *et al.*, 2008; 2009; 2011) and shows that (i) a high number of cases can be due to HAV food contamination, in endemic situations and due to the lack of protection of shellfish resources; (ii) foodborne cases are linked to a longer inter-epidemic period. In France it would be of interest to investigate the risk of an emerging epidemic due to particular level of food contamination, in regularly exposed population and in susceptible population. The latter situation currently seems more frequent, due to the decline of seroprevalence in France. For HAV, an individual based model with a discrete time pattern, would be useful, taking into account age and seroprevalence structure, asymptomatic carriers, contact rate estimates, food consumption, and contamination data. However the dose-response for HAV, which is a critical point for assessing the risk associated with a particular level of contamination in food, should be further validated or evaluated in real outbreak situations. In terms of precautionary principle, effectiveness of monitoring at sewage treatment plant, or in conjunction with shellfish monitoring, should be investigated. Because Hepatitis A has a long incubation period and because in many cases infectious individuals can remain asymptomatic, a dynamic model would help to explore the effectiveness of monitoring sewage water to prevent or detect the beginning of a human epidemic, and to prevent shellfish

contamination (in coastal areas), similar to what has been proposed for Poliovirus (Ranta *et al*., 2001).

V/ GENERAL DISCUSSION-CONCLUSION

5.1. MAJOR FINDINGS

The aim of this work was to better estimate viral foodborne cases in human population, focusing on the viruses with major sanitary impact in high level of hygiene countries, such as HAV and NoV. In order to evaluate prevention measures in a particular example, the study focused on shellfish, in particular oysters, because of their particular biology and bioaccumulation of viruses in tissues. The contamination is coming from coastal human population, via sewage system. Ministry of Agriculture, has recently raised questions for this particular food production, in relationship with virus contamination, in France. At international level, the question was also raised: "A challenging question is how much disease caused by norovirus can be attributed to foodborne spread" (EFSA, 2011; 2012).

The cost of closure of shellfish areas is hardly accepted by shellfish breeders and coastal populations. Therefore such a decision and the length of closure need to be well motivated and supported by scientific knowledge.

Fundamentally ecological factors are suspected to contribute to the selection of strains capable of spreading in terrestrial populations (Coburn *et al*., 2009; Henaux *et al*., 2010; Haven *et al*., 2012; Rohanni *et al.,* 2009; Roche *et al.,* 2010). Genotype II.4 of Norovirus is less associated with foodborne outbreaks and shellfish outbreaks (more associated with GI or GII.3 genotype) than with epidemics with interhuman transmission (Matthews *et al*., 2012). With the use of newly published biological data (Maalouf *et al*., 2010b, 2011) on shellfish, we investigate the foodborne transmission pathway of GI and GII during an epidemic.

From a methodological aspect the aim of this work was also to bridge the gap between Quantitative Risk Assessment (QRA), classically used for predicting foodborne cases and dynamic modeling, involving different ways of transmissions, through a stochastic approach.

For that purpose the first step was to define a complete stochastic risk assessment framework for foodborne viruses from food contamination to foodborne cases. This was applied to HAV (part II) and NoV (part II and III).

The stochastic QRA for HAV took into account the effectiveness of different monitoring and management strategies, in order to prevent primary (foodborne) cases in human population. In particular, the effectiveness of the closure duration of a shellfish area was evaluated under two scenarios of shellfish contamination, and compared with other strategies, such as a better sewage treatment. Effectiveness of monitoring transferred products from areas of production to other, as a current zootechnical practice in France, was also evaluated.

