Epidémiologie des diarrhées aiguës virales de l'adulte en médecine générale en France

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Résumé

L'épidémiologie des diarrhées aiguës (DA) hivernales a été peu décrite chez l'adulte. Ces DA sont principalement dûes à des virus entériques. Des virus influenza peuvent être détectés l'hiver dans les selles de patients grippés présentant des signes digestifs, mais on ignore s'ils peuvent être retrouvés chez des patients présentant exclusivement des troubles digestifs.

Durant les hivers 2010/2011 et 2011/2012, les médecins Sentinelles (Inserm-UPMC) ont inclus 192 patients adultes consultant pour une DA et 105 patients contrôles. Un prélèvement de selles était effectué pour la recherche de norovirus (génogroupes I et II), rotavirus du groupe A, adenovirus entérique humain, astrovirus et virus influenza A(H1N1)pdm2009, A(H3N2) et B.

Durant les hivers étudiés, l'incidence moyenne des DA chez l'adulte a été estimée à 3158 pour 100 000 adultes (IC 95% [2321 – 3997]). Un traitement était prescrit pour 95% des patients avec une DA, et un arrêt de travail pour 80% des patients actifs. Les examens de selles ont permis de détecter un virus entérique chez 65% des patients diarrhéiques, le plus souvent un norovirus (49%). Parmi les patients présentant une DA, 7,2% étaient positifs à un virus influenza, ces derniers n'ayant pas rapporté de signes respiratoires. Les symptômes décrits par les patients diarrhéiques adultes ne différaient pas en fonction de la présence ou absence d'un virus entérique. Les patients contrôles ne présentaient ni virus entériques ni virus influenza dans leurs selles. Aucun facteur risque évitable n'a été identifié, autre que le contact avec une personne malade au sein du foyer et/ou en dehors, rapporté chez 46,2% des patients ayant consulté pour une DA.

Mots clés : Diarrhée ; Médecine Générale ; Adulte ; Surveillance ; Grippe ; Virus entériques.

Laboratoire d'accueil : UMR S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique (dir. Dominique COSTAGLIOLA), Equipe Surveillance et Modélisation des maladies transmissibles (dir. Pierre-Yves BOËLLE), Inserm, Université Pierre et Marie Curie, Paris, France.

Abstract

Epidemiology of viral acute diarrheas in adults in general practice in France

The epidemiology of winter acute diarrheas (AD) has not been described in adults. These AD are mainly due to enteric viruses. In winter, influenza viruses can also be detected in stools of influenza patients with digestive signs, but we don't know if these viruses can be found in the stools of patients suffering from digestive disorders exclusively.

During the 2010/2011 and 2011/2012 winters, general practitioners (GPs) from the Sentinelles network (Inserm-UPMC) included 192 adult patients consulting for an AD and 105 control patients. Stool samples were collected and tested for norovirus (genogroups I and II), group A rotavirus, human enteric adenovirus, astrovirus and influenza viruses A(H1N1)pdm2009, A(H3N2) and B.

During the studied winters, the average incidence of AD in adults was estimated to be 3,158 per 100,000 adults (95% CI [2,321 – 3,997]). GPs prescribed a treatment in 95% of the patients with AD, and 80% of the working patients with AD could not go to work. Stool examinations were positive for at least one enteric virus in 65% of cases, with a predominance of noroviruses (49%). Of the patients suffuring from an AD, 7.2% tested positive for one influenza virus, none reported respiratory symptoms. Among the patients with AD, the reported clinical signs did not differ between adults with a virus in the stool sample and those with no virus found in the stool exam. None of the controls tested positive for one of the enteric and/or other influenza viruses.No preventable risk factor was identified, other than the contact with a sick person within and/or outside the household, reported by the patient in 46.2% of cases.

Keywords: Diarrhea; General Practice; Adult; Surveillance; Influenza; Enteric Viruses.

Host laboratory: UMR S 1136, Pierre Louis Institute of Epidemiology and Public Health (head: Dominique COSTAGLIOLA), team Surveillance and Modelling of communicable diseases (head: Pierre-Yves BOËLLE), Inserm, Pierre et Marie Curie University, Paris, France.

Sommaire

Abréviations utilisées

DA : Diarrhée aigue

SG : Syndrome grippal

CNR : Centre national de référence

ARN : Acide ribonucléique

ORF : Open reading frame

ADN : Acide désoxyribonucléique

RT-PCR : Reverse transcription polymerase chain reaction

HA : Hémagglutinine

MG : Médecin généraliste

MGL : Médecin généraliste libéral

InVS : Institut de veille sanitaire

OR : Odds-ratio

IC95% : Intervalle de confiance à 95%

1. Contexte et objectifs

La diarrhée aigue (DA) de l'adulte se définit par l'émission d'au moins trois selles molles à liquides par 24 heures, de survenue aiguë ou brutale, évoluant depuis moins de deux semaines [1]. Elle est généralement le symptôme d'une infection gastro-intestinale, qui peut être due à divers agents pathogènes comme des bactéries, parasites ou encore des virus, transmis la plupart du temps de façon interhumaine ou par l'ingestion d'eau ou d'aliments contaminés.

Bien que les DA sont une importante cause de décès et de morbidité dans les pays en voie de développement, leur impact dans les pays industrialisés reste non négligeable, notamment chez les personnes âgées [2, 3]. De nombreux travaux ont consisté à étudier les DA chez le jeune enfant en raison des conséquences importantes en termes de morbi-mortalité qu'elles peuvent engendrer. Chez l'adulte en bonne santé, la DA est généralement bénigne et a pu susciter moins d'intérêt, bien que son impact sur l'économie et le système de santé d'un pays ne soit pas négligeable [4-6].

En France, le réseau Sentinelles surveille le nombre de cas de DA vus en consultation de médecine générale depuis 1991 [7, 8], permettant de faire apparaitre un profil épidémiologique particulier avec une épidémie nationale chaque hiver [9]. Des études épidémiologiques ont été menées afin de rechercher l'étiologie de ces épidémies hivernales, ce qui a permis de conclure à une origine essentiellement virale [10, 11]. Une seule étude des facteurs associés à la survenue de ces DA hivernales en milieu communautaire a été menée en France, mais ne prévoyait pas d'identifier les agents pathogènes. [12]. A notre connaissance, une seule étude menée en 1999 aux Pays-Bas en milieu communautaire a cherché à identifier les facteurs de risque des infections entériques causées par des virus mais auprès d'une patientèle essentiellement pédiatrique [13].

La notion d'épidémies hivernales des diarrhées aigues et des syndromes grippaux (SG) a longtemps laissé croire que les virus grippaux pouvaient être responsables à la fois des SG et des DA, faisant même utiliser le terme de "grippes intestinales" [14]. Jusqu'à ce que les virus entériques soient eux-mêmes mis en cause [15-21]. Puis, plus récemment, des études ont montré que certains virus grippaux pouvaient entrainer, principalement chez l'enfant et dans un contexte de sévérité, des signes cliniques digestifs en plus des manifestations respiratoires, voire des signes exclusivement entériques [22-29] .

Les objectifs de ce travail de thèse étaient donc :

1) D'estimer au sein de la population adulte française, l'incidence des DA nécessitant une consultation en médecine générale durant les épidémies hivernales ; puis de décrire les caractéristiques cliniques et la prise en charge par le médecin généraliste des DA virales et enfin d'identifier les facteurs de risque susceptibles d'être associés à la survenue de ces DA virales de l'adulte.

2) De mesurer, parmi les patients adultes vus en consultation de médecine générale pour une DA (sans signes cliniques respiratoires), la proportion de patients chez lesquels un virus grippal est détecté dans les selles ; puis la proportion de patients chez lesquels sont détectés simultanément un virus grippal et un virus entérique.

2. Les diarrhées aiguës virales de l'adulte

Chez l'adulte, la diarrhée se définit comme l'évacuation d'au moins trois selles molles ou liquides en 24 heures. Elle peut être aiguë et durer de quelques heures à 14 jours, ou chronique et durer plus de 14 jours.

2.1 Physio-pathologie de la diarrhée aiguë

L'épithélium intestinal est constitué d'une couche de cellules (les entérocytes) possèdant des microvillosités qui forment une bordure en brosse. L'intestin a des fonctions multiples : l'absorption des nutriments au niveau des villosités intestinales, la sécrétion dans les glandes intestinales, le maintien de l'équilibre hydro électrolytique, un rôle protecteur contre les agressions. Pour remplir toutes ses fonctions, il lui faut une grande quantité d'eau, qui est apportée par le bol alimentaire et les sécrétions.

Quel que soit l'agent pathogène et quel que soit son type, la DA est due à un dysfonctionnement entérocytaire réalisant un défaut d'absorption d'eau et d'électrolytes, et son risque essentiel est la déshydratation [30, 31].

Dans les diarrhées virales, les virus prolifèrent au sein des entérocytes matures des villosités de l'intestin grêle, entraînant leur desquamation rapide et leur remplacement accéléré par des entérocytes immatures incapables de réaliser correctement leur fonction d'absorption [31].

2.2 Les principaux virus entériques responsables d'une diarrhée aiguë virale

Dès les années 1940, les virus ont été évoqués comme une cause importante de DA, bien qu'ils restaient alors non identifiés [16]. Mais il faudra attendre les années 1970 pour que soient identifiés des virus entériques comme cause de DA : le norovirus (Norwalk-like virus) en 1972 [15, 17], rotavirus en 1973 [18] puis l'astrovirus [19, 20] et l'adénovirus en 1975 [21].

En France, la surveillance des virus entériques est assurée par le Centre national de référence (CNR) des virus entériques (entérovirus exclus). Créé en 2002, il permet, entre autre, d'améliorer la recherche de ces agents lors de la survenue d'épidémies ou de toxi-infections alimentaires dont l'épidémiologie et la symptomatologie suggèrent une origine virale, et de caractériser les souches virales.

2.2.1 Les norovirus.

Les norovirus appartiennent au genre *Norovirus* d'une famille de virus dont le nom fait référence aux dépressions régulières en forme de calice observées sur leur surface, les caliciviridae [32, 33]. Les norovirus sont des petits virus non enveloppés, d'un diamètre de 27 nm. Ils sont les premiers virus responsables de gastroentérite identifiés chez l'homme, en 1972 à Norwalk (Ohio, États-Unis) [15, 17]. Leur génome est constitué d'un ARN simple brin et comprend trois cadres ouverts de lecture (ORF1-3) codant respectivement les protéines non structurales, la protéine majeure de capside (VP1) et une protéine structurale mineure basique (VP2) **(Figure 1)**.

