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Preliminary report: outbreak of Legionnaires' disease in the cities of Ulm and Neu-Ulm in Germany, December 2009 - January 2010

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Currently an investigation is ongoing to explore and control an outbreak of Legionnaires' disease, affecting 65 people as of 22 January 2010, in the cities of Ulm and Neu-Ulm, south-west Germany. A hitherto unidentified wet cooling system in these twin cities is considered as the most likely source of infection.

On 5 January 2010, Ulm University Hospital informed the local health office of a cluster of hospitalisations due to community acquired pneumonia caused by *Legionella pneumophila* serogroup (sg) 1. As of Friday 22 January 2010, 65 cases including five deaths were under investigation by the local and regional health authorities. With only a few exceptions all cases were living or working in Ulm or Neu-Ulm in south-west Germany. All cases are German residents aged between 27 and 96 years (median age 67 years) (Figure 1).

The majority of patients identified until 22 January 2010 had to be hospitalised, n=61. All patients and, if the clinical condition of the patient made an interview impossible, their family members, were interviewed using a standardised questionnaire to investigate potential sources of exposition, personal risk factors and the onset of symptoms. For 40 cases the onset of symptoms was during the last week of December 2009 (Figure 2). All but two patients who were admitted to our hospital had underlying diseases.

Clinical findings

In 41 of the patients who had been admitted to the University Hospital the following clinical symptoms were observed: fever in 35 of 41 patients, cough in 30 of 41 patients, abdominal pain and/or diarrhea in 11 of 41 patients, and symptoms relating to the central nervous system (confusion, somnolence or loss of consciousness, fainting) in 27 of 41 patients. All patients' chest radiographs showed an infiltrate. All patients were treated with standard antibiotic treatment consisting

of a macrolid (clarithromycin), or preferably a fluoroquinolone (levofloxacin) for at least 14 days [1]. Four patients required mechanical ventilation. Four patients, two of whom had received mechanical ventilation and who had been treated in the University Hospital, died between several hours and eight days after admission. The other patients responded well to treatment and the majority recovered promptly. The median length of hospital stay of the patients, who were discharged until 22 January 2010, was 9.9 days (range 4-16 days).

Laboratory investigations

For the investigation of the outbreak, the case definitions of the European Working Group on *Legionella* Infection were applied. Considering clinical and laboratory criteria, all cases were classified as confirmed by positive results from laboratory tests for urinary antigen or culture [2]. Microbiological investigations of the majority of patients' specimens were performed in the Institute of Medical Microbiology and Hygiene, University Hospital of Ulm, Germany. Rapid testing of urine specimens for *Legionella pneumophila* sg 1 soluble antigen using an immunochromatographic card assay (Inverness Medical) was performed in patients with clinically suspected Legionnaires' disease. Results were confirmed by retesting concentrated urine specimens with an enzyme immunoassay (Biotest). Expecterated sputum samples and other respiratory secretions were plated onto buffered charcoal yeast agar. Colonies suspicious for *Legionella* were serotyped using a latex agglutination assay (Oxoid). In addition, respiratory samples were processed for detection of *Legionella* specific DNA by nucleic acid amplification. DNA was isolated from respiratory samples using the Magna pure system (Roche) and a *L. pneumophila* specific real-time LightCycler PCR was run targeting the macrophage infectivity potentiator (mip) gene as described previously [5].

Results

All patients with a microbiological workup in our laboratory had a positive urinary antigen test, in the respiratory material of 10 patients PCR for *L. pneumophila* was positive. Four clinical isolates belonging to sg1 were further subtyped by using monoclonal antibodies (MAb). All these isolates were identified as monoclonal subtype Knoxville (carrying the virulence associated epitope recognised by MAb 3-1 monoclonal antibody) [3]. Molecular identification of cultured legionellae was achieved by 16S rRNA gene sequencing that showed a 99% homology to *L. pneumophila* in three isolates. So far, one isolate was genotyped and belongs to sequence type (ST) 62 [4].

Epidemiological investigations and findings

Epidemiological and environmental investigations are coordinated by the local authorities in Ulm and Neu-Ulm, with support of the state health offices in Baden-Württemberg and Bavaria. Investigations are

in progress to identify the potential source of this outbreak by comparing environmental isolates from the patients' domestic installations and wet cooling systems from the areas of both cities with the clinical ones. According to the patient interviews, the cases had no common exposure to water supplies in public buildings, hotels, sport facilities or similar sources. Only living in, working in, or visiting Ulm or Neu-Ulm appeared as a common characteristic of the affected persons. Further epidemiological investigations are planned.

Conclusions

This is the largest community associated outbreak of Legionnaires' disease recognised in Germany so far. In dealing with the event several important steps in outbreak detection and management were confirmed: the importance of a rapid clinical diagnosis and thorough microbiological confirmation and the immediate involvement of the health authorities. The latter is necessary to initiate investigations to detect the source of the outbreak, to raise awareness of the problem in the community and to optimise communication of all involved parties. Furthermore we realised that an outbreak of Legionnaires' disease most likely related to wet cooling systems is not restricted to the warm seasons.

Hospitals, general practitioners and the public have been informed of the situation by the local authorities on a regular basis, starting on 5 January 2010. As of 23 January 2010, health authorities have no indication that persons from other countries have been affected. The last clinical case was hospitalised on 13 January 2010. We would be grateful for any reports of cases from other countries which could potentially be linked to this outbreak.