However we identified weakness points in estimating a precise dose response and infectivity estimates for HAV. Dose-response was indirectly validated in previous study published study, but with surrogate virus data (Pinto *et al*., 2009), and specific assumptions (infectivity) were made. Data

about HAV shellfish contamination are also rare, and the seroprevalence level for human population, in coastal area is unknown (ANSES, 2010). Then the relative diminution of number of cases was only considered, not giving absolute number of cases estimates in coastal population, for each intervention strategy and each scenario of contamination investigated. Results showed that microbial monitoring system was not useful to prevent viral contamination risk . HAV (RT-PCR) monitoring at high frequency, by example twice a month can be efficient. However the most efficient strategy is to prevent or diminish contamination in coastal waters, using better sewage treatment systems. Sampling strategy is not taking into account spatial heterogeneity, introducing the need of several samples at each point of time. Monitoring transfers by HAV analysis, in case of contamination in the bay is shown to be also efficient. The effectiveness of waiting for 1 to 3 negative results, to reopen the area, increasing the duration of closure was also investigated. In case of homogenous, limited (rapidly stopped) and identified source of contamination, the need for waiting of three negative results is not always justified. In case of unidentified source of contamination, or endemic situation of contamination, the need for wait of three negative results is becoming necessary, in order to prevent foodborne cases.

For Norovirus situation, the diversity of strains and genotypes involved make the situation complex. At the beginning of this work we had data of dose-response from human trial, with watery matrix, for Norwalk virus (GI) (Teunis *et al*., 2008).

We had the opportunity to obtain outbreaks data associated with doses of each genotype that can be estimated. For one of these outbreaks, individual secretor status was known. This secretor status (driven by genetic character) was known to be associated with less susceptibility to infection (Teunis *et al*., 2008). Because the level of information was different between individuals and outbreaks, and because posterior distribution can be used for uncertainty estimate of dose-response parameters, a bayesian framework was used. Our results confirmed the very high infectivity of GI, as previously shown (Teunis *et al*., 2008), with a probability of disease with one copy around 0.13 [0.008-0.4] and show the same high infectivity of GII for secretor individuals with a probability of disease with one copy around 0.18 [0.018-0.42]. This high infectivity showed also that genome can be associated, for Norovirus, with an infectious virus with a high probability. Infectivity was much lower for non secretors, with a factor around 1000.

It was the first estimate of infectivity for GII, involved in winter gastroenteritis epidemic (with other viruses), and concerning a non negligible percentage of French population each winter (sentiweb, 2012). It is also a data gap for quantitative risk assessment for Norovirus, identified in several reports, which is fulfilled by this work, and opening the gate for QRA with norovirus in particular for shellfish.

The last part of the work is answering different questions. First, methodologically, we introduce foodborne QRA in dynamic modeling which was not done explicitly before, even for other pathogens such as Cholera and HAV (Righetto *et al.,* 2012; Ajelli *et al., 2*008, 2009, 2011) using human consumption data and dose-response estimates, as used for QRA analysis. We introduce QRA in a dynamic and stochastic framework in order to take into account variability of the dynamic of infection and variability in environmental transmission, and we use dose-response parameters and consumption data estimated previously.

For Norovirus this is the first dynamic model that takes into account, in the same framework foodborne and inter-human transmission, trying to attribute cases to each pattern of transmission. This work, with all the limitations due to the lack of available data, sensitivity analysis, validation criteria, justifies in some circumstances the closure of shellfish area in order to prevent cases in coastal population, even if this population is already exposed to a human epidemic. Finally this model explores the case of a coinfection by GI and GII, using recently published selective bioaccumulation results in shellfish (Maalouf *et al.,* 2011). Then the question of selection and competition of viral strains, considering two ways of transmission (food and inter-human), and with a marine reservoir selectivity, is explored for the first time for a human pathogen, in a time scale of a hundred days, and for a closed population (coastal). This would be helpful, especially when trying to investigate why foodborne outbreaks are more associated with genotype other than GII.4.

5.2. Limitations of this work and perspectives

All sort of limitations need to be mentioned for this work. Some data gaps were clearly identified. Quantitative data of HAV in shellfish, more precise meta-analysis of outbreaks data of HAV for estimating dose-response of HAV, seroprevalence data with age for coastal area (in particular in Paimpol area) were missing for HAV study. For both, HAV and NoV, consumption data is only known for fish consumers (Leblanc *et al.,* 2006), but representative percentage of population consuming shellfish in coastal areas was unknown. For both HAV and Norovirus infectivity of genome was not known. Nevertheless we show that this is not really a limitation while investigating efficiency of management strategies in coastal areas.

