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Detection of multiple enteric virus strains within a foodborne outbreak of gastroenteritis: an indication of the source of contamination

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SUMMARY

An outbreak of acute gastroenteritis of suspected viral aetiology occurred in April 2003 in the British Royal Fleet Auxillary ship (RFA) *Argus* deployed in the Northern Arabian Gulf. There were 37 cases amongst a crew of 400 personnel. Of 13 samples examined from cases amongst the crew, six enteric viruses were detected by reverse transcriptase polymerase chain reaction (RT–PCR). Five different viruses were identified including, three norovirus genotypes, a sapovirus and a rotavirus. No multiple infections were detected. A common food source was implicated in the outbreak and epidemiological analysis showed a statistically significant association with salad as the source of the outbreak, with a relative risk of 3·41 (95% confidence interval of 1·7–6·81) of eating salad on a particular date prior to the onset of symptoms. Faecal contamination of the salad at source was the most probable explanation for the diversity of viruses detected and characterized.

INTRODUCTION

Viruses associated with acute gastroenteritis include noroviruses (NV), sapoviruses (SV), astroviruses, rotaviruses and enteric adenoviruses. NV are an important cause of gastroenteritis and are mainly spread person to person. They are the commonest cause of outbreaks in semi-closed communities such as hospitals [1], nursing/retirement homes [1], hotels [2], and cruise ships [3] can be at particular risk. Other factors that aid the transmission of NV include environmental contamination [2], the ingestion of contaminated water [4] or food [5], particularly molluscan shellfish [6].

Within the military, large outbreaks of gastroenteritis due to NV have been reported on American naval ships. There were 777 cases amongst a crew of 5000 personnel of the USS *Forrestal* [7], and in another outbreak, 13% of 4500 personnel on another US aircraft carrier were ill with NV gastroenteritis [8].

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SV are most commonly seen in children (usually in those under 5 years of age) and occur as sporadic cases [9] and outbreaks of gastroenteritis in hospitals, nurseries, child day-care centres and schools [9]. Occasionally SV outbreaks occur in the elderly in nursing homes [10], but rarely in those under the age of 65 years, though an outbreak did occur among teachers in a school in the United States [11]. Sporadic cases of SV gastroenteritis in adults, occur occasionally and have been reported in Japan [12] and the United Kingdom (Gallimore et al., unpublished data).

Rotaviruses are a major aetiological agent in infants and young children. Most human rotavirus infections are caused by group A rotaviruses and infection occasionally occurs in adults [13].

Children have been shown to be infected simultaneously by one, two [9] or multiple enteric viruses [14] or different strains of the same virus [15]. Excretion of several different NV strains, has also been reported in a gastroenteritis outbreak in a party of canoeists infected from contaminated river water [4] and a person with oyster-associated gastroenteritis [16].

Sewage contamination of water supplies causing NV outbreaks, has been reported [17], but there have been few accounts of faecal contamination of salad crops by waste water or sewage sludge causing viral gastroenteritis. Gastroenteritis outbreaks due to washing, spraying or growing food in water contaminated with sewage has been reported [18]. Shellfish, and in particular oysters, cultured in faecal/sewage contaminated seawater have been responsible for many outbreaks of viral gastroenteritis.

More commonly, NV outbreaks are associated with the consumption of ready-cooked or fresh food (salad, etc.) in hotels and restaurants, following direct contamination during preparation by food handlers [19–21], who can be pre-symptomatic, symptomatic or post-symptomatic.

The Royal Fleet Auxillary (RFA) ship *Argus* was deployed to the Arabian Gulf in February 2003 in support of the Naval Task Group dispatched in response to the deteriorating situation in Iraq. The ship provided a sophisticated secondary medical care asset known as a Primary Casualty Receiving Facility (PCRF), and had a transport helicopter capability. The PCRF's diagnostic capability included a radiology department with CT scanner and a pathology laboratory able to provide clinical biochemistry, haematology, transfusion science and microbiology support to the level of a small hospital laboratory.

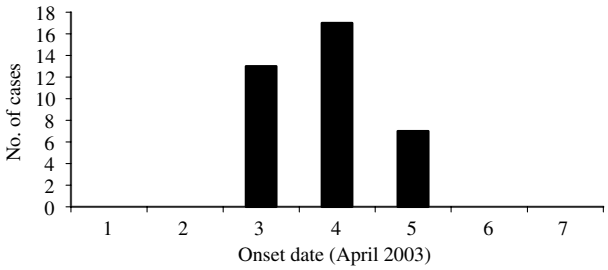

Fig. 1. Outbreak curve of onset date of cases of gastroenteritis amongst the crew in the RFA *Argus* outbreak 2003.