FIGURE 1

Age and sex distribution in patients with Legionnaires' disease, Ulm/Neu Ulm, Germany, as of 22 January 2010 (n=65)

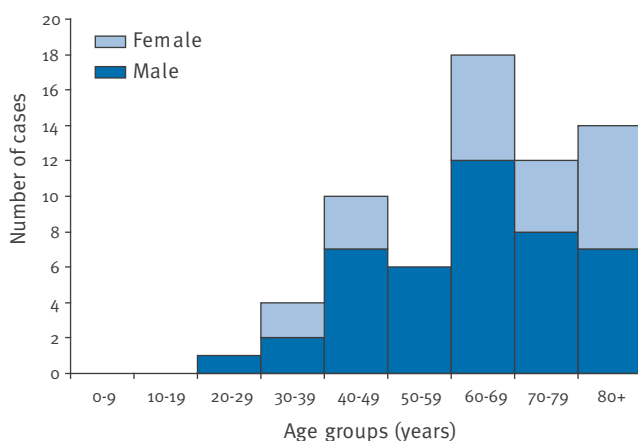
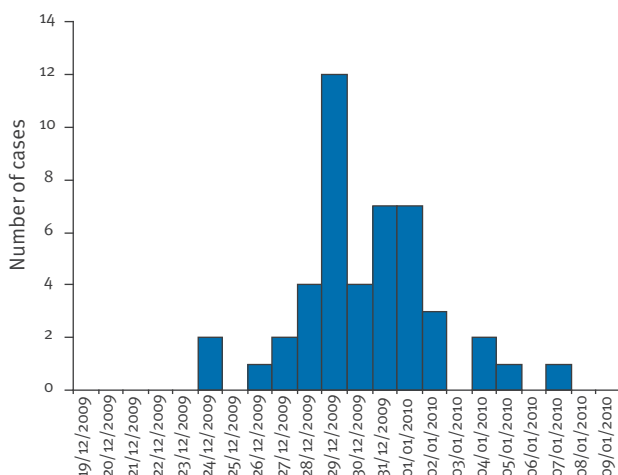


FIGURE 2

Onset of disease in patients with Legionnaires' disease, Ulm/Neu Ulm, Germany, information available as of 22 January 2010, (n=46)



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Avian influenza A(H5N1) in humans: lessons from Egypt

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Highly pathogenic avian influenza A(H5N1) has ravaged the Egyptian poultry population. Ninety human cases, including 27 fatalities have been recorded by 30 December, 2009. However, epidemiological information on the infection in humans in Egypt is scarce. We analysed the first three years of highly pathogenic avian influenza A(H5N1) in Egypt between 20 March 2006 and 31 August 2009) and found that more cases occurred in females than males, especially in 2006 and 2007. Women in the age group 20-39 years had the greatest tendency to be infected. It took an average of one day and 18 hours to seek medical assistance in patients who recovered and of six days in fatal cases. Children sought treatment much earlier than adults. On average, a patient died 11 days after the onset of symptoms. Exposure to infected poultry remained the most important risk factor.

Introduction

On 17 February 2006, highly pathogenic avian influenza A(H5N1) was first reported in the poultry population in Egypt [1]. Since that time, the infection had affected at least 21 governorates forcing over 1.5 million individuals to loose their source of livelihood [1]. Overall, 370 backyard poultry flocks, 850 farms, and four zoos have been affected, and more than 36 million birds (mainly chickens) have died or have been culled in Egypt at an enormous cost to the country [1]. Currently, the virus is endemic in the Egyptian poultry population.

The first human case of avian influenza A(H5N1) in Egypt occurred on 17 March 2006 [2], and to date (30 December 2009), the statistics of human infection and fatalities continue to rise. Specifically, 90 human cases (approximately one fifth of the total global count), including 27 fatalities (approximately one eleventh of the global count) have been recorded in Egypt as of 30 December 2009 [2]. These numbers rank Egypt third in the list of recorded human cases and fatalities in the world, after Indonesia and Vietnam, and remain by far the highest in Africa. The World Health Organization (WHO) had previously stated that “countries around the world had improved their defenses against bird flu, but the situation remained critical in Egypt and Indonesia where the risk of the H5N1 virus mutating into a major human threat remains high” [3].

Worrisome with the situation in Egypt is the frequency with which women and young people are being infected and the very current trend of rising infections in children: in 2009 alone, 79% of all infected individuals were under 10 years old. Between January and December 2009, 17 of the 34 recorded cases involved children between 12 and 30 months-old. Similarly, at the time of this report, human cases of 2009 pandemic influenza A(H1N1) had also been confirmed in the Egyptian population, which raises the possibility of co-infection and the emergence of reassortant viruses.

While the situation in Egypt remains critical, empirical evaluation of its peculiarities seem to be lacking, except for a very recent report by Dudley [4]. Assessment of the scientific literature and epidemiological data returned little or no concrete evidence from Egypt. However, the country has provided adequate records to international organisations like the WHO and the World Organization for Animal Health (OIE) and these reports have improved significantly since the first submission in terms of spatial and temporal data, and clinical records of affected persons.

In this study, we analysed the records on avian influenza A(H5N1) in Egypt between 20 March 2006 and 31 August 2009 and explain the epidemiological significance of our findings.