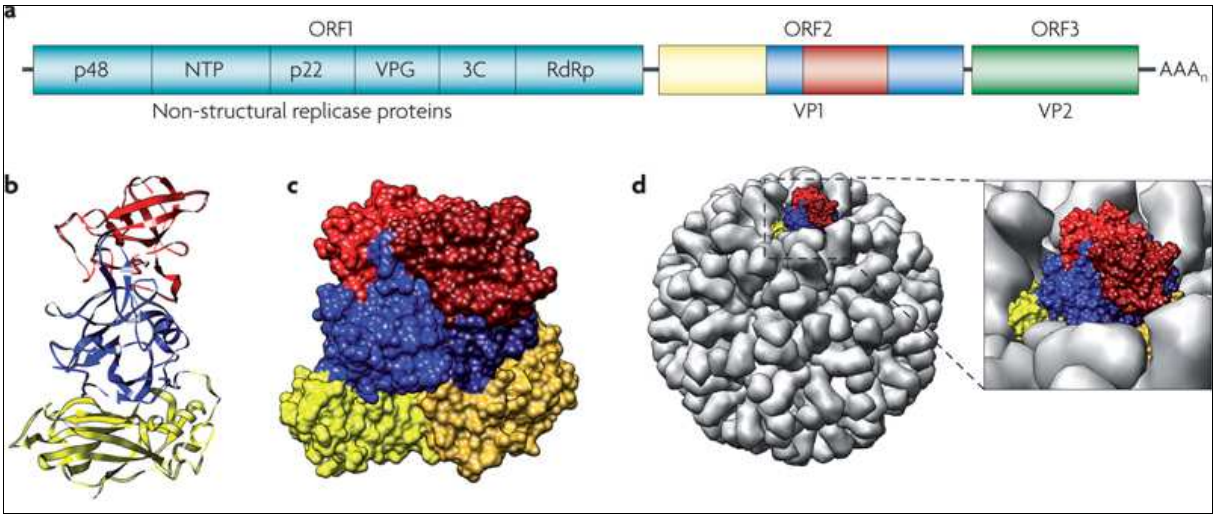

Figure 1: Structure des norovirus [34]

Les norovirus présentent une grande diversité génétique, variant d'une année à l'autre [35]. Les souches sont classées en génogroupes subdivisés en génotypes [36-38]. Actuellement, on distingue 5 génogroupes (I à V) mais seuls les génogroupes I, II et IV infectent l'Homme. Les génogroupes I et II sont les plus importants et sont divisés respectivement en 8 et 19 génotypes [39]. Ainsi la dénomination utilisée mentionne le génogroupe puis le génotype du norovirus : GGI.1 à GGI.8 et GGII.1 à GGII.19 **(Figure 2).**

Chez le sujet adulte, les norovirus sont les virus le plus souvent mis en cause lors des épidémies hivernales de DA en France, comme ce fut le cas pour les sujets âgés de 16-65 ans ayant participé à l'étude de Chikhi-Brachet et al. durant l'hiver 1998-1999 [10].

Figure 2 : Classification des norovirus [40]

2.2.2 Les rotavirus

Les rotavirus, de la famille des reoviridae, sont des virus non enveloppés de 70 nm de diamètre dont le génome est constitué de 11 segments d'ARN double brin, chacun codant pour une protéine. Cette segmentation à l'origine de réassortiments entre souches virales est mise à profit pour l'élaboration de souches vaccinales. Ces virus à symétrie icosaédrique sont constitués de protéines structurales organisées en trois couches concentriques : capside interne VP 2, capside intermédiaire VP 6 et externe VP 7 et VP 4 **(Figure 3).**

Le mode de classification actuel des rotavirus est basé sur les propriétés antigéniques majeures déterminées par les protéines de capside. Il existe ainsi sept sérogroupes (de A à G) qui se distinguent par les antigènes portés par la protéine de la capside intermédiaire VP6 [41]. Au sein de chaque sérogroupe, les deux protéines VP7 et VP4 déterminent respectivement les sérotypes G (G pour glycoprotéine) et P (P pour protéines sensibles aux protéases) [42].

Ces virus touchent toutes les tranches d'âges durant les épidémies hivernales mais ils constituent la première cause de DA chez les sujets âgés de 65 ans et plus [10].

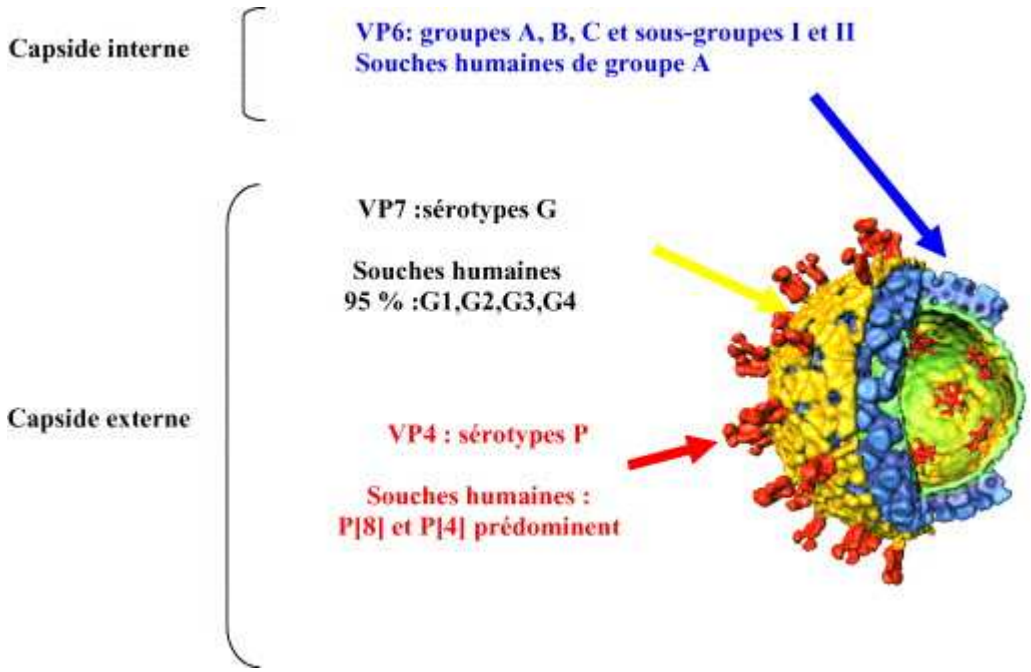

Figure 3 : Structure du rotavirus [43]

2.2.3 Les astrovirus

Les astrovirus sont de petits virus à ARN simple brin, non enveloppés, cultivables, identifiés en 1975 par microscopie electronique [19, 20]. Leur nom reflète leur morphologie en étoile à 5 ou 6 branches en microscopie électronique **(Figure 4)**. Chez l'Homme, huit types antigéniques ont été identifiés. La classification en génotypes à partir de l'analyse

phylogénique de la région codant la phase ouverte de lecture ORF 2 montre une bonne concordance avec les sérotypes, de 1 à 8.

Chez l'adulte, l'infection par les astrovirus est moins fréquente et touche plus souvent les personnes âgées ou immunodéprimées [11, 44].

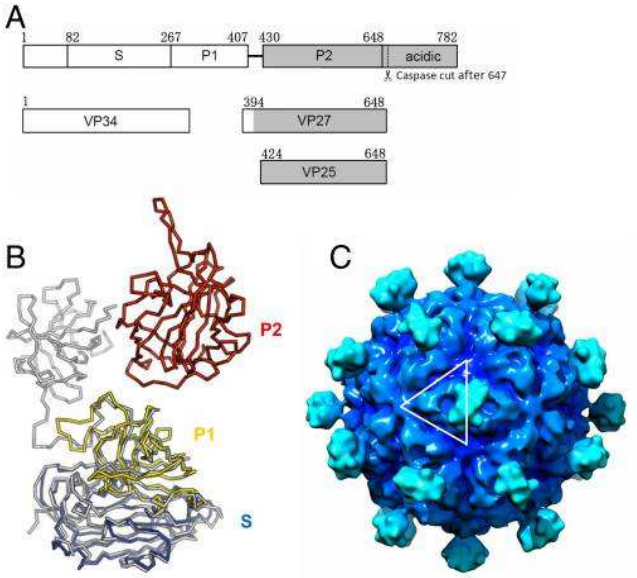

Figure 4 : Structure de l'astrovirus humain [45]

2.2.4 Les adénovirus entériques

Parmi les nombreux sérotypes d'adénovirus humains retrouvés dans les selles de patients ou d'individus sains, seuls les types 40 et 41 et beaucoup plus rarement les sérotypes 2, 3 et 31 sont indiscutablement des agents de DA [11]. Les adénovirus sont des virus à ADN double brin, non enveloppés, de structure icosaédrique composés de 20 facettes triangulaires et mesurant de 90 à 100 nm de diamètre. Ces virus possèdent treize protéines structurales, dont sept constituent la capside (**Figure 5**).

Les adénovirus sont relativement moins fréquents que les virus précédemment étudiés et chez l'adulte, ils touchent préférentiellement les personnes âgées de moins de 65 ans [10].

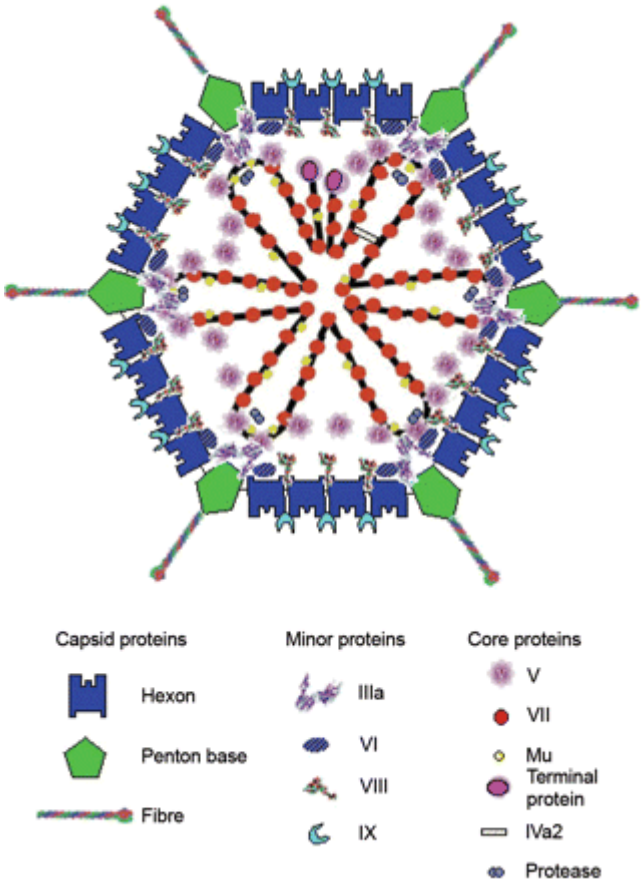

Figure 5 : Structure des adenovirus entériques [46]

2.3 Virus grippaux et infection digestive

De nombreux virus autres que les virus entériques présentés précédemment ont été proposés comme agents responsables d'épisodes de DA : coronavirus [47, 48], picobirnavirus [44, 49, 50], pestivirus [51] ou encore torovirus [52]. Les virus de la grippe (influenza virus) pourraient également provoquer des troubles gastro-intestinaux, tels que DA, vomissements ou encore douleurs abdominales [26-29, 53-56].

Si les virus influenza sont des virus à ARN ayant la faculté de se répliquer et d'infecter le système respiratoire [57], certaines études ont toutefois rapporté la détection, par RT-PCR en temps réel, de virus en grande quantité dans les selles et ont démontré la capacité de ces virus à se multiplier au niveau des cellules intestinales, notamment concernant le virus influenza A(H5N1) [58].