The ship's permanent staff included a Royal Navy Medical Officer to provide primary health care to all embarked personnel. During the deployment the ship's permanent company of RFA (civilian merchant fleet) and Royal Navy (mostly aviation) staff was significantly augmented, predominantly by medical personnel to man the PCRF, reaching some 400 members of crew.

This study describes an outbreak of gastroenteritis amongst the crew of the ship and how subsequently multiple viral pathogens were detected and characterized in the stools of the affected crew and how epidemiological data demonstrated a contaminated food source as the most likely cause.

METHODS

Clinical details

Thirty-seven personnel from throughout the ship's company presented with abrupt onset of a gastrointestinal illness with onset dates between 3 and 5 April 2003. The number of affected personnel represented approximately 10% of the ship's company. The presentation was of sudden onset of watery diarrhoea, colicky abdominal pain and nausea with or without vomiting. The illness was short lived with resolution within 24 h in most cases. Patients were managed by being 'stood down' until free of symptoms for 24 h with a fluids-only diet (with dioralyte as required) for the first 24 h.

Thirteen cases presented on 3 April with a further 17 cases on 4 April at which time an outbreak control meeting was convened. A further seven cases presented on 5 April (Fig. 1).

The outbreak was controlled using standard infection control protocols highlighting hand-washing hygiene and the provision of appropriate quantities of toilet paper, paper hand towels and soap, thorough

cleaning of toilet facilities with particular attention to door handles and segregation of toilets for affected personnel.

Primary laboratory investigation

Thirteen stool specimens were submitted to the pathology laboratory in the PCRF within 24–48 h after onset of gastroenteric symptoms. Routine bacteriological examination was negative for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Escherichia coli* and *Vibrio* spp. Following a discussion between the Consultant Microbiologist for the PCRF and the Defence Medical Services Consultant in Communicable Disease Control based in the United Kingdom, the stool samples together with 17 acute serum samples were dispatched to the United Kingdom on 8 April for virological examination at the Enteric Virus Unit of the Enteric, Respiratory and Neurological Virus Laboratory, Specialist and Reference Microbiology Division of the newly formed Health Protection Agency, Colindale, London, UK under the auspices of a pre-deployment agreement between Ministry of Defence (MOD) and the (then) Public Health Laboratory Service (PHLS).

Epidemiology data

Following the initial cases of acute diarrhoea on 3 and 4 April, clinical information was collected from each of the patients who had presented with illness. Simple descriptive analysis indicated that those affected were distributed across all areas of the ship. Onset times of illness suggested that a point source outbreak was likely, rather than person-to-person spread from a previously infected individual (Fig. 1). A working case definition was made of 'diarrhoea and/or vomiting onset after 2 April 2003'.

This was followed up with a more detailed questionnaire distributed to members of the PCRF. This constituted just over 50% of the ship's company, but included 75% of the cases, and was felt to be representative of the population at risk. At the time the ship was still active operationally and access to other crew members was impractical. Exposure history focused on the period 48 h prior to onset of symptoms in the first cases. Accurate food information was derived from menu cards provided by the ship's caterers for 2 and 3 April 2003. Data was transmitted electronically to the Defence Medical Services Department in the United Kingdom and analysis was performed using the Epi-Info version 6.0 statistical software (CDC, Atlanta, GA, USA).

Virological investigation

Faecal samples for reverse transcriptase polymerase chain reaction (RT–PCR) and PCR for gastroenteric viruses were processed as a 10% faecal suspension and extracted using the guanidinium isothiocyanate-silica method [22]. RNA was eluted in 40 μl of nuclease-free water (Promega, Southampton, UK) and complementary DNA (cDNA) was prepared by, as previously described [23]. Samples were screened for NV, SV, astroviruses, group A rotaviruses and adenovirus types 40 and 41 by RT–PCR and PCR. The serum samples were not tested.