Materials and Method

The Egyptian government reports to the WHO, available on the WHO website [2], were the primary source of data for these analyses. We considered all laboratory-confirmed human cases of avian influenza A(H5N1) reported to the WHO from Egypt between 20 March 2006 and 31 August 2009. All positive samples reported and used in these analyses had earlier been confirmed by microneutralisation assay on serum or by PCR on respiratory tract specimens as reported [5]. Similar confirmatory tests were done in the Egyptian national reference laboratory and at the WHO reference laboratories for diagnosis of influenza A(H5) infection, including the United States Naval Medical Research Unit 3 in Cairo, Egypt [4].

The parameters included in our analysis were: date of exposure, date of onset, course of symptoms, and time

from hospitalisation until death/recovery, as listed in the WHO situation reports on avian influenza [6].

In the absence of complete information, reports were based on approximate dates and times from the reports. However, in cases of ambiguity arising from the records, such data were excluded from the calculations. In total, 85 confirmed cases were reported during the study period, of which 27 were fatal. After the exclusion of ambiguous data, only 63 of the 85 reported cases and 20 of the 27 fatal cases were evaluated for symptoms and hospitalization; and 44 of the 58 cases who recovered or were stable were analysed for symptoms and recovery. Analyses were performed using StatGraphics v2.0. Distributions were compared using chi-square test, and medians were compared using Fisher's exact test.

Results

Demographic characteristics

In the period under analyses, 85 cases were evaluated, 32 of whom were male and 53 were female. Eighteen cases had been reported in 2006, 25 in 2007, eight in 2008 and 34 to date (31 August) in 2009, including a total of 27 human fatalities over the three and a half-year period.

The youngest cases were one year of age (two boys), and the oldest case was a 75 year-old woman. The median age of all confirmed cases was six years. The age of the cases ($n=85$) ranged from 12 months to 75 years, with a mean of 13 years and two months. The median age of all fatalities ($n=27$) was 25 years (range: four to 75 years) and the mean was 26 years and three months. The median age of the female cases ($n=53$) was 15 years, (range: 14 months to 75 years) and the mean was 16 years and 10 months, while the median age of the male cases ($n=32$) was four years (range: two months to 32 years) and the mean seven years and two months (Table, Figures 1 and 2).

The overall sex ratio (male:female) was 0.6 and the annual sex ratios were 0.4 (2006), 0.4 (2007), 1.0 (2008) and 0.9 (2009). By age, the sex ratios (male:female) were 1.1 (<10 years), 0.3 (10-19 years), 0.1 (20-29 years), 0.2 (30-39 years), 0 (40-49 years; no case) and 0 (>50 years; all cases female), with $\chi^2=11.87$ in Pearson's chi-square test, $p=0.001$ in Fisher's exact test, and degree of freedom (DF)=1 (see Table).

Intervals

The number of days from onset of symptoms to hospitalisation (S-H) for all cases was calculated for 63 of the 85 cases. The median was two days (range: 12 hours to 11 days), the mean was two days and 19 hours. For the fatal cases (17 of 27 were included in the analysis), the median (S-H) was six days (range: two to 11 days) with a mean of six days. Among the recovered cases (47 of 58 were included in the analysis), the median (S-H) was one day (range: 12 hours to five days), and the mean was one day and 18 hours.

The time from onset of symptoms to death had a median of nine days (range: five to 30 days) and a mean of 11 days, while the time from hospitalisation to death had a median of four days (range: one to 25 days) and a mean of six days. The S-H in children and teenagers between the ages of 10 and 19 years ($n=51$) had a median of one day (range: 12 hours to eight days) and a mean of two days and 12 hours, in contrast to the adults over 20 years of age ($n=12$), in whom the median was four days (range: 12 hours to 11 days) and the mean was four days. Many (19) of the adults did not present with full hospital records and were not included in the analysis for hospitalisation.

Mortality

The overall case fatality rate was 32% (27/85). It was much lower in male (3/32) than in female (24/53) cases. According to age, the case fatality was two of 49 in the under 10-year-olds, eight of 13 in the 10-19-year-olds, seven of nine in the 20-29-year-olds, eight of 12 in the 30-39-year-olds, and two of two in the over 50-year-olds (there were no cases among the 40-49-year-olds). In the years under review, the case fatality was 10 of 18 for 2006, nine of 25 for 2007, four of eight for 2008 and four of 34 for 2009, with $\chi^2=10.81$ in Pearson's chi-square test and DF=4).

Discussion

This study is subject to some limitations. We conducted our analyses based on the limited data available for scrutiny. We suspect that cases have been missed because of the current surveillance system in humans which targets only severe infections backed by laboratory confirmation [7]. If this is so, Egypt may have had many more cases and possibly fatalities than reported and used in this work. People trying to avoid hospitalisation, especially among the adults, may also have contributed to underreporting.

In this analysis, the female cases had a wider age window (14 months to 75 years) than the male cases (12 months to 32 years). Since exposure to poultry remains the most important risk factor for human infection in Egypt, this may reflect the fact that across all age groups, more women than men are involved in poultry-related activities. All infected individuals with the exception of three (whose exposure status was uncertain) had been exposed to infected poultry or poultry products or to slaughtered or defeathered infected birds. In children and young adults, however, infection was more prevalent among males, although it is not clear why. Although infections in children peaked in the years 2007 and 2009, the reason for this is not yet clearly understood. Strong peaks of infection usually appear to follow periods of relaxation of preventative measures [7].