Chez le sujet adulte présentant un syndrome grippal dû à une infection par un virus influenza, il a été montré que des troubles gastrointestinaux étaient présents chez 21 à 29% d'entre eux [26, 59]. La présence d'ARN viral dans les selles pourrait varier de 25 à 46%, selon des études menées essentiellement auprès de patients adultes hospitalisés pour une grippe grave [28, 29, 60-63]. Dans certaines études, les virus ont pu être isolés et mis en culture, soulevant la question de leur éventuelle viabilité et donc d'une possible transmission oro-fécale [27, 60]. La physiopathologie de ces manifestations digestive reste mal connue. Un rôle direct du virus influenza sur le tube digestif a été évoqué, mais reste débatu [61].

Afin de se fixer puis de pénétrer dans les cellules humaines, via la glycoprotéine de surface hémagglutinine (HA), les virus influenza saisonniers humains utilisent préférentiellement un type de récepteurs cellulaires, le SA α 2,6-Gal (acide sialique couplé à

du galactose en position 2-6). Alors que ce récepteur est bien présent à la surface des cellules épithéliales du système respiratoire, on trouve un autre récepteur sur les cellules épithéliales du tube digestif, le SA α 2,3-Gal [61, 62]. Des travaux récents ont montré que les virus influenza A(H1N1)pdm2009 et A(H5N1) avaient la capacité à se fixer au récepteur SA α 2,3-Gal [61]. De plus, des études menées in vitro ont mis en évidence la possibilité pour les deux types de récepteurs de s'exprimer à la surface des cellules épithéliales différenciées de l'intestin [64-66]. Il a été également montré que certaines mutations dans la structure génétique de l'HA pouvaient faire varier la capacité à se fixer à ces différents récepteurs [67].

Une autre hypothèse pour expliquer la détection de virus influenza dans les selles pourrait simplement être la déglutition de sécrétions nasopharyngées contenant du virus influenza, ou encore la diffusion digestive du virus par voie hématogène, à travers les cellules dendritiques ou les macrophages infectés dans les cas de grippe graves caractérisées par une charge virale élevée [68].

Ainsi, les données disponibles montrent que le virus de la grippe peut être détecté dans les selles de patients grippés, sans qu'il soit possible de distinguer entre circulation passive du virus et infection avérée du tube digestif par le virus. Qu'en est-il de la détection de virus influenza chez des patients présentant exclusivement des signes digestifs ? Le travail présenté dans la section 4 de cette thèse a cherché à répondre à cette question.

2.4 Cycle de transmission des virus entériques

La transmission interhumaine est le mode principal de transmission des DA hivernales. De nombreuses épidémies par transmission de personne à personne ont été rapportées dans des hôpitaux, des services de long séjour et des maisons de retraite, et également en centres de séjour de vacances comme des hôtels et des croisières. La transmission par les mains du personnel joue alors un rôle important, de même qu'une contamination persistante de l'environnement en particulier pour les norovirus [69].

Le mode de transmission des virus entériques est essentiellement de type oro-fécal, direct par les mains, ou indirect par les surfaces ou les objets, aliments ou eaux souillés. Si la transmission par les sécrétions pharyngées n'a jamais été démontrée, la transmission par aérosols à partir des matières fécales, de vomissements, ou de linges contaminés est possible. Elle est favorisée par l'abondance des particules virales dans les selles en phase aiguë de la maladie, un taux d'attaque élevé, un taux de portage prolongé dans les selles, jusqu'à près de 3 semaines, et une grande résistance des virus, qui gardent notamment leur pouvoir infectieux sur les surfaces sèches et les mains [11]. Ainsi, concernant le rotavirus, plus de 15% d'une charge virale déposée sur les doigts peut encore être transmise après 20 minutes [70].

2.5 La surveillance des diarrhées aiguës en médecine générale en France

Le réseau Sentinelles est un réseau d'environ 1300 médecins généralistes libéraux (MGL) (soit 2,2% de la totalité des MGL en France métropolitaine), volontaires, répartis sur le territoire métropolitain français. Il est coordonné par l'équipe "Surveillance et Modélisation des maladies transmissibles" de l'Institut Pierre Louis d'Epidémiologie et de Santé Publique (UMR S 1136, anciennement UMR-S 707) de l'Inserm et de l'Université Pierre et Marie Curie, en collaboration avec l'Institut de veille sanitaire (InVS) (http://www.sentiweb.fr). Il permet la constitution de grandes bases de données sur plusieurs maladies, dont les diarrhées aiguës, avec la description de cas individuels vus en consultation de médecine générale, à des fins de veille sanitaire et de recherche.

Chaque semaine, depuis 1990, les médecins Sentinelles transmettent via Internet le nombre de patients ayant consulté, sur une période donnée, pour une DA répondant à une définition de cas clinique : « Diarrhée aiguë récente (au moins 3 selles liquides ou molles par jour datant de moins de 14 jours) motivant la consultation ». Il est alors possible d'estimer le taux d'incidence hebdomadaire et de suivre son évolution dans le temps et dans l'espace. Pour estimer le taux d'incidence hebdomadaire ou annuel national, le nombre moyen de cas par médecin Sentinelles (normalisé en fonction de leur participation et leur répartition géographique) est multiplié par le nombre total de médecins généralistes en France, et le résultat est ensuite divisé par la population française pour obtenir un taux d'incidence [7, 8].

Bien que le réseau Sentinelles ne prévoie pas de recherche étiologique chez les cas rapportés, aucune « fausse alerte » épidémique n'a été donnée en 20 ans de surveillance, à

partir de cette surveillance clinique. Chaque année, une épidémie hivernale est détectée, liée à l'augmentation de la circulation des virus entériques, principalement les norovirus et le rotavirus de groupe A [10, 71, 72]. Lors de ces épidémies saisonnières, l'incidence est alors en moyenne de 1,4 millions de personnes (min : 188 000 ; max : 3,6 millions). Quant à la durée des épidémies, elle varie de 1 à 18 semaines, avec une durée moyenne de 7 semaines. La date moyenne de début des épidémies est fin décembre et la date moyenne de fin d'épidémie est mi-février. Quant aux personnes touchées, environ 13% ont moins de 5 ans, près de la moitié est âgée de 15-59 ans et 10% ont 60 ans ou plus. L'âge médian observé chaque année est proche de 25 ans et le sex-ratio très proche de 1 (bilans annuels du réseau Sentinelles).

Le travail présenté dans la section 3 de la tèse s'est appuyé sur les données issues de la surveillance des DA par le réseau Sentinelles Inserm-UPMC.

2.6 Les facteurs de risque associés à la survenue d'une diarrhée aiguë en médecine générale

Très peu d'études, uniquement menées en France et en Europe, se sont intéressées aux facteurs de risque associés à la survenue d'une DA.

Une première étude avait déjà été conduite en France avec les médecins généralistes du réseau Sentinelles Inserm-UPMC durant l'hiver 1995-1996 [12]. Dans ce travail, aucune documentation microbiologique n'a été effectuée chez les patients présentant une DA et l'analyse ne portait pas spécifiquement sur la population adulte. Ce travail a permis de montrer que le risque de survenue d'une DA lors des épidémies hivernales était significativement augmenté chez les personnes :

- Ayant eu un contact récent avec une personne atteinte de DA, que ce soit :
  - au sein du foyer familial : Odds Ratio OR = 5,0 ; IC 95% = [3,4 – 7,3] ;
  - sur le lieu de travail : OR = 3,1 ; IC 95% = [1,6 – 6,3] ;
  - ou autre lieu : OR = 2,7 ; IC 95% = [1,2 – 5,8] ;
- Vivant avec des enfants âgés de deux ans et moins : OR = 1,6 ; IC 95% = [1,1 – 2,4]) ;
- Ayant reçu récemment un traitement par :
  - Pénicilline : OR = 1,9 ; IC 95% = [1,1 – 3,3] ;
  - Ou Céphalosporine : OR = 2,5 ; IC 95% = [1,1 – 5,9].

Le risque n'était en revanche pas significativement augmenté chez les personnes ayant récemment consommé des huîtres crues (OR = 1,1 ; IC 95% = [0,9 – 1,4] ou d'autres fruits de

mer ou encore chez les personnes qui ont régulièrement consommé de l'eau du robinet plutôt que de l'eau en bouteille (OR = 0.8 ; IC 95% = [0,6 – 1,1].

Une seconde étude a été menée en France, toujours auprès des médecins généralistes du réseau Sentinelles Inserm-UPMC, durant l'été 1996 [73]. Le risque de survenue d'une DA était significativement augmenté chez les personnes :

- Vivant loin de leur résidence principale : OR = 3,0 ; IC 95% = [1,6 – 5,7] ;
- Ayant été en contact avec un cas de DA : OR = 2,0 ; IC 95% = [1,3 – 3,1].

A notre connaissance, une seule étude menée en 1999 aux Pays-Bas en milieu communautaire a cherché à identifier les facteurs de risque des gastroentérites d'origine virale, et plus précisément des infections à norovirus, sapovirus et rotavirus A [13]. Les facteurs de risque significativement associés aux gastroentérites à norovirus étaient alors :

- La présence d'au moins 2 membres du foyer atteints d'une gastroentérite : OR = 10,9 ; IC 95% = [2,0 – 60,5] ;
- Le contact en dehors du foyer avec un cas de gastroentérite : OR = 12,7 ; IC 95% = [3,1 – 51,8].

Dans cette même étude, il a été montré que les facteurs de risque significativement associés aux gastroentérites à rotavirus du groupe A étaient :

- Le contact en dehors du foyer avec un cas de gastroentérite : OR = 12,9 ; IC 95% = [1,2 – 133,6] ;
- Le manque d'hygiène lors de la préparation des repas : OR = 1,5 ; IC 95% = [1,1 – 2,1].

Toutefois, 86-92% des sujets inclus dans cette étude étaient âgés de moins de 10 ans et, ici encore, aucune analyse ne s'intéressait spécifiquement aux adultes.

3. Diarrhée aiguë chez les adultes consultant un médecin généraliste en France durant l'hiver : incidence, caractéristiques cliniques, prise en charge et facteurs de risque.

3.1 Contexte

Quelques études françaises et européennes ont cherché à étudier l'étiologie virale ou les facteurs de risque associés à ces épidémies hivernales détectées en médecine générale mais aucune ne portait spécifiquement sur une population adulte. Chaque année, une épidémie hivernale de DA est détectée par le réseau Sentinelles, touchant en moyenne 1,4 millions de personnes, parmi lesquelles près de la moitié est âgée de 15-59 ans et 10% ont 60 ans ou plus. Cette surveillance nationale ne prévoit pas de recherche étiologique.

3.2 Objectif

L'objectif de l'article présenté ci-dessous était de déterminer l'incidence de la DA chez l'adulte consultant un médecin généraliste en période épidémique hivernale ; mais aussi de décrire les caractéristiques cliniques de la DA d'origine virale, la façon dont les médecins généralistes français la prennent en charge et enfin d'identifier les facteurs de risque susceptibles d'être associés à leur survenue.

3.3 Méthode

L'incidence de la DA chez l'adulte a été estimée à partir des données recueillies en continu par le réseau Sentinelles pendant deux hivers consécutifs (de Décembre 2010 à Avril 2011 et de Décembre 2011 to Avril 2012). Durant ces deux hivers, un échantillon de médecins Sentinelles a inclus des patients adultes qui se présentaient en consultation pour une DA. Les patients devaient alors compléter un questionnaire et effectuer un prélèvement de selles pour l'investigation virologique qui consistait à rechercher les virus entériques suivants : astrovirus, rotavirus du groupe A, adenovirus entérique humain, et norovirus des génogroupes I et II. Des patients témoins appariés sur l'âge et le sexe ont également été inclus, ce qui a permis d'effectuer une analyse cas–témoins afin de déterminer les facteurs de risque de la DA virale.