Quantitative excretion data of humans and the link with transmission should be further investigated for both viruses.

For both data at the human population level are also missing for this work. Efficient contact rates in French general population and in coastal areas, by age or sociodemographic characteristics for those diseases are not known, and the epidemic data at lower scale for Norovirus, in coastal-shellfish areas of production in comparison with non coastal areas (with less consumption of shellfish), should be further investigated, including by example genotype or strain characteristics. It would be helpful when trying to fit the model to real data. For other parameters, such as dilution factor in the environment, expert elicitation can help for calibration the model (Albert *et al., 2*012).

Sensitivity analysis is missing for part I and IV, and is partially made for part III. It could be an important point to do in future, in order to better know key factors linked to the output, and prioritize data provision. Second order risk analysis was made for QRA part, and could be done in future for part IV, showing the effect of uncertainty estimates on results. However this approach is limited to the range of values chosen for describing uncertainty of parameters (and shape of distribution) and need to be compared with other model structure.

For Norovirus, whenever sensitivity analysis is missing for prioritizing parameters, it was particularly difficult to set immunological parameters. Long and short term acquired immunity is not known, and

should be defined, probably at cluster level and strain level. Innate immune protection, in this work takes into account secretor status, but is neglecting ABO blood type, that could be linked with strain effect in particular for GII (Le Pendu *et al.,* 2006; Tan *et al.,* 2008). For dose-response it could be an interesting perspective, with more documented outbreaks, to investigate ABO blood type effect with strain and genotype. And in a general manner to investigate the effect of dose, not only with infection and disease risk, but also with incubation time, duration and level of excretion, and severity of symptoms, for both pathogens (HAV and NoV).

The effect of co-infection is partly seen at genotype level for GI and GII (part III and IV). More data are required to inform about the co-infection effect of strain by the same pathogen, and between pathogens. Very often microbial contamination of fecal origin is involving several pathogens (AFSSA, 2007 ;Le Guyader *et al.,* 2008). And during winter epidemic, several pathogens are involved and can interfere each other (Chiki-Brachet *et al*., 2002; Arena *et al*., 2012). The winter seasonal pattern of Norovirus (Lopman *et al*., 2009) raised questions about infectivity and epidemic potential from a foodborne outbreak in summer times, which could be further explored. One environmental explanation could be the lack of survival of virus to ultraviolet rays or drought in summertime (Yang and Marr, 2011), but should be confirmed by further research, and interfere with other possible explanation (change of contact rate, change of susceptibility in human population). For investigating foodborne cases risk linked to shellfish, it is interesting to see that bioaccumulation in shellfish is found to be seasonal (Maalouf *et al., 2*011).

As further objective to be considered is the risk of an emerging epidemic, in coastal areas originally caused by food contamination, as it was observed in China (Halliday and al., 1991). For this purpose, a dynamic model for HAV, without further validation of dose-response, without an estimate of seroprevalence and contact rate is unrealistic. But for HAV it could be an interesting perspective to see if monitoring at sewage plant (and /or shellfish) can be an interesting strategy to prevent cases, and an epidemic (with more data) .

As management strategies investigated in this work, we focused on oyster monitoring in the area of production (sea), on diminishing the level of contamination and effectiveness of closure of shellfish areas. We don't investigate the monitoring at sewage plant (in influent or effluent), knowing that sewage plant systems (residence time, treatment) are different from one coastal area to another area. We don't investigate, and it would be interesting to do so, the efficiency of combination of surveillance systems, by example in water sewage at sewage plant, in sea waters, in shellfish in the area of production, and in shellfish sold for human consumption (at breeder or at the seller level). Spatial heterogeneity of contamination in oyster production areas can be also further explored, taking into account hydrodynamic modeling, for differentiating different risk areas, in time, from known sources of contamination.