NV primers (Ni/E3 and SG1/D1) were broadly reactive and amplified a region of ORF1 [24, 25], SV primers (SR80/JV33) amplified a region of the RNA polymerase gene [9, 11], astrovirus primers (Mon268/Mon270) amplified a region of the capsid gene [26], rotavirus primers (VP6-F/VP6-R) amplified a region of the VP6 gene [27] and adenovirus primers (Adeno-F/Adeno-R) amplified a region of the long fibre gene of adenovirus 40 and 41 strains [28]. Additional primers GIFF-1, -2, -3/GISKR and GIIFB-1, -2, -3/GIISKR which amplify the ORF1/ORF2 junction of genogroup I (GI) and GII NV respectively, were used to genotype NV-positive samples [29].

PCR amplicon cloning and sequencing

PCR amplicons for NV and SV generated from faecal samples were cloned using a TA cloning system (TOPO®, Invitrogen, Paisley, UK), using methods previously described [30]. Generation of contiguous sequences and pairwise alignments of the 109-bp inter-primer region (SG1/D1) of the NV ORF1 sequences, the 425-bp inter-primer region (GIIFB/GIISKR) of the NV GII ORF1/2 sequences, the 555-bp inter-primer region (GIFF/GISKR) of the NV GI ORF1/2 sequences, and the 280-bp inter-primer region (SR80/JV33) of the SV ORF1 sequences, was performed using Genebuilder and Clustal in Bionumerics version 2.5 (Applied Maths, Kortrij, Belgium).

Screening multiple clones

Twenty clones for each of the NV SG1/D1 PCR amplicons from the ORF1 region (patient nos. 3

Table 1. *Enteric viruses detected in RFA* Argus *ship outbreak*

Patient no.	Virus	Genotype [31]	Strain designation
1	Rotavirus	Group A rotavirus	
2	Nvd		
3	Norovirus	GII-6 (Seacroft/1990/UK)	Argus-3/2003/IQ
4–7	Nvd		
8	Norovirus	GI-6 (Sindlesham/1995/UK)	Argus-4/2003/IQ
9	Sapovirus	Argus-2/2003/IQ*	Argus-2/2003/IQ
10	Nvd		
11	Norovirus	GI-3? (Desert Shield/1990/SA)	Argus-1/2003/IQ†
12	Nvd		
13	Norovirus	GI-6 (Sindlesham/1995/UK)	Argus-5/2003/IQ

Nvd, No virus detected.
* Nearest neighbour on GenBank is Stockholm/1997/SE (AF194182) at 80% identity.
† Nearest neighbour on GenBank is Saitama KU8GI/99/JP (AB058547) at 96% identity in the 5′-end of the Orf2 region.

and 8) were screened by DNA sequencing for evidence of mixed NV infections.

RESULTS

RT–PCR and PCR for enteric viruses

A group A rotavirus was detected by RT–PCR in one patient only, NV was detected in four patients using SG1/D1 primers (all samples were negative when tested with Ni/E3 primers) and a SV was detected in one patient using primers SR80/JV33. All samples were negative by RT–PCR for astroviruses and PCR for enteric adenovirus strains 40 and 41. No virus was detected in seven patients (Table 1).

Enteric virus strain typing

Three different NV strains were detected in the patients in this outbreak. Patient nos. 8 and 13 both were infected with a NV GI strain (designated Argus-4 and Argus-5/2003/IQ respectively), and was genotyped [31] as a GI-6, Sindlesham/1995/UK (AJ277615) strain, by Orf2-5′-end sequencing. Patient no. 11 also had a GI strain, and was designated Argus-1/2003/IQ, this strain clustered with Saitama KU8GI/99/JP (AB058547) with 96% identity and is a probable GI-3, Desert Shield/1990/SA (U04469) strain. Patient no. 3 had a NV GII strain, designated Argus-3/2003/IQ, and was genotyped as a GII-6, Seacroft/1990/UK (AJ277620) strain (Fig. 2).

The SV strain detected in the faeces of patient no. 9 was designated Argus-2/2003/IQ and had only 80% identity to Stockholm/1997/SE (AF194182) in a small region of the RNA polymerase gene and was not typed further.

Multiple clone screen of NV PCR amplicons

Twenty clones of NV amplicon derived from the faeces of patient nos. 3 and 8, were analysed by DNA sequencing of a region of the ORF1 and were identical to the original cloned strain in each case, Halle445/1999/DE-like and Fin/H/Vesi/G1-like polymerases respectively, indicating that symptomatic crew were not infected with a mixture of NV strains.