3.4 Résultats et discussion

Durant les deux hivers étudiés, l'incidence moyenne de la DA de l'adulte consultant un médecin généraliste a été estimée à 3 158 pour 100 000 adultes français (IC 95% [2 321 – 3 997]). Le signe clinique le plus rapporté était la douleur abdominale (91,1%), la diarrhée aqueuse (88,5%), et la nausée (83,3%). Les médecins généralistes ont prescrit un traitement à 95% des patients inclus pour une DA, et 80% des patients diarrhéiques qui étaient en activité professionnelle ont bénéficié d'un arrêt de travail. L'analyse virologique des prélèvements de selles a permis de détecter au moins un des virus entériques recherchés chez 65% des patients diarrhéiques (IC 95% [57 – 73]), le virus le plus souvent retrouvé étant le norovirus (49%). Les facteurs de risque significativement associés à la survenue d'une DA virale étaient le contact avec une personne qui avait souffert d'une DA dans les 7 derniers jours, que ce soit au

sein du foyer familial ou à l'extérieur, et le fait d'avoir une activité professionnelle (ou d'être étudiant).

Ainsi, la DA hivernale est une maladie fréquente chez l'adulte et le norovirus en est le plus souvent la cause. Aucun facteur de risque lié à un comportement individuel sur lequel on puisse agir n'a été identifié, sinon le contact avec une personne malade. Ainsi, à ce jour, le renforcement de l'éducation des patients concernant les règles d'hygiène en cas de contact constituerait la seule façon de limiter le poids de cette maladie.

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RESEARCH ARTICLE Open Access

Acute diarrhea in adults consulting a general practitioner in France during winter: incidence, clinical characteristics, management and risk factors

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Abstract

Background: Data describing the epidemiology and management of viral acute diarrhea (AD) in adults are scant. The objective of this study was to identify the incidence, clinical characteristics, management and risk factors of winter viral AD in adults.

Methods: The incidence of AD in adults during two consecutive winters (from December 2010 to April 2011 and from December 2011 to April 2012) was estimated from the French *Sentinelles* network. During these two winters, a subset of *Sentinelles* general practitioners (GPs) identified and included adult patients who presented with AD and who filled out a questionnaire and returned a stool specimen for virological examination. All stool specimens were tested for astrovirus, group A rotavirus, human enteric adenovirus, and norovirus of genogroup I and genogroup II. Age- and sex-matched controls were included to permit a case–control analysis with the aim of identifying risk factors for viral AD.

Results: During the studied winters, the average incidence of AD in adults was estimated to be 3,158 per 100,000 French adults (95% CI [2,321 – 3,997]). The most reported clinical signs were abdominal pain (91.1%), watery diarrhea (88.5%), and nausea (83.3%). GPs prescribed a treatment in 95% of the patients with AD, and 80% of the working patients with AD could not go to work. Stool examinations were positive for at least one enteric virus in 65% (95% CI [57 – 73]) of patients with AD with a predominance of noroviruses (49%). Having been in contact with a person who has suffered from AD in the last 7 days, whether within or outside the household, and having a job (or being a student) were risk factors significantly associated with acquiring viral AD.

Conclusions: During the winter, AD of viral origin is a frequent disease in adults, and noroviruses are most often the cause. No preventable risk factor was identified other than contact with a person with AD. Thus, at the present time, reinforcement of education related to hand hygiene remains the only way to reduce the burden of disease.

Keywords: Diarrhea, General practice, Adults, Surveillance, Gastroenteritis, Norovirus, Rotavirus

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Background

In industrialized countries, acute diarrhea (AD) is a major cause of morbidity and medical expenses, particularly in vulnerable populations, such as elderly patients who are more often hospitalized, stay in the hospital longer and die more often than younger individuals when AD occurs [1]. Infectious AD can be caused by various microbiological pathogens such as bacteria, parasites or viruses. AD occurs year-round but exhibits a pronounced winter peak, related to an increase in AD of a viral origin, mainly due to noroviruses and group A rotavirus infections [2-4].

Few studies have described the epidemiology and management of viral AD in adults during the winter. During the winter of 1998–1999 in France, human caliciviruses were shown to be the most frequently encountered viruses in 16- to 65-year-old patients consulting a general practitioner (GP) for AD, while group A rotavirus predominated in patients 65 years of age and older [2]. During the 1995–1996 winter, risk factors shown to be associated with AD in France included contact with a person with AD, living with a child ≤2 years of age, and recent treatment with oral penicillin or cephalosporin [5]. However, in this study, microbiological investigations were not required, and the results were presented for all age groups and not specifically for adults. In the Netherlands, hand hygiene and contact with a sick person were identified as risk factors for viral gastroenteritis related to caliciviruses and group A rotavirus infections, but approximately 90% of the included patients were <10 years of age [6]. The management of viral AD in general practice was studied for rotavirus infections in children [7], but to our knowledge, such data are not available for viral AD occurring in adults.

Thus, data describing the epidemiology and management of viral AD in adults seen in general practice are scant. The objective of this study was to identify the clinical characteristics, management and risk factors associated with the occurrence of viral AD in French adults consulting a GP.

Methods

Study design

In France, continuous surveillance of AD is conducted by the French *Sentinelles* GPs network (www.sentiweb.fr) [8,9]. *Sentinelles* GPs' characteristics, such as regional distribution, proportion in rural practice, type of practice and types of main clinical skills, are comparable to those of all French GPs [10].

The study was conducted over two consecutive winters from the 49th week of 2010 (2010w49) to 2011w17 and then from 2011w49 to 2012w17.

The *Sentinelles* GPs reported (via the Internet) information regarding all adult individuals (≥18 years old) presenting with AD, which was defined as "at least 3 daily watery (or nearly so) stools, less than 14 days". The age and sex of the patients were documented.

A sample of *Sentinelles* GPs participated in a complementary survey with the aim of investigating clinical characteristics, virology, and management of AD occurring in adults. They were asked to recruit one AD case per week. To ensure that the selection of patients remained random, the GP had to include the first patient seen in consultation and who met the inclusion criteria in that particular week. Patients with inflammatory bowel disease and patients with an obvious non-viral etiology of diarrhea (traveler's diarrhea, recent use of antibiotics, colchicine, non-steroidal anti-inflammatory drugs or laxatives, or recent administration of chemotherapy or radiotherapy) were excluded.

Sentinelles GPs were also asked to include one age- and sex-matched patient per AD case for a nested case–control study. The study's aim was to identify the risk factors associated with the occurrence of viral AD. This matched individual presented just after the AD case for a non-gastrointestinal disease and did not report any gastrointestinal symptoms during the month preceding the consultation.

The GPs completed and sent a case report form for all patients included in the complementary survey by postal mail. The case report included collected data on gender, age and potential risk factors. The studied risk factors were factors related to lifestyle (professional status, educational level, presence in the household of children ≤2 years of age, contact with pets or farm animals, hand hygiene, suffering from a chronic disease), and exposure during the last 7 days (contact with persons with AD in and/or outside the household; having eaten an unusual meal; consumption of tap water, oysters, mussels or shellfish; having used public transport; and/or having gone to a swimming pool). Data on reported symptoms, medications, days of missed work, additional medical examinations, or required hospitalizations were also collected for each AD case.

Patients included in the complementary survey were asked to collect and send stool specimens by postal mail in triple packaging (according to the United Nations class 6.2 specifications). They were also asked to return a follow-up questionnaire the week after enrollment to indicate the duration of symptoms (AD patients) and to ascertain whether an AD had occurred or not (non-AD patients).

Virological analysis

All stool specimens were tested for four enteric viral pathogens (astrovirus, group A rotavirus, human enteric adenovirus, and norovirus of genogroup I - NoVGI - and genogroup II - NoVGII) using the Seeplex® Diarrhea-V ACE assay (Seegene) according to the manufacturer's instructions. A recent study showed that the Seeplex® Diarrhea-V assay is a sensitive, specific, convenient and reliable method to simultaneously detect several viral

pathogens found directly in stool specimens from patients with gastroenteritis [11].

Statistical analysis

The AD cases reported via the Internet by the *Sentinelles* GPs allowed the estimation of winter incidence rates for mainland France by age group (18 – 39 years, 40 – 59 years, 60 – 79 years and ≥80 years). The winter incidence rate was calculated as follows: the average number of cases notified by *Sentinelles* GPs (adjusted for participation and geographic distribution) was multiplied by the total number of private GPs practicing in France and then divided by the French population [12,13]. Confidence intervals were estimated by assuming that the distribution of the number of reported cases followed a Poisson distribution.

The data collected during the complementary survey were entered twice to ensure consistency. Data analysis was performed using STATA (version 11.2, StataCorp LP, Texas, USA). Quantitative variables were described by using medians [interquartile range IQ] and means ± standard deviations and were compared by the Wilcoxon test. Qualitative variables were described by using proportions and compared using a chi-square or Fisher's exact test if the chi-square test were not applicable; the results were presented as odds ratio with 95% confidence intervals (OR [95% CI]).

For the nested case–control study, a *case* was a patient with AD in which at least one enteric virus was identified; a *control* was a matched patient without AD in which no enteric virus was identified. Univariate analyses were conducted using the McNemar test. A conditional logistic regression model was used to study the independent effects of risk factors that were associated in the univariate analyses (p-value of <0.20). Variables for the model were chosen through automatic backwards selection using a significance level of 0.05. Assuming a control-to-case ratio of 1:1, an exposure rate of 15% among controls, a two-tailed level of significance of 5% and a power level of 80%, 87 cases were needed to detect a minimal odds ratio (OR) of 3.

Ethics statement

Oral consent was obtained from the patients at the time of inclusion for their participation in the study and for the publication of the clinical and virological data.

The Hospital Ethics Committee (CHU Saint-Antoine, Paris, France) approved the study.

Results

Incidence rates in general practice

During the two winters studied, 370 GPs participated in the electronic surveillance, and 10,415 AD cases were reported. Figure 1 shows the weekly incidence rates, and Table 1 presents the winter incidences and incidence rates by age groups. The median age of adult patients seen by the *Sentinelles* GPs over the two consecutive winters was 37 years (IQ = [27 – 52]) and 36 years (IQ = [27 – 51]), respectively; the proportion of men was 46.2% and 45.4% over the two winters, respectively.

Clinical characteristics, management and virology

Among the 100 *Sentinelles* GPs who agreed to participate in the complementary survey, 65 enrolled 192 adult patients who were seen for AD. Their median age was 36 years (IQ = [28 – 52]), and 111 (57.8%) were men. The reported clinical signs are presented in Table 2.