We don't investigate interventions in the human population. For HAV, target vaccination strategy in endemic(coastal) areas was found to be efficient in Italy (Ajelli *et al*., 2011), in order to diminish seroprevalence and number of cases. This strategy is not investigated for France, in fact endemic areas with important size of population and cases, which act as a reservoir for other areas do not exist in France. However this strategy could be investigated in some situations, as a limitation of spread of disease, in a case of emerging and local epidemic, remembering that in France HAV

vaccination is recommended for most exposed groups (InVS, 2009). It could of use to assess quantitatively the effectiveness of sentinel surveillance for NoV and HAV, for comparing/fitting results with modeling predictions. For HAV, taking into account asymptomatic cases known to be involved in transmission, and long incubation of the disease to investigate the interest of a complementary monitoring in the environment (sewage plant) to prevent an epidemic spread, as previously done for Poliovirus (Ranta *et al.,* 2001). The diversity of strains and mechanisms of emerging and persistence in human population, in association with lack of immunity data, is making vaccination strategy for NoV more complex (Lindesmith *et al*., 2008; Karst, 2010; Atmar *et al*., 2011).

Other strategies, as social distancing, were shown to be counterproductive for HAV, increasing risk for older population with more severe symptoms (Ajelli *et al*., 2011). However analysis of efficiency of measures such as improvement of hygiene practices or social distancing should be studied in a particular context, and for a specific population. Improvement of hygiene to prevent Norovirus risk in hospitals, or closed environment was shown to be effective (Heijne *et al.,* 2011).

We investigated two ways of transmission, the foodborne and inter-human. In fact we approach the question in a simple manner, because as seen in part I, other food products than oyster can be contaminated, food preparation can be contaminated (Mokhtari *et al.,* 2009) and also because this coexposure can occur at the same period for the same individual. The same analysis can be made in what we called "inter-human transmission". This inter-human transmission include individuals contacts (by hand by example), but concerns different infectious material for NoV (fomites and feces excretion)(Marks *et al*., 2003; O'Neill and Marks, 2005), and also indirect transmission by contamination of walls, carpets, (..) and in summer time by bathing is sea or river. All these different ways of transmission could be considered for future investigation. Our study cannot be extrapolated to all other foodborne viruses. Hepatitis E virus (HEV), another foodborne virus is different with those that are developed here, and need specific study. In high hygiene countries, it is the only known zoonotic virus for human by food, with reservoir in animals (in particular in pigs who are multiplying the virus) and the disease is not known to be transmitted from human to human (AFSSA, 2007).

As a last point the dimension of our analysis in time and space was limited to duration of one epidemic (one season, one epidemic period or a year) and to a limited population (neglecting migration). Results of QRA, and foodborne risk linked to shellfish, are linked to food consumption habits, that are known to be seasonal and heterogeneous in space (coastal and non coastal). However comparison can be done for characterizing risk between population areas (coastal and non coastal).

Long term effect and effect for a coastal population, then for a larger population taking into account the exchanges network, could be an interesting challenge to explore strains and genotypes dominance for Norovirus. A final framework, for a coastal population, illustrated how it could work out, introducing the dynamic of strains change, including other reservoirs as sediment (Grodzki *et al*., 2012) or other shellfish species (Figure 33). Heterogeneity in population (by age or food habit) can be studied with other model structure (SEIRS, or SEIS) (Figure 33).