It also appears that especially in the group of the 20-39-year-olds, women had a greater tendency to be infected and more women died post infection. Fifteen of 21 infected women in this age group died.

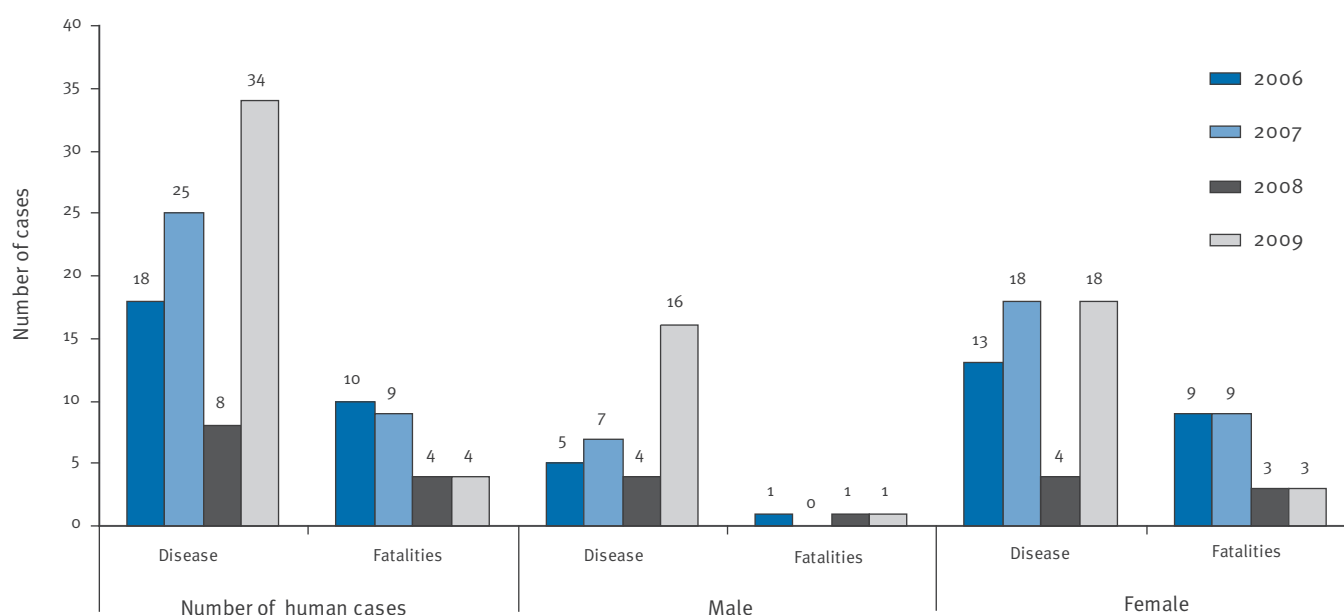
TABLE

Human cases and fatalities associated with avian influenza A(H5N1) in Egypt, 20 March 2006–31 August 2009 (n=85)

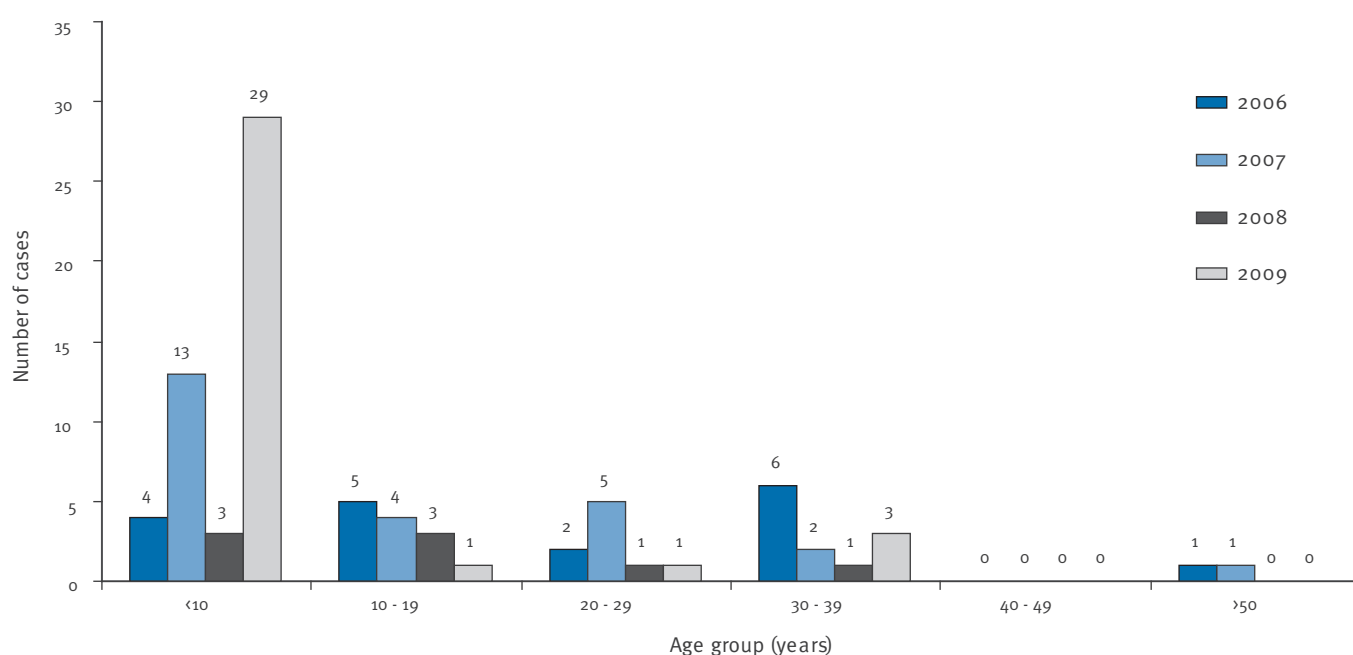
Year	Human cases and fatalities distributed according to sex						Human cases (disease) distributed according to age					
	Number of human cases						Age range of human cases (years)					
	Total		Male		Female							
	Disease	Fatalities	Disease	Fatalities	Disease	Fatalities	<10	10-19	20-29	30-39	40-49	>50
2006	18	10	5	1	13	9	4	5	2	6	0	1
2007	25	9	7	0	18	9	13	4	5	2	0	1
2008	8	4	4	1	4	3	3	3	1	1	0	0
2009	34	4	16	1	18	3	29	1	1	3	0	0
Total	85	27	32	3	53	24	49	13	9	12	0	2