Overall, 183 (95.3%) patients received a drugs prescription, which were mostly intestinal antisecretory drugs (N = 98, 53.6%), antiemetics (N = 96, 52.4%), antispasmodics (N = 72, 39.3%), intestinal adsorbents (N = 65, 35.5%), analgesics/antipyretics (N = 54, 29.5%) and regulators of intestinal motility (N = 54, 29.5%). Among

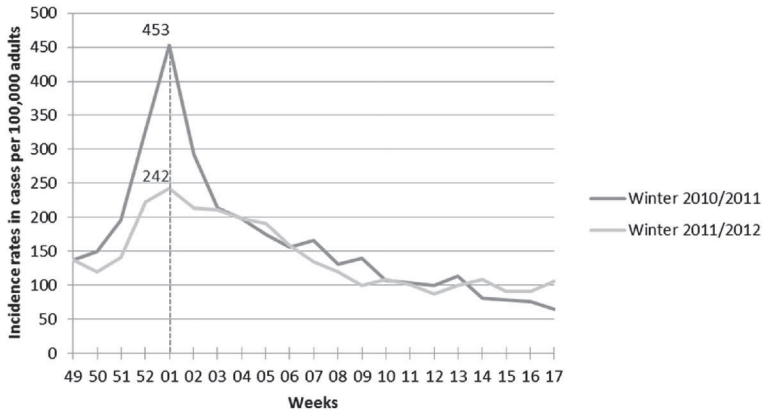

Figure 1 Weekly incidence rates of acute diarrhea in adults (≥18 years old) consulting a GP in France (estimated using the French *Sentinelles* GPs network).

Table 1 Incidence rates of acute diarrhea in France by age group per 100,000 cases estimated by the French *Sentinelles* GPs network during two consecutive winters

	Winter 2010/2011		Winter 2011/2012	
	2010w49 – 2011w17		2011w49 – 2012w17	
	Incidence [IC 95%]	Incidence rate per 100,000 [IC 95%]	Incidence [IC 95%]	Incidence rate per 100,000 [IC 95%]
18 years of age and older	1,691,959 [1,287,372 – 2,096,954]	3,471 [2,641 – 4,302]	1,472,351 [1,060,343 – 1,885,771]	3,002 [2,162 – 3,845]
18 – 39 years	953,943 [784,323 – 1,123,563]	5,388 [4,430 – 6,346]	859,608 [678,755 – 1,040,461]	4,920 [3,885 – 5,956]
40 – 59 years	470,312 [351,703 – 588,921]	2,771 [2,072 – 3,470]	390,424 [273,691 – 5 07,157]	2,290[1,606 – 2,975]
60 – 79 years	194,142 [122,101 – 266,248]	1,761 [1,107 – 2,415]	162,982 [90,142 – 235,822]	1,420 [785 – 2,055]
≥ *80 years*	73,562 [29,245 – 118,222]	2,200 [875 – 3,536]	59,337 [17,755 – 102,331]	1,668 [499 – 2,876]

the 146 patients who reported having a job, 116 (79.5%) benefited from stopping work for a median duration of 3 days (IQ = [2 – 3]), regardless of gender.

Stool samples from 145 patients with AD (75.5%) were returned. The median age of those patients was 37.5 years (IQ = [30 – 54]), and 80 were men (55.2%). Stools tested positive for at least one of the four enteric viruses investigated in 94 cases (65%). The detailed results from the virological investigation are presented in Table 3. Among the patients with AD, the reported clinical signs did not differ between adults with a virus in the stool sample and those with no virus found in the stool exam, neither in frequency nor in severity (Table 4). Thus, the management of patients with AD who tested positive for a virus was not different from the management of patients who tested negative (data not shown). None of the cases required hospitalization.

Table 2 Reported clinical signs in adult patients consulting a GP for acute diarrhea (complementary survey)

	Patients with acute diarrhea (N = 192) (%)
Average time before consultation ± sd (days)	1.6 ± 1.8
Average duration of diarrhea ± sd (days)	2.0 ± 1.8
Average number of stools in the last 24 h ± sd	5.7 ± 2.8
Average max. number of stools per day ± sd	6.0 ± 2.9
Mucous diarrhea	29 (15.1%)
Bloody diarrhea	2 (1.0%)
Watery diarrhea	170 (88.5%)
Abdominal pain	175 (91.1%)
Nausea	160 (83.3%)
Vomiting	119 (62.0%)
Average duration ± sd (days)	*1.0 ± 1.1*
Fever	83 (43.2%)
Average body temperature ± sd (°C)	*38.4 ± 0.5*
Average duration ± sd (days)	*1.4 ± 1.3*
Dehydration	8 (4.2%)
Other symptoms	8 (4.2%)

Risk factors for viral AD

The GPs enrolled 101 matched individuals for the nested case–control study. Among them, 95 patients mailed back a stool specimen. Of the stools examined, 4 tested positive (4.2%) for one enteric virus (NoVGII) and were excluded from the case–control study. Thus, 91 pairs (51 male and 40 female) were included in the analysis. The median age was 36 years (IQ = [28 – 50]) for the cases and 37 years (IQ = [29 – 53]) for the controls. Viral acute diarrheas were independently associated with having been in contact with a person who has suffered from an AD in the last 7 days, either within or outside the household, and having a job or student (Table 5). The contact of cases with sick people

Table 3 Results from the virological investigation of adult patients consulting a general practitioner for acute diarrhea in France from week 2010w49 to week 2011w17 and from week 2011w49 to week 2012w17 (complementary survey)

Viruses detected	Patients with acute diarrhea (N = 145) (%)
Norovirus GII	59 (40.7)
Norovirus GI	17 (11.7)
Astrovirus	5 (3.5)
Rotavirus	2 (1.4)
Adenovirus 40/41	0 (0.0)
Coinfections	11 (8.0)
Norovirus GI + GII	*5 (3.5)*
Norovirus GI + Rotavirus A	*1 (0.7)*
Norovirus GI + Astrovirus	*1 (0.7)*
Norovirus GII + Astrovirus	*4 (2.8)*
At least one virus detected	94 (64.8)
No virus detected	51 (35.2)

Table 4 Reported clinical signs in adult patients consulting a GP for acute diarrhea in virus-positive and virus-negative stool samples (complementary survey)

	Patients with acute diarrhea		
	At least one virus detected (N = 94) (%)	No virus detected (N = 51) (%)	p-value*
Average age ± sd (years)**	40.4 ± 15.1	44.3 ± 17.8	0.16
Men**	52 (55.9%)	28 (57.1%)	0.89
Average time before consultation ± sd (days)	1.6 ± 1.9	1.7 ± 1.9	0.92
Average duration of diarrhea ± sd (days)	1.6 ± 1.5	2.1 ± 1.8	0.23
Average number of stools in the last 24 h ± sd	5.4 ± 2.7	6.3 ± 3.3	0.09
Average max. number of stools per day ± sd	5.7 ± 2.5	6.5 ± 3.3	0.13
Mucous diarrhea	10 (11.0%)	8 (17.4%)	0.30
Bloody diarrhea	1 (1.1%)	1 (2.2%)	0.63
Watery diarrhea	83 (91.2%)	39 (84.8%)	0.26
Abdominal pain	85 (93.4%)	42 (91.3%)	0.66
Nausea	77 (82.8%)	36 (78.3%)	0.52
Vomiting	61 (66.3%)	24 (52.2%)	0.11
Average duration ± sd (days)	*0.8 ± 0.9*	*1.3 ± 1.4*	*0.12*
Fever	42 (46.2%)	15 (33.3%)	0.16
Average body temperature ± sd (°C)	*38.3 ± 0.4*	*38.5 ± 0.7*	*0.42*
Average duration ± sd (days)	*1.2 ± 1.2*	*1.7 ± 1.7*	*0.30*
Dehydration	3 (3.3%)	1 (2.2%)	0.71
Other symptoms	5 (5.5%)	1 (2.2%)	0.39

*Logistic regression: adjustment for age and sex.
**Not adjusted for age and sex.

Table 5 Factors associated with viral acute diarrhea (cases) in 91 pairs of adult patients consulting a GP

	Cases (N = 91) (%)	Controls (N = 91) (%)	OR uni [95% CI] (p-value)*	OR multi [95% CI] (p-value)*
Professional status (employed or student/non employed or retired)	80 (87.9%)	67 (73.6%)	4.25 [1.43 – 12.63] (0.01)	4.10 [1.27 – 13.21] (0.02)
Educational level (high school and above/middle school)	80 (87.9%)	69 (75.8%)	2.83 [1.12 – 7.19] (0.03)	2.37 [0.86 – 6.57] (0.10)
Children ≤2 years in household (yes/no)	20 (22.0%)	9 (9.9%)	2.57 [1.07 – 6.16] (0.03)	1.87 [0.69 – 5.09] (0.22)
Being in contact with pets or farm animals (yes/no)	45 (49.5%)	48 (52.8%)	0.80 [0.48 – 1.58] (0.65)	n.i.
Washing hands before cooking (never-sometimes/often-always)	10 (11.6%)	13 (16.1%)	0.58 [0.23 – 1.48] (0.26)	n.i.
Washing hands after using the toilet (never-sometimes/often-always)	7 (7.7%)	8 (8.8%)	0.86 [0.29 – 2.55] (0.78)	n.i.
Washing hands after attending public places (never-sometimes/often-always)	45 (52.3%)	35 (40.7%)	1.50 [0.76 – 2.95] (0.24)	n.i.
Suffering from a chronic disease (yes/no)	28 (30.8%)	29 (31.9%)	0.94 [0.48 – 1.86] (0.86)	n.i.
Contact with persons with AD in the household (yes/no)	31 (34.1%)	10 (11.0%)	5.20 [2.00 – 13.50] (0.01)	4.18 [1.54 – 11.33] (<0.01)
Contact with persons with AD outside household (yes/no)	22 (24.2%)	9 (9.9%)	3.60 [1.34 – 9.70] (0.01)	3.31 [1.03 – 10.63] (0.04)
Having eaten an unusual meal (yes/no)	33 (36.3%)	28 (30.8%)	1.39 [0.68 – 2.83] (0.37)	n.i.
Having consumed oysters, mussels, or shellfish (yes/no)	27 (29.7%)	29 (31.9%)	0.90 [0.48 – 1.70] (0.75)	n.i.
Having consumed tap water (yes/no)	69 (75.8%)	73 (80.2)	0.76 [0.37 – 1.58] (0.47)	n.i.
Having used public transportation (yes/no)	20 (22.0%)	14 (15.4%)	2.00 [0.75 – 5.33] (0.17)	2.57 [0.71 – 9.39] (0.15)
Going to a public swimming pool (yes/no)	4 (4.4%)	5 (5.5%)	0.80 [0.22 – 2.98] (0.74)	n.i.

*Conditional logistic regression: matched for age and sex.
OR: odds-ratio; Uni: univariate; Multi: multivariate; CI: confidence interval; n.i: not included in the multivariate model.

outside the household had taken place either at work (59%) or other place (41%). The median duration between the contact with a sick person and the onset of the symptoms was 2 days (IQ = [1 – 4]).

Discussion

This study presents the first analysis of the global burden of AD in adults who consulted a GP in France. Winter incidences, clinical characteristics, virological investigation, management and risk factors for viral AD were investigated.

Incidence rates in general practice

During the two studied winters, 3,471 and 3,002 cases per 100,000 French adults consulted a GP for an AD in winters 2010/2011 and 2011/2012, respectively. The data on AD incidences vary from country to country because of differences in case definition, surveillance systems, and/or the period of study. In France, a telephone survey estimated the incidence rate of acute gastroenteritis at 0.33 cases/person-year [14]. In the Netherlands, a population-based study conducted in 1998/1999 estimated that the gastroenteritis incidence was 283 per 1,000 person-years [15]. In both studies, the incidence rate peaked in children and then decreased in adults.