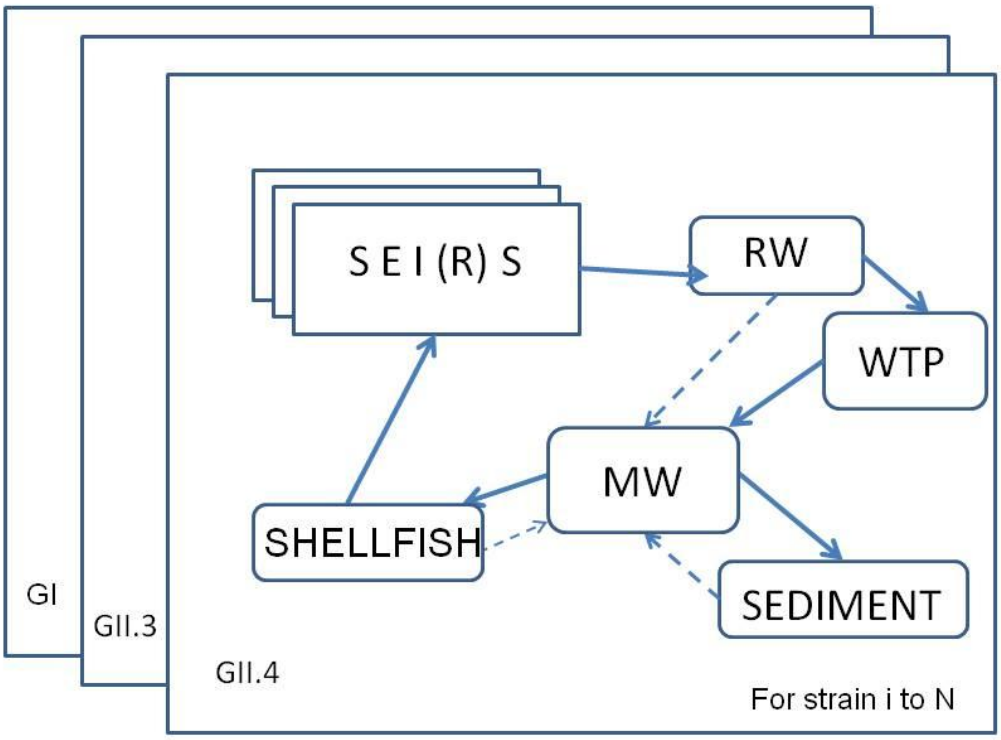


FIGURE 33: DYNAMIC MODEL FOR LONG TERM EFFECT OF NOROVIRUS SHELLFISH FOODBORNE TRANSMISSION FOR COASTAL POPULATIONS

Legend: S: susceptible, E infected, I infectious, R immune, RW raw reject of sewage water, WTP treated reject by water treatment plant, MW, sea water, GI genogroup I, GII.4 (cluster)genotype II.4, GII.3 (cluster) genotype II.3 of Norovirus

Arrows: environmental and foodborne transmission pathway; dashed arrows: rare or accidental pathway, tempest (sediment to MW), heavy rainfall (RW to MW), heavy mortalities or releasing (shellfish to MW)(not observed)

Rectangle human population (with different compartments describing heterogeneity of transmission), rectangle with round corners, environmental compartments

5.3. CONCLUSION

Specificity of virus, as foodborne pathogens were took into account in this work, with application to NoV and HAV. Secondary cases, mechanisms of resistance and immunity, detection and quantification were taken into account. This work provides help for virus risk assessors, in particular in giving a dose-response based on outbreaks data for norovirus. It provides help for risk managers, involved in the closure of shellfish areas or monitoring, in order to prevent primary cases of HAV (part II), and comprehensive mechanisms of primary and secondary cases of Norovirus (part IV). It provides a contribution to scientific knowledge and raises epidemiological concerns, with estimates of high infectivity of norovirus GII, and confirmation by outbreak data for GI (Teunis *et al*., 2008) for secretor individuals, and the very low infectivity for non secretors. A new scientific question is raised, trying to explain high level of non GII.4 oyster foodborne outbreaks, in exploring mechanisms involved in genotype selectivity for human coastal epidemic and foodborne outbreaks. And finally this work, initiated conceptually by previous work (Codeço *et al*., 2001; Eisenberg *et al*., 1998, 2004, 2005) bridge the gap, for Norovirus, by linking two disciplines, with their own conceptual framework, quantitative microbial food risk assessment and dynamic modeling, that could be extended to other foodborne viruses, such as HAV and other foodborne pathogens.

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