FIGURE 1

Distribution of human cases of avian influenza A(H5N1) by sex, Egypt, 20 March 2006–31 August 2009 (n=85)

**FIGURE 2**

Distribution of human cases of avian influenza A(H5N1) by age group, Egypt, 20 March 2006–31 August 2009 (n=85)



These groups face the highest risk of exposure as it is mainly they who are involved in home slaughtering and defeathering of chicken and preparation of food, farm work and visits to infected farms. A recent study has analysed the age and sex bias with regards to the situation in Egypt [4], and it has been reported that farmers from other infected African countries believe that there is little or no risk of infection from culling, defeathering, home slaughtering and visit to infected premises [8,9]. In addition, failure of the government to pay compensation in Egypt for culled birds and the practice of keeping of poultry on rooftops and in close association with humans may have played a role. Although no association has yet been established between the level of exposure to avian influenza A(H5N1) and fatalities in Egypt, reports on workers in Asia showed that a high prevalence of infection in the poultry population is associated with a higher incidence of infection in humans, and that controlling such outbreaks of H5N1 influenza in the poultry flocks can stop human infection [7,10,11]. In addition, genetic characterisation of viruses from both the human and avian populations in Asia revealed that the viruses from both species were very similar [9,10].

According to our analysis, early hospitalisation following infection increased the chances of recovery. Children tend to be hospitalised earlier than adults and this may have contributed to the significantly lower death rate in the children (only two cases in children under the age of 10 years were fatal). Similarly, although 62 of the 85 cases were under 19 years old, this does not represent national demography since only approximately 32% of the population are 15 years and younger [12]. In most parts of Africa, people are known to visit a hospital less frequently as they advance in age, and supposedly non life-threatening conditions such as seasonal influenza are often treated at home and therefore underreported [8].

The overall case fatality in this study was 32% (27/85). This percentage may appear small when compared with statistics from other places, for example 82% in Indonesia (115/141), 68% in Thailand (17/25), 66% in China (25/38) and 50% in Vietnam (56/111). Nevertheless, with the exceptional surge in number of cases (especially in children) arising in Egypt in 2009 and the recent reoccurrence of human cases of avian influenza A(H5N1) in China and Vietnam despite an intensive control programme in the poultry populations, the pandemic potential of this virus is still very evident. Case fatality was significantly higher in females compared with males, but whether this is related to exposure dose can not be confirmed in this analysis.

As previously suggested by Briand and Fukuda [9], public health guidelines in Egypt will need to be tailored to meet the local situation taking into consideration the agricultural practices and the people's perceptions. It will also be necessary to conduct more studies on

human H5N1 influenza infection in Africa to evaluate the situation of asymptomatic carriers and unreported cases.

Finally, as evident in this analysis, exposure to infected poultry remains the only common denominator and an important risk factor for the spread of avian influenza A(H5N1) in humans in Egypt. Other workers had identified and reported the same risk factor exposure to sick poultry previously [10,11].

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Campylobacteriosis and other bacterial gastrointestinal diseases in Sofia, Bulgaria for the period 1987-2008

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Campylobacter is the most commonly reported enteric bacterial pathogen in humans. We still do not have any systematic data concerning campylobacteriosis in Bulgaria. For that reason, we present data of the thermophilic *Campylobacter jejuni* and *Campylobacter coli* in the aetiology of diarrhoeal diseases in Sofia, for the period from 1987 to 2008. The study included patients from 0 to over 65 years old. A total of 51,607 faecal specimens were screened for *Campylobacter*. *C. jejuni* and *C. coli* were detected in 3.58% (1,847) of the strains, with the highest percentage in 1988 (7.5%) and the lowest in 2006 (0.3%). Campylobacteriosis occurred most frequently in the wet months of March, April, May and June, with 105, 102, 124 and 141 cases, respectively, and was rare in January with 25 cases. The most affected groups were children between 0 and 4 years of age (52%) and between five and 14 years of age (30%). *Campylobacter* infection occurred in 22% of all bacterial gastrointestinal diseases in the city of Sofia during the study period. *Salmonella* was the most frequently identified pathogen with 32%, followed by *Shigella* (30%), *Campylobacter* (22%) and diarrhoeagenic *Escherichia coli* (16%). The study shows that *Campylobacter* plays an important role as a bacterial cause of enterocolitis in Sofia, Bulgaria.

