are of equal significance. Substantially more data are available for the specimens analysed, e.g. patient's age, clinical diagnosis, sampling date and onset of disease. First isolations of influenza virus and particularly an increase in their incidence may be predictive of the very beginning of an epidemic even before any change can be detected in the clinical morbidity rates. Routine detection of other viral respiratory pathogens yields complementary data which are useful in monitoring general trends in morbidity. Summary data are informative enough of the circulation of different agents in the population throughout the year. The virological results are also sometimes used to validate the clinical reports. For example, during the 2003-2004 season there were two ARI morbidity peaks in the Czech Republic [FIGURE 1]. This was caused by two regional influenza epidemics in different parts of the Czech Republic when the fast transmission was interrupted by the Christmas holidays [FIGURES 3,4].

The ARI / ILI reporting system of the Czech Republic is a modern and efficient surveillance system based on the collection of high quality data. The whole ARI / ILI reporting system is essential for pandemic planning in the Czech Republic. It can be linked with the system for crisis management to enable reporting and analysis on a daily basis. For efficient information at all levels, high quality local and national surveillance is necessary. Since using an internet-based platform, the reporting system in the Czech Republic as well as the EISS are easily accessible and provide timely information.

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ORIGINAL ARTICLES

Surveillance report

SURVEY OF THE CONTAMINATION OF FOODSTUFFS OF ANIMAL ORIGIN BY SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* SEROTYPE O157:H7 IN BELGIUM FROM 1999 TO 2003

A Chahed [1], Y Ghafir [1], B China [1], K Dierick [2], L De Zutter [3], D Piérard [4], G Daube [1].

A survey of the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) of O157 serotype in foodstuffs of animal origin (beef, veal, pork, chicken, fish) from 1999 to 2003 in Belgium was performed. STEC strains were only isolated from beef with a prevalence of 0.73%. This percentage is low in comparison with the prevalence in other countries. Among the 76 isolated STEC O157 strains, 75% belonged to the serotype O157:H7 and 25% to the serotype O157 non H7. Moreover, the most frequent pathotype was *eae stx2 ehxA* (74%).

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Key words: Escherichia coli, Foodstuff, pathotype, Shiga toxin

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Introduction

Two legal texts Published by the European Parliament in November 2003 are dedicated to the survey and management of zoonoses and zoonotic agents in European Union (EU): Directive 2003/99/EC [1] on the survey of zoonoses and zoonotic agents and Regulation 2160/2003/EC [2] on the control of salmonella and other zoonotic agents present in food chain. These texts repeal the directive 92/117/CEE [3] concerning the survey of zoonoses and zoonotic agents in EU and indicate that each member country must collect relevant data concerning the major zoonotic agents and must report this to the European Commission. Among these zoonotic agents to be surveyed, the directive mentions verocytotoxigenic *Escherichia coli* (VTEC). These pathogens are a public health problem in Belgium: 46 pathologic cases associated with Shiga toxin-producing *Escherichia coli* (STEC) were identified in 2002 [4].

Enterohaemorrhagic *E. coli* (EHEC) are VTEC or STEC which can cause a broad spectrum of human diseases, including diarrhoea, haemorrhagic colitis, and the haemolytic uraemic syndrome (HUS).

The O157 serotype has been responsible for numerous outbreaks worldwide involving fatal cases [5], and therefore EHEC O157 are deserving of to a very careful survey. It remains the principal serotype involved in HUS in Europe. Vulnerability to EHEC infection depends on several factors.

1. Age: children aged 15 years and younger, and people aged over 65 years were more exposed.
2. Immunity: the presence of antibodies seems to protect people from a O157 challenge.
3. Gastrointestinal modifications: a diet poor in proteins is a risk factor.
4. Blood group: Shiga toxins seem to be transported by the red blood cells. The O group and people with red blood cells lacking the P antigen were more sensitive.
5. Ingested dose: the more bacteria were ingested the more severe disease occurred.
6. Previous antibiotic treatment is a risk factor suggesting a protecting role for the intestinal flora. Moreover, an antibiotic treatment of the disease increases the risk of HUS appearance [6].

Their major virulence factors are the Shiga-toxins Stx1 and Stx2 responsible for the kidney problems and the intimin encoded by the pathogenicity island LEE (Locus of enterocyte effacement) and involved in diarrhoea. Moreover, EHEC O157:H7 bacteria produce an enterohaemolysin encoded by the plasmidic *ehxA* gene [7].