Clinical characteristics, management and virology

In this study, more than 80.0% of patients reported abdominal pain, watery diarrhea, and/or nausea, while vomiting and fever were reported by 62.0% and 43% of patients, respectively. These results are in agreement with other French studies [2,14].

Adults are less likely to consult a GP for gastroenteritis compared with children, as it remains a self-limiting disease [14]. Patients with more severe symptoms are more prone to consulting a GP, which is illustrated by the fact that 80% of working adult cases had to stop working. Although no cases required hospitalization, the economic burden of AD related to outpatient visits could be significant, because the average annual incidence of AD in adults is 1 million cases (www.sentiweb.fr). In addition to the cost of outpatient visits, medical treatment and missed work days increase the heavy burden of viral AD cost in adults. Indeed, 95% of the patients in this study received a drug prescription. The management of AD is most likely amenable to a more appropriate drug prescription in France. For example, antiemetics are prescribed in a majority of cases, whereas their efficacy in this indication has never been validated, and their side effects may be serious [16].

The feces samples were not screened to rule out bacterial and parasitic infections. However, we included patients in whom there was a very high suspicion of viral diarrhea (and a very low risk of bacterial or parasitic infection), as inclusions were done during winter and cases with an obvious non-viral etiology of diarrhea were excluded. During the winter, viral AD is predominant, but the reason is not clear. Hypotheses for these findings include that the clustering of people indoors during the winter months facilitates person-to-person transmission and the enhanced persistence of noroviruses at low temperatures [17]. Noroviruses have been described as the leading cause of winter AD [18], and the GII genogroup strains have been previously shown to predominate during winter, although the reason for this remains unclear [19]. In this study, the proportion of adult patients with AD who were positive for at least one enteric virus (65%) was higher than in previous studies in general practice performed in France or Europe (15-39%) [2-4]. However, unlike these studies, the aim of our study was to generate a sample of patients who were positive for a virus; thus, patients with obvious non-viral diarrhea were excluded. In the patients included here, the clinical characteristics of AD, and thus its management, were not different for adults with or without an identified virus in the stool. It is possible that a study with more statistical power would have identified some clinical differences, such as more frequent occurrence of vomiting [20].

Risk factors for viral AD

Being previously in contact with an individual presenting with AD was identified as a risk factor for developing AD. Norovirus and rotavirus are among the most communicable pathogens responsible for AD. Experimentally, an inoculum as low as 500 (and even less) viable organisms is sufficient to establish an infection, and the virus is environmentally stable [20]. Thus, enteric viruses have a high potential for person-to-person spread. The increased risk in people who have had a contact with a sick person in the household is consistent with this already well-known mode of transmission [21]. In 1995 and 1996 in France, Lettrillard et al. [5] showed that the risk of developing AD was 5 times higher in patients who had been in contact with a person suffering from AD in their household. However, the study included patients whose AD etiology was unknown (no stool sample). Studies in Germany [4] and the Netherlands [6] have confirmed this observation and estimated adjusted ORs ranging from 1.9 to 12.9 based on viral detection; however, the results of these studies were not stratified by age. In this study, patients who reported having a job and students were significantly more likely to suffer from viral AD than those who were unemployed or retired. Among the studies that have tried to identify the risk factors for acquiring an AD, none have investigated professional status. The result obtained in this study seems quite relevant and suggests that this population has increased contact with sick people, which is the main risk factor for infection. The acquisition of a viral AD may be associated with other factors that were not identified in

this study. For example, De Wit et al. showed that norovirus AD risk was increased in people with poorer hand hygiene (OR = 1.3 [1.0 – 1.7]) [6], which was not observed here. It has also been shown that living with children ≤2 years of age increases the risk of developing AD in the winter, regardless of the children's health status (AD or not) [5]. The association between developing AD and living with children ≤2 years that was identified in our univariate analysis did not persist after adjusting for other variables. No association between viral AD and tap water use, seafood consumption or an unusual meal was found.

Conclusions

During the winter, AD of viral origin is a frequent disease in adults with a significant burden in the population. Noroviruses are mainly responsible for the disease. Other than contact with a person suffering from AD, no other preventable risk factor was identified. Thus, at the present time, education related to hand hygiene remains the only way to reduce the burden of disease.

Abbreviations

AD: Acute Diarrhea; GP: General Practitioner; NoVGI: Norovirus of Genogroup I; NoVGII: Norovirus of Genogroup II; (RT)-PCR: (Reverse Transcription)-Polymerase Chain Reaction; IQ: InterQuartile range; OR: Odds Ratio; 95% CI: 95% Confidence Interval.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CA, JPA, VV, KAB, RCB, NJDS, LV, JA, TB, AF and TH co-conceived the study. CA and TH collected the epidemiological and microbiological data. AF, LV and KAB designed the microbiology experiments. AF performed the microbiology experiments and analyzed and interpreted the data. CA and CS analyzed and interpreted the statistical data. CA, JPA, VV, KAB, RCB, NJDS, LV, JA, CS, TB, AF and TH contributed to writing the paper and approved the final manuscript.

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4. L’investigation simultanée des virus influenza et entériques dans les selles de patients adultes consultant un médecin généraliste pour une diarrhée aiguë

4.1 Contexte

Il existe chaque hiver en France une épidémie de DA et une épidémie de syndromes grippaux. Ces deux épidémies ont le plus souvent lieu à des périodes similaires, posant la question d’une éventuelle interaction entre les virus responsables de chacune de ces épidémies. En effet, les virus entériques sont les principaux virus responsables des DA de l’adulte mais les virus grippaux, comme les virus entériques, peuvent provoquer des troubles gastro-intestinaux, tels que DA, vomissements ou encore douleurs abdominales. On connaît mal l'excrétion fécale de virus de la grippe saisonnière et pandémique. A notre connaissance aucune donnée sur les adultes consultant en médecine générale et aucune donnée comparative avec des contrôles n'ont été publiées.

4.2 Objectif

L’objectif du travail présenté dans l’article ci-dessous était donc de rechercher la présence de virus grippaux dans les selles de patients adultes qui consultaient leur médecin généraliste pour une DA sans complication. L’investigation a également consisté à déterminer la fréquence des coinfections par des virus entériques et grippaux.

4.3 Méthode

Durant l'hiver 2010/2011 (de Décembre 2010 à Avril 2011), un échantillon de médecins Sentinelles a inclus des patients adultes qui se présentaient en consultation pour une DA. Les patients devaient alors compléter un questionnaire et effectuer un prélèvement de selles pour l'investigation virologique. Les virus entériques recherchés étaient les suivants : astrovirus, rotavirus du groupe A, adenovirus entérique humain, et norovirus des génogroupes I et II. Cette investigation virologique était complétée d'une recherche des virus influenza A (virus saisonnier A(H3N2) et virus pandémique A(H1N1)pdm2009) et virus influenza B. Des patients témoins appariés sur l'âge et le sexe ont également été inclus.

4.4 Résultats et discussion

Parmi les 138 cas de DA inclus, 10 (7,2%) ont été testés positifs à un virus influenza alors qu'ils ne présentaient pas de signes respiratoires. Chez 5 d'entre eux (3,6% des patients inclus dans l'étude), un virus influenza était isolé alors que chez les 5 autres un virus entérique était détecté en association avec un virus influenza.

Chez huit des dix patients porteurs de virus influenza dans les selles, il s'agissait d'un virus influenza B, alors que pour les deux autres patients il s'agissait d'un virus influenza A(H1N1)pdm2009 ou A(H3N2).

Aucun virus entérique ni grippal n'a été détecté chez les 93 témoins inclus.

Cette étude a montré que des virus grippaux sont retrouvés dans les selles de patients vus en médecine générale pour une DA de façon non exceptionnelle. Dans la moitié des cas, le virus grippal est associé à un autre virus entérique. Ces résultats soulèvent la question du mécanisme biologique par lequel les virus influenza peuvent être excrétés dans les selles mais

également sur la possible infection du tube digestif et, par conséquent, d'une possible transmission fécale-orale des virus grippaux. Les réponses qui pourraient être apportées à ces questions permettraient de mieux contrôler et prévenir les épidémies de grippe et de DA.

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Simultaneous investigation of influenza and enteric viruses in the stools of adult patients consulting in general practice for acute diarrhea

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Abstract

Background: Gastrointestinal symptoms are not an uncommon manifestation of an influenza virus infection. In the present study, we aimed to investigate the presence of influenza viruses in the stools of adult patients consulting their general practitioner for uncomplicated acute diarrhea (AD) and the proportion of concurrent infections by enteric and influenza viruses.

Method: A case-control study was conducted from December 2010 to April 2011. Stool specimens were collected and tested for influenza viruses A (seasonal A/H3N2 and pandemic A/H1N1) and B, and for four enteric viruses (astrovirus, group A rotavirus, human enteric adenovirus, norovirus of genogroups I – NoVGI - and genogroup II - NoVGII).

Results: General practitioners enrolled 138 cases and 93 controls. Of the 138 stool specimens collected, 92 (66.7%) were positive for at least one of the four enteric viruses analysed and 10 (7.2%) tested positive for one influenza virus. None of these 10 influenza positive patients reported respiratory symptoms. In five influenza-positive patients (3.6%), we also detected one enteric virus, with 4 of them being positive for influenza B (2 had co-detection with NoVGI, 1 with NoVGII, and 1 with astrovirus). None of the 93 controls tested positive for one of the enteric and/or other influenza viruses we investigated.

Conclusions: In this study we showed that the simultaneous detection of influenza and enteric viruses is not a rare event. We have also reported, for the first time in general practice, the presence of seasonal and pandemic influenza viruses in the stools of adult patients consulting for uncomplicated AD. A simultaneous investigation of enteric and influenza viruses in patients complaining of gastrointestinal symptoms could be useful for future studies to better identify the agents responsible for AD.

Keywords: Influenza virus, Enteric virus, Stools, Co-infection, General practice

Background

Gastrointestinal (GI) symptoms are not an uncommon manifestation of an influenza virus infection [1-3]. However, little is known about the GI pathogenesis of influenza viruses. It is possible that GI symptoms developed during the clinical course of influenza could either be a part of disease manifestation, due to the side effects of antibiotic treatment, or a co-infection with other diarrheal pathogens. Gastrointestinal manifestation associated with seasonal influenza has been recognised for more than 30 years [4]. During the influenza A epidemic of 1988 in Australia several children developed hemorrhagic gastritis of varying severity after a typical Influenza-like illness (ILI) [5]. Similarly, during the two epidemics in 1973 and 1974, influenza virus B was detected in hospitalised children who had abdominal pain, often severe enough to require differentiation from acute appendicitis, as a dominant symptom [1]. Less severe GI symptoms have been reported to occur in 20-30% of children with an influenza B infection [4,6,7].

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Early epidemiologic study of the pandemic influenza A/H1N1 2009 virus suggested that it produced diarrhea, vomiting, or both, in ≈ 25% of case-patients [8].