Introduction

Campylobacteriosis is an infectious disease caused by thermophilic members of the bacterial genus *Campylobacter*. *C. jejuni* and *C. coli* are among the most important enteropathogens that cause gastroenterocolitis. The rate of *Campylobacter* infections worldwide is increasing, with the number of cases often exceeding those of salmonellosis and shigellosis [1,2]. These reported numbers of campylobacteriosis in many countries have revealed that this infection is emerging and becoming a major public health problem. According to the World Health Organization (WHO) *Campylobacter* is one of the most frequently isolated bacteria from stools of infants with diarrhoea in developing countries [3]. Despite the fact that campylobacteriosis is a notifiable disease in Bulgaria, there is no systematic data concerning this infection. In this report, we present data on the role of *C. jejuni* and *C. coli* compared to the

other bacterial agents of diarrhoeal diseases in Sofia, Bulgaria.

Methods

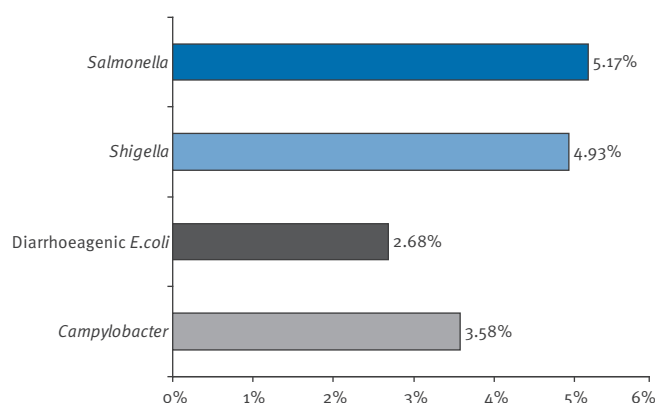
The study covered a period from 1987 till 2008 in Sofia, Bulgaria. Sofia has a population of about 1.5 million inhabitants. A total of 51,607 faecal specimens obtained from patients with enterocolitis were investigated for *Campylobacter*, *Salmonella*, *Shigella* and diarrhoeagenic *Escherichia coli*, i.e. enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) *E. coli*. Data were provided from the department of epidemiology at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, based on isolations of these bacterial pathogens by the Regional Inspectorate of Public Health Protection and Control, Sofia, and by five hospital and private laboratories in Sofia. The age of the patients ranged from 0 to over 65 years.

Culture media

The laboratory methods for *Salmonella*, *Shigella* and diarrhoeagenic *E. coli* were done according to the national standard method for diagnosis of enteric bacteria [4]. Faecal specimens for *Campylobacter* were

FIGURE 1

Percentage of bacterial enteropathogens isolated from 51,607 faecal samples collected in Sofia, Bulgaria from 1987 to 2008



inoculated on selective media containing 10% defibrinated sheep blood agar with *Campylobacter* selective supplement (BUL BIO-NCIPD, Bulgaria) and five antibiotics (vancomycin, trimethoprim, cefalotin, rifampicin and nystatin). The inoculated selective media were incubated for 48 hours in microaerophilic atmosphere with 10% CO₂ and 5-8% O₂, generated from packages Helico-Campy Pack (BUL-NCIPD, Bulgaria).

Results

Isolates

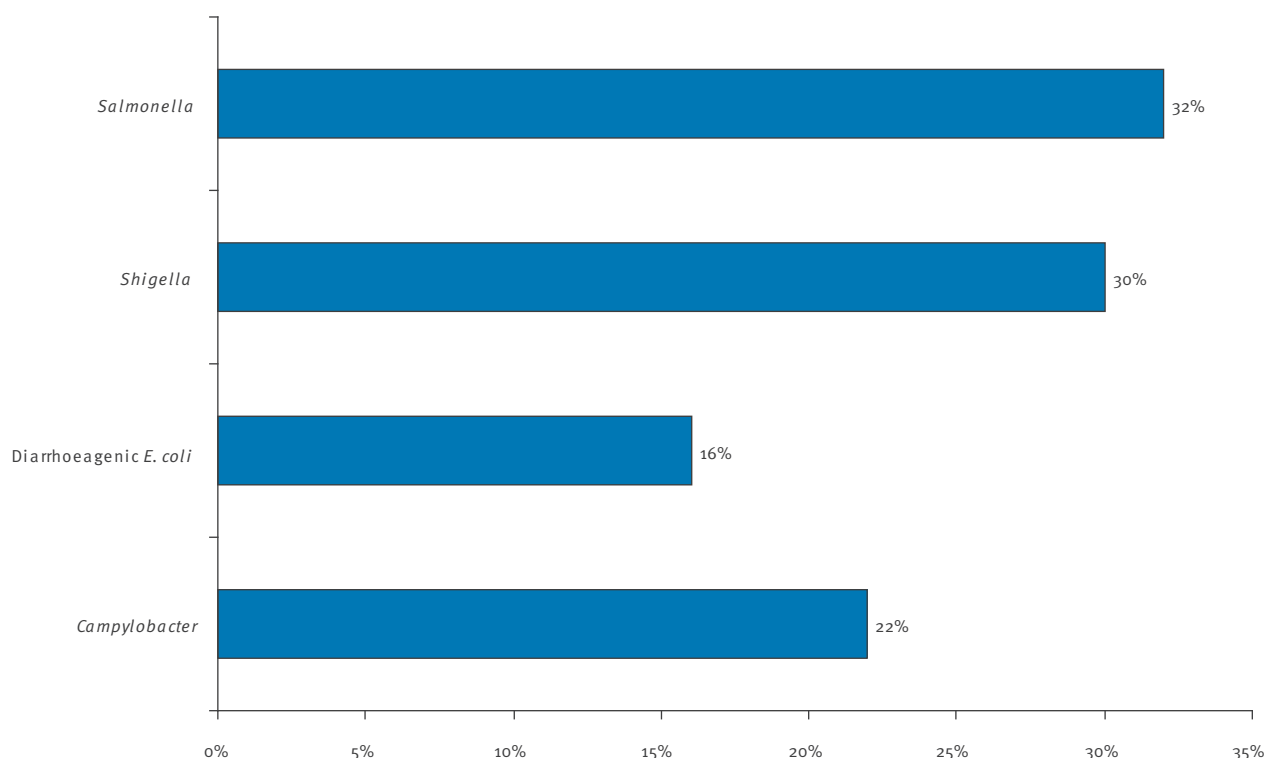
From the 51,607 investigated stool specimens, 1,847 isolates of *Campylobacter* (3.58%) were obtained. Of