Epidemiological data analysis

Analysis of the evaluable food history questionnaires, representing 90% of those distributed, indicated a significantly increased risk of subsequent illness associated with the consumption of salad on 2 April [risk ratio (RR) 3·41, 95% confidence interval (CI) 1·70–6·81] and with consumption of salad on 3 April (RR 2·35, 95% CI 1·022–5·39) (Table 2).

Subsequent investigation indicated that the ship had been re-supplied at sea on 2 April with fresh produce. The supplies had been sourced from Bahrain, but had originated in another country in the Middle East. Crew members that were shown to be excreting gastroenteric viruses were amongst those that had gastroenteric illness from eating salad vegetables.

DISCUSSION

This investigation has demonstrated the excretion of multiple enteric viruses in the stools of the crew of

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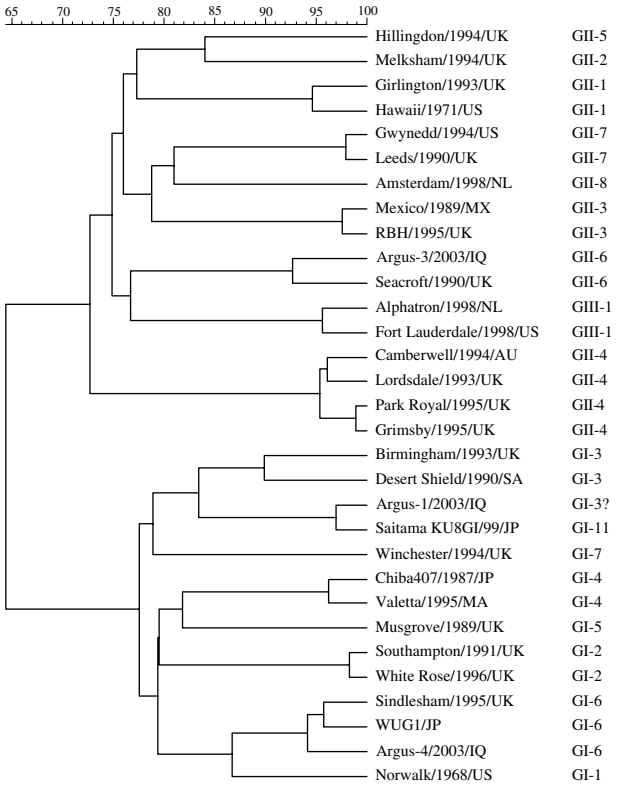

Fig. 2. Dendrogram of 5′-end region of the ORF2 of norovirus strains co-circulating in RFA *Argus* outbreak 2003. GenBank strains are Hillingdon/1994/UK (AJ277607), Melksham/1994/UK (X81879), Girlington/1993/UK (AJ277606), Hawaii/1971/US (U07611), Gwynedd/1994/US (AF414409), Leeds/1990/UK (AJ277608), Amsterdam/1998/NL (AF195848), Mexico/1989/MX (U22498), RBH/1995/UK (AJ277617), Seacroft/1990/UK (AJ277620), Alphatron/1998/NL (AF195847), Fort Lauderdale/1998/US (AF414426), Camberwell/1994/AU (U46500), Lordsdale/1993/UK (X86557), Park Royal/1995/UK (AJ277613), Grimsby/1995/UK (AJ004864), Birmingham/1993/UK (AJ277612), Desert Shield/1990/SA (U04468), Saitama KU8GI/99/JP (AB058547), Winchester/1994/UK (AJ277609), Musgrove/1989/UK (AJ277614), Chiba407/1987/JP (AB022679), Valetta/1995/MA (AJ277616), Sindlesham/1995/UK (AJ277615), WUG1/JP (AB081723), Norwalk/1968/US (M87661), Southampton/1991/UK (L07418), and White Rose/1996/UK (AJ277610). Sequence of Argus strains from this study can be obtained by contacting the corresponding author.

the RFA ship *Argus* during an outbreak of gastroenteritis and the statistically significant association with salad as the cause. The outbreak was of a relatively short duration, but it did affect at least 10% of the crew (although it was only feasible to question 50% of the ship's company with the resources available and the time constraint, it was assumed that all those that had symptoms would have reported sick, given the acute nature of the illness and the high working intensity of the operational environment), similar to the 13% attack rate seen previously in a US aircraft carrier [8].