However, fecal excretion of pandemic and seasonal influenza viruses has rarely been studied, and the lack of reports of co-infection among influenza and enteric viruses is probably because of reporting bias. Consequently it remains unknown whether co-infection with influenza pathogens in patients with GI symptoms represents rare events. Previous studies reported the detection of seasonal influenza in the stools of pediatric patients presenting concurrent acute diarrhea (AD) and ILI [9], and in the stools of hospitalised and outpatients presenting both GI and respiratory symptoms [10,11]. Influenza viral RNA was also detected in the stools of A/H1N1 2009 positive patients hospitalised due to the progression of acute gastroenteritis [12]. Previous studies showed that the avian influenza A/H5N1 virus can be detected in stools [13], and the presence of this virus was further demonstrated in the biopsy of the small and large intestines of fatal cases [14,15]. Other respiratory viruses have been found in stools, such as respiratory syncytial virus [16], SARS coronavirus [17], adenovirus [18] and bocavirus [19]. But, to our knowledge, there are no studies reporting the detection of Influenza viruses in the stools of adult patients consulting in general practice for acute diarrhea.

In the present study we aimed to investigate the presence of pandemic and seasonal influenza viruses in the stools of General Practitioners' (GPs) adult patients presenting exclusively GI symptoms and the proportion of concurrent infections by enteric and influenza viruses by using a case control design.

Results

Samples collected

General practitioners enrolled 175 adult patients consulting for AD and 101 non diarrheal individuals, but we received stools samples from 138 cases and 93 controls. The two populations (cases and controls) presented similar demographic characteristics: median age of cases was 37 years [28 - 54] versus 39 years [29 - 54] for controls ($p = 0.62$); the proportion of women in the cases group was 45.9% versus 47.3% in the control group ($p = 0.85$).

Virological findings

Of the 138 stool specimens collected, 92 (66.7%) were positive for at least one of the four enteric viruses analysed. Ten (7.2%) tested positive for one influenza virus, eight of them being positive for influenza virus B and two positive for influenza virus A (1 A/H1N12009 and 1 A/H3N2). Five influenza-positive patients (3.6%) showed a co-detection of one enteric virus (3 NoVGI, 1 NoVGII, and 1 astrovirus) (Table 1). None of the 93 controls were positive for either enteric and influenza viruses. Influenza viral concentration ranged from $2.5x10^4$ to $4.2x10^6$ PCR copies per gram of stool (Table 1).

Characteristics of patients

In Table 2 we reported the median age, proportion of females, median duration of the enrolment diarrhea episode, proportion of patients presenting fever, and the median duration of fever after enrolment for 5 groups of patients: the ones who tested positive for both enteric and influenza viruses (n = 5), the ones who were positive only for influenza (n = 5), the sum of the two preceding groups, that is, the ones positive for the influenza virus with or without the co-detection of an enteric virus (n = 10), the ones who were only positive for at least one enteric virus (n = 87), and finally the ones who were negative for both enteric and influenza viruses (n = 41).

Any significant differences, concerning demographical data (age and sex) have been pointed out between the five groups of patients described on Table 2. Concerning the clinical data reported in Table 2, patients with the detection of at least one enteric viruses (n = 87) seem to have a duration of fever after enrolment which is lower than patients who were positive for influenza, with or without co-detection of an enteric virus (n = 10) (OR = 0.49 [0.26-0.91]; $p = 0.02$). This significant difference holds true when we compare the same first group (n = 87) and the group of patients who tested positive for both enteric and influenza viruses (n = 5) (OR = 0.35 [0.13-0.91]; $p = 0.03$). Any significant differences for the duration of fever after enrolment have been highlighted between patients who tested positive for two enteric viruses (n = 11) with respect to patients who were positive for one enteric virus (n = 76) ($p = 0.11$).

None of the 10 influenza positive patients reported any respiratory symptoms.

Two influenza-positive patients declared the duration of enrolment fever episode of six days. The first one was a 24 year old man without underlying conditions and with the duration of enrolment diarrhea episode of 3 days, and who tested positive for the influenza virus A/H3N2 (viral concentration of $2.8x10^4$ PCR copies per gram of stool). The second one was a 54 year old woman without underlying conditions and with the duration of enrolment diarrhea episode of 5 days and who concomitantly tested positive for the influenza B and astrovirus (viral concentration of $4.2x10^6$ PCR copies per gram of stool).

Discussion

The present study found evidence of the presence of seasonal and pandemic influenza viral RNA in 7.2% of adult patients (≥18 years old) consulting their GP for the

Table 1 Demographical, clinical and virological data of the Influenza virus-positive patients

Sex/Age	No of days from onset to stool collection	Mo of collection	Influenza Viral concentration (PCR copies/g stool)	Underlying medical conditions	Gastrointestinal symptoms	Consistency of stool specimen	Duration of the enrolment diarrhea episode (days)	Duration of the enrolment vomiting episode (days)	Duration of fever episode (days)	Supplementary medical examen	Influenza virus	Enteric virus
M/24	2	January	$2.8x10^4$	None reported	Abdominal pain, nausea, vomiting	Watery	3	3	6	None reported	A/H3N2	Negative
F/56	1	February	$3.5x10^6$	Hypertension, osteoporosis, polymyalgia rheumatica	Abdominal pain, nausea	Watery	1	None reported	No fever	None reported	B	Negative
NA/>18	1	January	$2.5x10^4$	None reported	Abdominal pain, nausea, vomiting	Loose	2	3	1	None reported	B	Norovirus GI
F/54	2	January	$4.2x10^6$	None reported	Abdominal pain, nausea, vomiting	Watery	5	2	6	Fiberscope	B	Astrovirus
F/79	0	December	$2.5x10^4$	Hypertension, osteoporosis	Abdominal pain, nausea, vomiting	Watery	1	1	1	None reported	B	Negative
M/22	7	January	$3.2x10^4$	None reported	Abdominal pain, nausea	Watery	N/A	None reported	NA	None reported	B	Norovirus GI
F/19	3	February	$4.0x10^4$	None reported	Abdominal pain, nausea	Watery	1	None reported	0	None reported	B	Negative
F/30	1	January	$2.2x10^5$	Migraine	Abdominal pain, nausea	Watery	1	None reported	No fever	None reported	B	Negative
M/86	1	December	$2.7x10^4$	None reported	Nausea	Watery	2	None reported	No fever	None reported	A/H1N1 2009	Norovirus GI
F/74	1	January	$3.8x10^5$	Hypertension, osteoporosis, asthma	Abdominal pain, nausea, vomiting	Watery	2	1	1	None reported	B	Norovirus GII

Table 2 Demographical and clinical data of patients

Demographical and clinical data	Patients positive to influenza virus			Patients positive to enteric virus only	Patients negative to enteric and influenza viruses
	Patients positive to both influenza and enteric viruses	Patients positive to influenza virus only	Patients positive to at least one influenza virus		
	(n = 5)	(n = 5)	(n = 10)	(n = 87)	(n = 41)
Median age (years) [IQ]*	64 [38 - 80]	30 [24 - 56]	43 [24 - 74]	35 [28 - 50]	44 [32 - 56]
Females (%)	2 (50.0%)	4 (80.0%)	6 (60.0%)	43 (49.4%)	18 (43.9%)
Median duration of the enrolment diarrhea episode (days) [IQ]	2 [2 - 3.5]	1 [1 - 1]	2 [1 - 2]	1 [1 - 2]	2 [1 - 4]
Patients suffering of fever (%)	4 (80.0%)	3 (60.0%)	7 (70.0%)	33 (37.9%)	9 (21.9%)
Median duration of fever after enrollment (days) [IQ]	1 [1 - 6]	1 [0 - 6]	1 [1 - 6]	1 [0 - 2]	1 [1 - 2]

*** [IQ] = [Interquartile range].**

typical and uncomplicated symptoms of AD during the ILI and AD outbreaks in France (http://www.sentiweb.fr). We have also reported the detection of enteric viruses in half of the patients who tested positive for influenza viruses. The most frequent combination was a co-detection with two agents, primarily influenza virus B plus NoVGI.

It is to be noted that in our study the most prevalent Influenza virus was influenza virus B, detected in 8 of 10 stool specimens positive for influenza viruses. These results seem to be in agreement with previous studies about the detection of influenza virus B in patients complaining of GI symptoms. The presence of influenza virus B in gastric mucosa has been previously reported among patients with GI symptoms without concurrent respiratory symptoms [20]. Similar results have been reported among hospitalised children infected with the influenza B virus for which abdominal pain was a dominant symptom, especially in older children [1]. As highlighted by Kaji et al. [21], GI symptoms were significantly more common in adult patients with a positive throat swab for the influenza B virus (GI = 23%), and with respect to the influenza A virus (GI = 6% for A/H3N2 and 4% for A/H1N1). Previously, the influenza B virus has been reported [9] in 81% (17/21) of influenza positive stools of pediatric patients (<6 years of age) with concurrent respiratory and GI symptoms. Interestingly, one of the influenza virus B strains detected among these pediatric patients was viable [9].

In this study we have also reported the detection of A/H3N2 and A/H1N1 2009 viral RNA in the stools of two patients with AD. The detection of the A/H3N2 virus in stool samples has been previously reported in six high-risk influenza adult patients [10] and in three young children [9] reporting ILI and diarrhea. Seasonal influenza viruses detection by RT-PCR in stools has also been reported in very young children presenting with ILI and AD between the ages of 5 weeks and 9 months [7]. Influenza virus A/H1N1 2009 was recovered from 16 (24.6%) stools of A/H1N1 2009 positive patients who were hospitalised due to the progression of acute gastroenteritis [12]. In another study, the authors showed a positive viral culture for A/H1N1 2009 in the stool of four patients presenting the highest viral load [22], suggesting the fecal shedding of viable pandemic viruses.

In this study, the overall proportion of co-detection of influenza and enteric viruses was 3.6%. We detected one enteric virus in 5/10 stool specimens of influenza-positive patients. Among them, four tested positive for the influenza B virus and one enteric virus (2 NoVGI, 1 NoVGII, and 1 astrovirus), and one for influenza A/H1N1 2009 (concomitantly with NoVGI). It is to be noted that although our sample was not large enough to make conclusions that are statistically approved, we can observe that patients who tested positive for both influenza and enteric virus were older (64 years [38-80]) than patients showing a single detection of influenza viruses (30 years [24-56]) and those ones positive for enteric viruses only (35 years [28-50]). To our knowledge, until now a co-detection of influenza viruses and enteric pathogens has rarely been reported. Co-infections between rotavirus and influenza viruses (6 influenza B and 1 influenza A) have been previously reported among 2.2% of hospitalised young children with gastroenteritis [23]. One case of co-infection with influenza A/H3N2 virus and norovirus has been reported in an elderly patient who developed diarrhea since day 3 and passed 3–4 episodes of watery/loose stool per day up to day 13 [24]. In the present study, the duration of fever seems to be shorter among patients who tested positive for at least one enteric virus with respect to patients positive for both enteric and influenza viruses. It is difficult to interpret this result given the low number of influenza-enteric co-detections and the low number of the 'pure' influenza-positive cases.

Finally, the explanation of the presence of seasonal (A and B) and pandemic A/H1N1 2009 influenza viruses RNA in the stools is not clear. As previously known, the avian influenza virus prefers to bind the α-2, 3-sialic acid receptor, while human Influenza viruses frequently bind the α-2, 6-sialic acid receptor. Recent evidence indicates that both types of receptors are expressed on the surfaces of *in vitro* differentiated intestinal epithelial cells [25-27], suggesting that both avian and human influenza viruses have the potential to infect and replicate in human intestinal epithelial cells. Recent data confirmed that human intestinal epithelial cells can be infected by the pandemic (H1N1) viruses and H9N2 viruses isolated from both humans and birds [28]. On the other hand, a recent study on adult hospitalised patients showed that a direct intestinal infection by seasonal influenza A viruses seems an unlikely explanation for the fecal detection of viral RNA in the patients reported [11]. Alternative explanations of influenza virus detection in stools could be the swallowing of virus-containing nasopharyngeal secretion or extrapulmonary virus dissemination via hematogeneous circulation.