these, 75% were *C. jejuni*, 22% were *C. coli* and 3% belonged to other species. *Salmonella* was isolated most frequently, from 5.17% of the samples, followed by *Shigella* (4.93%), *Campylobacter* (3.58%) and diarrhoeagenic *E. coli* (2.68%) (Figure 1).

For the period of the study, *Campylobacter* infection occurred in 22% of all the bacterial gastrointestinal diseases in the city of Sofia. *Salmonella* was the most frequently isolated pathogen with 32%, followed by *Shigella* (30%), *Campylobacter* (22%) and diarrhoeagenic *E. coli* (16%) (Figure 2).

FIGURE 2

Distribution of the pathogenic enteric bacteria isolated from faecal samples collected in Sofia, Bulgaria from 1987 to 2008 (n=8,396)



TABLE

Proportion of pathogenic enteric bacteria isolated from 30,033 faecal samples in Sofia, Bulgaria, 1987-1997 (n=4,235)

Year	Bacterial pathogen (%)			
	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Diarrhoeagenic E.coli</i>
1987	6.20	4.00	2.00	2.00
1988	7.51	4.95	2.66	2.17
1989	5.00	4.55	0.67	2.67
1990	5.00	3.30	2.08	1.20
1991	2.47	3.10	3.13	2.37
1992	3.30	3.10	3.30	4.40
1993	6.17	2.61	3.00	3.30
1994	1.66	2.04	11.80	2.87
1995	5.17	2.19	6.55	1.75
1996	5.54	2.33	2.37	1.98
1997	6.49	1.59	1.70	2.90
Average (%)	4.95	3.07	3.57	2.51

Although *Salmonella* was on average the predominant enteric pathogen during the study period as a whole, *Campylobacter* predominated in the years 1987 (6.42%), 1988 (7.61%), 1989 (5.00%), 1990 (5.00%), 1993 (6.17%), 1996 (5.54%), 1997 (6.49%), 1999 (4.20%), 2000 (2.70%) and 2001 (4.90%). The highest proportion of *Campylobacter* was found in 1988 (7.50%) and the lowest in 2006 (0.30%).

In our previous study of 30,033 faecal specimens from the patients with enterocolitis in the period from 1987 to 1997, *Campylobacter* ranked first (4.95%) among the bacterial causes of enterocolitis [5], followed by *Shigella* (3.57%), *Salmonella* (3.07%) and diarrhoea-genic *E. coli* (2.51%) (Table).

Seasonal distribution

The peak of *Campylobacter* infection in our study was in the wet months of spring and summer: on average 105 cases in March, 102 cases in April, 124 cases in May and 141 cases in June.

Age distribution

An analysis of the age-specific incidence (Figure 3) showed that children up to the age of four years were the age group most affected by campylobacteriosis in Sofia (52%), followed by the group of 5-14-year-olds (30%), the group above the age of 65 years (6%), the 15-24-year-olds (5%), the 45-64-year-olds (4%) and the 25-44-year-olds (3%). In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%.

Discussion

Diarrhoeal diseases are a major problem for many countries in the world. The determination of the aetiological agent is an important step in the prophylaxis and the prompt treatment of enterocolitic infections.

In our study of 51,607 stool specimens, *Salmonella* was isolated most frequently, which correlates with reports of increasing incidence of human salmonellosis in Europe and the United States (US) in recent years [1,5]. The distribution of the different enteropathogenic bacteria among the positive faecal samples in our study was also similar to that observed in the US, where *Campylobacter* was isolated from 4.4%, *Salmonella* in 2.3% and *Shigella* in 0.9% of faecal samples in the same time period [7]. Campylobacteriosis was the leading cause of bacterial gastroenteritis reported in Belgium, Canada, Finland, Sweden, Central and South America, and southern states of Australia [8-10] during the time of our study.

In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%. These data correlate with findings of other authors [1,10].