Between 1994 and 2002 in Belgium, 398 STEC strains were isolated from human patients. Of these, 195 (49%) were from serotype O157, but only 182 were classical EHEC (positive for *eae, stx* and *ehxA* PCR) [4].

The major foodstuffs from animal origin have been randomly sampled among the national production in order to evaluate the prevalence of EHEC O157 and the major incriminated pathotype.

Material and methods

Sampling of beef and pork carcasses was performed in slaughterhouse by swabbing on a half-carcass 2 to 4 hours postmortem. For each half-carcass, 4 surfaces were sampled corresponding to a total surface of 600 cm²:

(a) the internal face of the jam (100 cm²),
(b) the posterior part of the internal pelvis (100 cm²),
(c) the sternum and the sternocephalic muscles (300 cm²),
(d) the posterior face of the anterior member (100 cm²).

For beef, a 1600 cm² surface corresponding to 4 zones of 400 cm² was swabbed:

(a) the postero-external face of the thigh (400 cm²),
(b) the flank (400 cm²),
(c) the thorax (400 cm²),
(d) the posterior face of the anterior member (400 cm²).

For pork, the number of analysed carcasses was 163 in 2000 representing 0.0015% of the Belgian annual production. For beef, the number of carcasses studied was, 1984 in 1999, 1501 in 2000, 1388 in 2001, 1215 in 2002 and 1479 in 2003 representing 0.35 %, 0.25%, 0.19%, 0.26 % of the annual Belgian beef production, respectively. One hundred and fifty seven veal carcasses samples were analysed in 2000.

Chicken carcasses were sampled at the slaughterhouse exit or at the distribution level. Twenty five grams of carcass skin were removed at the neck and the front neck level. Two hundred and forty three carcass samples were analysed in 2001, representing 0.0007% of the annual Belgian chicken production. Minced meat and cuts of beef and pork were sampled at the production stage or at the distribution stage. Raw chicken minced meat preparations were sampled at the distribution level with a minimum sample of 100 g. Chicken fillets (without skin or bones) were sampled at the exit of the production chain or at the consumer distribution level. For fish, 25g of flesh and skin were sampled from entire fish at the abattoir exit.

The isolation protocol for *E. coli* O157:H7 involved a pre-enrichment at 42°C in mTSB broth, supplemented with novobiocin during 6 at 7 hours, followed by an enrichment in MacConkey broth supplemented with cefixime-tellurite and incubated at 37°C for 18 hours. An O157 immunoassay (VIDAS ECO) and an immunoconcentration if the immunoassay was positive (VIDAS ICE or Dynabeads O157) were performed. In the case of a positive immunoassay, a plating on sorbitol-MacConkey agar supplemented with cefixime-tellurite was performed and incubated for 18 hours at 42°C, followed by a confirmation by latex agglutination (Oxoid) and by biochemical gallery (Api20E, Biomérieux) [8]. If this confirmation step was not conclusive, the result was considered to be negative. The presence of the H7 antigen was investigated using H7 antiserum-sorbitol fermentation medium [9]. Finally the presence and of the virulence genes *(eae, stx1, stx2, ehxA)* were investigated by PCR[10].

The statistics (contingency table, $\chi 2$ calculation) were performed using the InStat2.01 software.

Results

One hundred and sixty three pork carcasses were analysed in 2000

Table 1

Prevalence of STEC O157 in foodstuffs of bovine origin, Belgium

Matrix	Analysed amount	Year	1999	2000	2001	2002	2003	Total	Statistics ($\chi 2$)*			
Beef carcass	1600 cm²	n p %	1984 25 1.3	1501 6 0.5	1388 13 0.9	1215 13 1.1	1479 10 0.68	7567 67 0.89	Significant	Significant		Non significant
Statistics ($\chi 2$)*			Non significant									
Minced meat	25 g	n p %	974 1 0.1	487 1 0.2	298 0 0	297 0 0	285 2 0.7	2341 4 0.17			Significant	
Statistics ($\chi 2$)*			Non significant									
Cut	25 g	n p %	NT	NT	NT	222 0 0	298 5 1.68	520 5 0.96				Non significant
Statistics ($\chi 2$)*						Non significant						
Isolated strains		n p %	2958 26 0.88	1988 7 0.35	1686 13 0.77	1734 13 0.74	2062 17 0.82	10428 76 0.73				
Statistics ($\chi 2$)*			Non significant									

n = number of analysed samples
* ($p<0.05$)
p: number of positive samples
NT: non tested

with 145 cut samples and159 minced meat samples. All these samples were negative for the presence of EHEC O157.