This study has several limitations. First, the total proportion of viral co-detection was likely underestimated because we did not test other diarrheal pathogens. Thus, some cases of single infection in our study could be classified as multiple infections in studies which would include these other pathogens. Second, influenza virus cultures were not performed. However, to help us evaluate whether PCR signals were false positives, positive and negative controls were included in each PCR performed. The detection of influenza B has been performed by using two different primer pairs for the NS gene, and we detected influenza A by using two independent PCR

assays for the detection of M gene and H gene. Third, respiratory samples were not collected. It is to be noted that the enrolment of patients was blind to any type of information related to respiratory tract infection, thus preventing potential bias.

Conclusion

In conclusion, in this study we showed that the simultaneous detection of influenza and enteric viruses is not a rare event. We have also reported, for the first time in general practice, the presence of seasonal and pandemic influenza viruses in the stools of adult patients consulting for uncomplicated AD. This result could support the idea that the influenza virus could, on some occasions, be a responsible cause of gastroenteritis given the presence among some patients of diarrhea and the absence of any respiratory symptoms along with the absence of co-pathogens in 50% of them. More focused screening of fecal samples for the detection and isolation of influenza viruses in patients presenting with gastroenteritis will be required to demonstrate this additional potential disease association. The possible presence of infectious influenza viruses in fecal samples could create problems concerning infection control and highlights the importance of contact precaution when handling stools. Whereas influenza viruses are usually regarded to spread via direct contact with respiratory droplets, the possible fecal–oral transmission of influenza viruses has to be elucidated. This would have a number of implications for GP management of influenza virus infected patients, especially among patients at risk of severe influenza, in order to limit inadvertent human-to-human transmission. These cases are reported to highlight the potential clinical and infection control benefits of precisely knowing the true etiology of gastroenteritis-like symptoms. A simultaneous investigation of enteric and influenza viruses of patients complaining of GI symptoms could be useful for future studies in order to better identify the agents responsible for AD and to understand the potential mode of transmission and interaction of these viruses, especially during epidemic ILI and AD outbreaks.

Methods

Study design

A case–control study was conducted from December 2010 to April 2011. Sixty-three GPs from the French Sentinel Network [29] collected stools from adult patients (≥18 year old) consulting for AD (Sentinel network case-definition for AD: at least three daily watery or nearly so stools dating less than 14 days), and from controls. General practitioners had to enroll two patients per week, one case and one control. We excluded from the cases group: patients with inflammatory bowel disease, and patients with an obvious non-infectious etiology of diarrhea (recent use of antibiotics, colchicines, non-steroidal anti-inflammatory drugs, laxatives, recent administration of chemotherapy or radiotherapy). Controls were patients consulting their GP for non-GI diseases and not reporting GI symptoms during the month preceding the consultation. Data on the time of the onset of symptoms, reported symptoms, physical findings, gender, age, previous treatment, and medical attention before enrolment were collected by completing a case report form (CRF) for all participants who met the case definition. In addition, cases and controls sent a follow-up questionnaire the week after enrolment to indicate the duration of symptoms (for cases) and to ascertain whether an AD had occurred or not (for controls). The Hospital Ethic's Committee (CHU Saint-Antoine, Paris, France) approved the study. Oral consent was obtained from the patients at the time of inclusion, for their participation in the study and for the publication of the clinical and virological data.

Sample analysis

Patients collected and sent, by postal mail, stool specimens in a triple packaging according to the instructions of the French National Reference Center for Enteric Viruses: the primary receptacle was a labeled primary watertight, leak-proof receptacle containing the specimen and without a transport medium. The receptacle was wrapped in absorbent material to absorb all fluid in case of breakage. A second durable, watertight, leak-proof receptacle was used to enclose and protect the primary receptacle. This secondary receptacle was placed in an outer shipping package bearing the United Nations packaging symbol (UN3373). In order to prevent the stool contamination by the patients' respiratory secretions, general practitioners insisted on precautions when collecting the stool and this information was also indicated on the information letter we gave to patients.

All stool specimens were tested for influenza virus A (A/H1N1 2009 and A/H3N2) and Influenza B and for four enteric viral pathogens (astrovirus, group A rotavirus, human enteric adenovirus, and norovirus of genogroup I - NoVGI - and genogroup II - NoVGII).

Stool specimens were homogenised (20% wt/vol) in sterile water, centrifuged for 10 minutes at 3000 rpm and 200 μl of the clarified supernatants were subjected to nucleic acid extraction, using a QIAmp MinElute Virus Kit® (QIAGEN, Courtaboeuf, France). Total nucleic acid was eluted in a final volume of 40 μl, of which 5 μl was used for PCR amplification. The efficiency of nucleic acid extraction was measured by real-time PCR amplification of the human GAPDH gene [30]. Influenza viruses A and B were detected by using two different real-time RT-PCRs [31]. Virus sub-typing (A/H1N1 2009 and A/H3N2) was performed by two real-time RT-PCRs [32,33].

Positive and negative controls were included in each RT-PCR. The copy number of influenza A and B viral RNA was determined against 10-fold serial dilution of external plasmid standards (from 2 x 10^8 down to 2). The enteric viruses were detected by simultaneous amplification of nucleic acid through using the Seeplex® Diarrhea-V ACE assay (Seegene), and according to the manufacturer's instructions. A recent study showed that the Seeplex® Diarrhea-V assay is sensitive, specific, convenient and reliable for the simultaneous detection of several viral pathogens found directly in stool specimens from patients with gastroenteritis [34].

Statistical analysis

Continuous variables were described by median [interquartile range] and dichotomous data were described by proportions. Groups were compared by the Student test or Mann–Whitney test (as appropriate) for continuous variables. The Chi-2 or Fisher's exact test (as appropriate) was used to compare dichotomous variables between groups, and the results are presented as Odds Ratios with their 95% confidence intervals (OR [95% IC]). All statistical analyses were two-tailed with a significance level (P value) of <0.05. Analyses were performed using STATA software (version 11.0, StataCorp LP, Texas, USA).

Abbreviations

GI: GastroIntestinal; ILI: Influenza Like-Illness; AD: Acute Diarrhea; GP: General Practitioner; CRF: Case Report Form; NoVGI: NoroVirus of Genogroup I; NoVGII: NoroVirus of Genogroup II; GAPDH gene: GlycerAldehyde 3-Phosphate DeHydrogenase gene; OR: Odd Ratio; NS gene: Non Structural gene; M gene: Matrix gene; H gene: Hemagglutinin gene.

Competing interests

The authors declare no conflict of interest.

Author contributions

CA, JPA, VV, KB, RCB, LV, JA, TB, FC, TH, and AF co-conceived the study. CA and TH collected epidemiological and microbiological data. AF, LV and KB designed microbiology experiments. AF performed, analyzed and interpreted microbiology data. CA analyzed and interpreted epidemiological data. CA, JPA, VV, KB, RCB, LV, JA, TB, FC, TH, and AF contributed to writing the paper and approved the final manuscript.

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5. Conclusion et perspectives

Ces travaux ont permis de confirmer le rôle prédominant du norovirus dans les DA de l'adulte durant les épidémies hivernales. Durant ces épidémies, rien ne distingue cliniquement les cas de DA de l'adulte chez qui un virus a été retrouvé dans les selles, des cas de DA pour lesquels la recherche de virus était négative (ce qui pourrait suggérer pour ces derniers cas qu'il s'agisse également de diarrhées virales pour lesquelles le diagnostic virologique a été pris en défaut). Les facteurs de risque susceptibles d'être associés à l'apparition d'une DA d'origine virale chez les sujets adultes vus en médecine générale en France métropolitaine durant les périodes d'épidémies hivernales sont constitués essentiellement par les situations d'interaction (contact) entre les individus. D'autres facteurs tels que les comportements alimentaires, les interactions avec les animaux et les habitudes de lavage des mains n'étaient pas associés au risque de survenue d'une DA de l'adulte. Ce constat vient donc appuyer le fait que, à l'heure actuelle, les moyens de prévenir la DA et de réduire l'importance des épidémies chez les adultes restent limités. Le renforcement des mesures d'hygiène a montré une efficacité pour la prévention des infections digestives essentiellement dans les populations pédiatriques de pays à bas niveau d'hygiène. [74, 75]. Dans les pays développés, l'efficacité des mesures d'hygiène pour la prévention des diarrhées hivernales d'origine virale, survenant en milieu communautaire, chez l'adulte, reste à confirmer. En effet, dans ce contexte de pays développés, il est possible que l'effort supplémentaire à fournir pour observer une efficacité, comparativement au niveau d'hygiène déjà élevé et au lavage habituel des mains, soit trop important pour être facilement démontrable et applicable à large échelle.

Chaque hiver, en France, lors des épidémies de DA, l'incidence estimée par le réseau Sentinelles au sein de la population adulte est près de 2 fois inférieure à celle estimée chez les sujets âgés de moins de 18 ans (bilan du réseau Sentinelles). Mais l'impact médico-économique de la DA de l'adulte reste non négligeable. En effet, les résultats rapportés dans

cette thèse montrent que la prise en charge médicale des cas de DA implique une prescription médicamenteuse quasi systématique et un arrêt de travail dans près de 80% des cas chez les personnes actives. Ces résultats permettent d'estimer que chaque hiver en France, l'épidémie de DA est reponsable d'un coût d'absentéisme au travail de 250 millions d'euros[1]. A ce coût de l'absentéisme et des soins médicaux doit s'ajouter celui de l'automédication, sachant que 60 à 70% des adultes ne consultent aucun médecin en cas de diarrhée [76]. La fréquence élevée des DA chez l'adulte, l'absence de facteurs de risque maitrisable autre que la prévention des contacts avec une personnes malade (pas toujours faisable) et l'importance des coûts de prise en charge de la maladie montrent l'intérêt potentiel des vaccins anti-norovirus en cours de développement [77, 78].

Bien que les virus entériques soient les principaux virus retrouvés, il est possible de détecter des virus influenza dans les selles de patients consultant pour une DA en l'absence de syndrome grippal. Ce résultat soulève la question d'une possible infection pathogène du tube digestif par le virus influenza.

Les résultats des travaux rapportés dans cette thèse pourront être utiles à la mise en place d'analyses de l'impact de futures stratégies vaccinales des adultes contre les infections à Norovirus. Par ailleurs, ils ont amené à initier de nouvelles recherches ayant pour objectifs de déterminer la prévalence des virus influenza dans les selles des patients consultant, non plus pour une DA mais pour un syndrome grippal, puis d'identifier les facteurs de risque cliniques et sociodémographiques associés. Ces travaux, actuellement en cours au sein de l'équipe d'accueil Bioscope Corse-Méditerranée (EA7310, Université de Corse), tenteront également d'éclaircir les phénomènes biologiques par lesquels les virus influenza sont excrétés dans les selles.

[1] Ce calcul prend en compte les données de l'Insee pour le revenu journalier moyen des Français et les données d'incidence du réseau Sentinelles

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