Table 2. *Foodstuffs eaten by the crew of the RFA* Argus *prior to the gastroenteritis outbreak*

	Eaten		Not eaten			
Food	Ill	Not ill	Ill	Not ill	RR	95% CI
2 April 2003						
Fruit juice	8	45	17	62	0·70	0·33–1·51
Fried eggs	5	17	16	80	1·36	0·56–3·32
Soup	14	38	12	58	1·57	0·79–3·11
Cod in cheese	9	25	15	72	1·54	0·74–3·17
Jelly royale	2	19	22	82	0·45	0·11–1·77
Prawn biryani	9	56	15	54	0·64	0·30–1·35
Peppered pork	17	54	11	35	0·78	0·40–1·53
Pineapple cake	13	46	14	61	1·18	0·60–2·31
Salad	16	23	10	73	3·41*	1·70–6·81
Mixed vegetables	1	10	23	94	0·46	0·07–3·11
Fresh fruit	13	40	5	37	2·06	0·80–5·32
Cheese	5	24	16	77	1·00	0·40–2·50
3 April 2003						
Chilled apple	10	34	12	73	1·61	0·76–3·43
Eggs	3	8	17	91	1·73	0·60–5·00
Sauté kidney	2	10	20	94	0·95	0·25–3·58
Soup	8	44	10	56	1·02	0·43–2·39
Savoury mince	6	57	11	42	0·46	0·18–1·16
Manchester tart	3	26	16	68	0·54	0·17–1·73
Kidney Tobago	5	11	16	90	2·07	0·88–4·87
Roast beef	15	95	7	15	0·43	0·20–0·93
Yorkshire pudding	17	89	5	20	0·80	0·33–1·97
Apple pie	7	52	15	56	0·56	0·25–1·29
Salad	8	22	10	78	2·35*	1·02–5·39
Mixed vegetables	1	13	20	93	0·40	0·06–2·78
Fresh fruit	10	35	10	39	1·09	0·50–2·37
Cheese	6	24	16	79	1·19	0·51–2·76

RR, Risk ratio; CI, confidence interval.
* Food implicated with statistical significance.

Effective infection control measures including thorough cleaning of toilet facilities, segregation of toilets for affected personnel and cleaning of adjacent surfaces to minimize environmental spread [2, 32] were instigated as soon as the outbreak was recognized. This prompt action may have reduced the spread of the virus, thus limiting the number of crew affected.

Statistical analysis of data from questionnaires returned, revealed that fresh produce delivered to the ship on 2 April, was the most probable source of the outbreak. Analysis confirmed a significant association between the consumption of fresh salad on 2 and 3 April and subsequent gastroenteric illness, and in conjunction with the virological data suggests faecal contamination of salad vegetables was the likely source of infection [33].

Investigation of this outbreak highlighted difficulties with procuring fresh food items at sea for the Royal Navy ships on operational deployments. It identified possible problem areas, with implications for all other ships in the fleet. An immediate review of procurement procedures was undertaken, and revised policies are now in place.

Foodborne enteric infections may be associated with contamination of food during cultivation, harvest or by food handlers during preparation. Contamination with and transmission of multiple enteric viruses would suggest contamination during cultivation when food crops, particularly salad vegetables, may be exposed to sewage sludge or sewage contaminated water [34].

In sewage-contaminated water outbreaks, multiple enteric pathogens have been demonstrated with *Campylobacter* spp., *Shigella* spp. and NV occurring in one outbreak [35]. In other waterborne outbreaks, faecal coliforms (indicating sewage contamination) and *E. coli* were detected, the gastroenteritis was shown to be caused by NV [36, 37] and in a recent waterborne outbreak in the United States, only NV were detected [38].

Although in the United Kingdom, the use of untreated sewage sludge on land used to grow food crops is prohibited, this is not the case for all countries. Those sourcing food crops, particularly those that are eaten raw, should be aware of the agricultural practices in the region of supply.

In the absence of reliable and sensitive methods for the detection of viral contamination of foodstuffs, the examination of faecal samples for multiple viral pathogens can provide evidence for foodborne transmission. Therefore, microbiological investigation in tandem with the collection and analysis of epidemiological data can provide firm evidence of foodborne transmission. The burden of foodborne NV is difficult to define and these data suggest that the application of multiplex viral detection assays to outbreaks may have a role in defining more accurately the scale of foodborne disease.

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