For chicken, 243 broiler skin samples, 181 fillets samples, and 152 hen skin samples were analysed in 2001. All samples were negative.

For veal, the 157 carcasses samples analysed in 200 were negative. All of the 153 aquaculture fish samples analysed in 1999 were negative.

However, of the 7567 beef carcasses samples analysed from 1999 to 2003, 67 (0.89%) were positive [Table 1]. Of the the 520 beef cuts analysed in 2002 and 2003, 5 (0.96%) were positive. Of the 2341 beef minced meat samples analysed between 1999 and 2003, 4 (0.17%) were positive. A statistical analysis indicated that the diference in prevalence between carcasses and cuts was not significant but the differences of prevalence between minced meat and the two other matrices were significant. Moreover, there was no significant difference between years for a particular matrix or for all the matrices taken together.

The 76 STEC O157 strains isolated from bovine samples were analysed for the presence of the H7 antigen and for the presence of virulence genes [Table 2]. Of these strains, 75% expressed the H7 antigen and 25% did not. Moreover, 74% harboured the *stx2* gene, 20% the *stx1* and *stx2* genes, and 6% the *stx1* gene (only for strains isolated in 1999). Finally, the *ehxA* and the eae genes were present in all strains. The most frequent pathotype was: *eae stx2 ehxA* (62%).

Table 2

Characteristics of isolated STEC strains, Belgium

Serotype	Pathotype	1999	2000	2001	2002	2003	Total
O157:H7	*eae stx2 ehxA*	19	0	6	12	10	47*
O157:H7	*eae stx1 stx2 ehxA*	0	0	2	0	6	8
O157:H7	*eae stx1 ehxA*	2	0	0	0	0	2
O157 non H7	*eae stx1 stx2 ehxA*	0	1	4	1	1	7
O157 non H7?	*eae stx2 ehxA*	2	6	1	0	0	9
O157 non H7	*eae stx1 ehxA*	3	0	0	0	0	3
Total		26	7	13	13	17	76

* This pathotype is significantly more frequent than the other ($p<0.01$)

Discussion

The surveillance plans developed in Belgium to follow the prevalence of EHEC O157:H7 in the major foodstuffs of animal origin indicate that only beef samples were positive.

Nevertheless, the sample number for other groups (veal, pork, chicken, fish) was low. Nevertheless, foodborne diseases due to EHEC in pork meat were rare Actually, in pork meat, several studies indicated that the *E. coli* O157:H7 prevalence in fresh pork raw meat was lower than 2% [11]. This study confirms that the prevalence of STEC O157: H7 in pork meat is low. Indeed, Bouvet et al. (2002) showed that 15% of the carcass swabs examined contained STEC, but that none of these STEC were from serotype O157:H7 [12].

For chicken, no foodborne disease involved STEC O157:H7 in chicken meat or eggs was reported. Nevertheless, a French study found 4/110 chicken meat samples positive for *E. coli* O157, although not producing Shiga toxins [13]. An American study found 4% of carcasses to be contaminated with EHEC O157:H7 [11]. Therefore, the prevalence of STEC O157 in chicken seems to be generally low. As for fish, an O157:H7 strain was isolated from an outbreak in Japan in 1998. Moreover, fish consumption appeared to be a risk factor for EHEC infection in Belgium [14].

For beef, the average prevalence for the 5 years was 0.73% whereas the calf samples were negative. Data from the literature indicate the absence of STEC O157:H7 in carcasses of veals analysed in the United States and in Europe whereas the prevalence in adult bovine was 4% [11]. It is difficult to compare our data with the data from the literature, since sampling and isolation methods differ between studies. Nevertheless, for bovine carcasses, the prevalence in our study was 0.89%. Danish, Czech and British studies have shown similar prevalences of 0.7%, 1% and 1.4%, respectively [15,16,17] . However an Irish study has shown a prevalence of 11% of STECO157: H7, an Italian study has shown a prevalence of 12% of STEC O157, and a French study has shown a STEC O157 prevalence of 10.7% [18, 19, 20]. Some studies have shown an intermediate STEC O157:H7 proportion, for example, a Turkish study which showed a prevalence of 3.6% [21]. Interestingly, an American study indicated that the prevalence of STEC O157 decreased on carcasses at the slaughterhouse during processing: 87% positive at the pre-evisceration stage, 57% in the post-evisceration stage, and 17% at the post-processing stage [22]. Since our samples were taken at the post-processing level, this may partly explain the low contamination level observed. The prevalence of STEC O157 in minced beef meat observed in our study was 0.17%. A low prevalence was also observed in English and French studies with 0.35% and 0.11%, respectively [17, 11]. For beef cuts, few data are available, although a Danish study performed in 2001 indicated that none of the cuts examined (n=543) was positive [15]. An American study performed in 2002 indicated that 0.2% of the beef cuts examined (n=1014) were positive for STEC O157:H7 [23].

Most of the isolated strains belonged to the O157:H7 serotype with a higher prevalence for strains harbouring the *stx2* gene in comparison to the strains harbouring *stx1* and *stx2* genes or to the strains with only the *stx1* genes. Similar results were obtained in France [20]. Moreover, the *stx2* positive strains are the most virulent EHEC O157:H7 strains [24]. Consequently, even with a low prevalence, the potential implication of these EHEC strains in human pathology must be monitored.

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ORIGINAL ARTICLES

Surveillance report

HOSPITAL PREPAREDNESS AND MANAGEMENT OF PATIENTS AFFECTED BY VIRAL HAEMORRHAGIC FEVER OR SMALLPOX AT THE LAZZARO SPALLANZANI INSTITUTE, ITALY

G Ippolito, E Nicastri, M Capobianchi, A Di Caro, N Petrosillo, V Puro

The US cases of anthrax in 2001 and the recent severe acute respiratory syndrome outbreak have heightened the need for preparedness and response to naturally emerging and re-emerging infections or deliberately released biological agents.

This report describes the response model of the Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani (INMI), Rome, Italy for managing patients suspected of or affected by smallpox or viral haemorrhagic fever (VHF) either in the context of an intentional release or natural occurrence.

The INMI is Italy's leading hospital in its preparedness and response plan to bioterrorism-related infectious agents. All single and double rooms of INMI are equipped with negative air pressure, sealed doors, high efficiency particulate air (HEPA) filters and a fully-equipped anteroom; moreover, a dedicated high isolation unit with a laboratory next door for the initial diagnostic assays is available for admission of sporadic patients requiring high isolation. For patient transportation, two fully equipped ambulances and two stretcher isolators with a negative pressure section are available. Biomolecular and traditional diagnostic assays are currently performed in the biosafety level 3/4 (BSL 3/4) laboratories.

Continuing education and training of hospital staff, consistent application of infection control practices, and availability of adequate personnel protective equipment are additional resources implemented for the care of highly infectious patients and to maintain the readiness of an appropriately trained workforce to handle large scale outbreaks.

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Key words: biological agents, bioterrorism, haemorrhagic fever, Italy, preparedness, smallpox

Introduction

The cases of anthrax in Florida and New York City in 2001, following the terrorist events in New York City and Washington, D.C. [1] and the recent severe acute respiratory syndrome (SARS) outbreak [2] have heightened the need for preparedness and response to emerging and re-emerging infections or deliberately released biological agents [3,4]. Smallpox [5] and haemorrhagic fever viruses (VHF) [6] pose the greatest concern because of their potential ease of dissemination or transmission, major public health impact (e.g., high mortality), panic and social disruption [4].

This report describes the model of response for the Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani (INMI), Rome, Italy in managing patients suspected of or affected by smallpox or VHF either in the context of an intentional release or natural occurrence.

The Institute

Since its foundation in 1936, the Lazzaro Spallanzani hospital has been devoted to the prevention, diagnosis and care for infectious diseases. Over the years, its focus has changed in relation to the evolving patterns of infection threat. In particular, the hospital was heavily involved in the control of hepatitis B and C epidemic in the '70s, and the human immunodeficiency virus (HIV) and tuberculosis spread in the mid '80s and early '90s.

In 1982, after smallpox vaccinations in Italy were discontinued, the Italian Ministry of Health identified the Lazzaro Spallanzani hospital as the place that would receive suspected cases and a negative-pressure Gelman's containment bed isolator was purchased. The isolator was rigid, uncomfortable and unacceptable to patients, although it gave the nursing and medical staff a high degree of protection. However, not all routine nursing and medical procedures could be carried out due to this rigid physical barrier and it was also not practical to perform mechanical ventilation or haemodialysis.

In 1994, a new three floor hospital complex was completed for a total of 256 beds in 7 wards, 48 beds in day hospital care, and 20 intensive care beds.

The building has an air conditioning system that is able to provide up to 12 air changes per hour to all single and double rooms. In addition, the system also allows changes from negative to positive room pressure and vice versa, enabling the rooms to be used for airborne isolation or as a protective environment. All rooms have

Instituto Nazionale per le Malattie Infettive Lazzaro Spallanzani, Rome, Italy