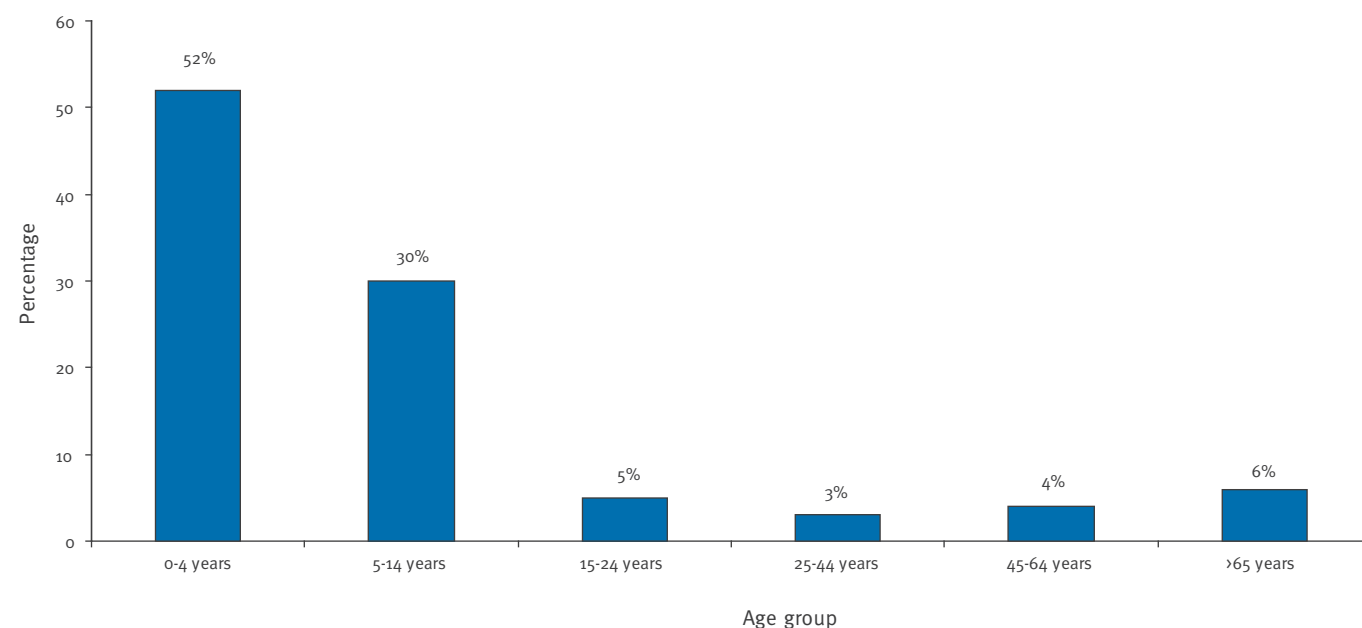
The diagnosis of *Campylobacter* in Sofia for the second decade in the study period, 1998-2008, was limited due to a shortage of data from hospital and private laboratories where the investigations are episodic.

Campylobacter enteritis has no seasonal preference in developing countries. In contrast, epidemics occur in summer and autumn in developed countries [1,11]. According to other authors in countries with moderate climate such as Bulgaria, *Campylobacter* is isolated most frequently in May, June and July [1,5], while the peak of *Campylobacter* infection in our study was in the wet months of spring and summer.

Our study provides data only for one region of Bulgaria, Sofia, although campylobacteriosis is notifiable disease in Bulgaria. The study showed the importance of thermophilic *Campylobacter* as a food-borne pathogen

FIGURE 3

Age distribution of patients with campylobacteriosis in Sofia, Bulgaria, 1987-2008



and underlines the need to strengthen surveillance of *Campylobacter* in Bulgaria. A lot of effort is needed to improve surveillance of campylobacteriosis in our country. Only a small number of laboratories are currently reporting *Campylobacter* cases. The main reason of the underreporting of campylobacteriosis in Bulgaria is the limited laboratory capacity for *Campylobacter* detection. The National Centre of Infectious and Parasitic Diseases in Sofia provides training in practical and theoretical courses on the diagnosis, treatment and epidemiology of campylobacteriosis. *Campylobacter* should be included in the set of enteric pathogens (*Salmonella*, *Shigella*, diarrhoeagenic *E.coli*, *Yersinia*) tested for in cases of diarrhoea.

In conclusion, the results of our investigation for the period of 1987–2008 show that *Campylobacter* plays an important role as a bacterial pathogen that causes enterocolitis in Sofia, Bulgaria. The most affected group were 0-14-year-old children. Despite the fact that campylobacteriosis is a notifiable disease, the investigations are episodic and there is no systematic data for our country. For that reason we consider it an urgent need to introduce systematic surveillance of this infection in Bulgaria.

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Phage typing of *Salmonella* Typhimurium – is it still a useful tool for surveillance and outbreak investigation?

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Phage typing has for decades been useful as a phenotypical, definitive method for epidemiological characterisation of *Salmonella* Typhimurium. The system recommended by the World Health Organization (WHO) Collaborative Centre for phage typing of *Salmonella* has, however, become rather complex, and the present study illustrates the challenges of sufficient standardisation of the interpretation of lysis results to make sure that the same strain is assigned to the same phage type in different laboratories. Even though molecular typing methods will replace phenotypic characterisation methods in the future, it is our opinion that phage typing will remain for some time a useful tool to strengthen global *Salmonella* surveillance. Therefore, improved standardisation and quality assurance is essential to obtain a robust and harmonised method that allows comparison of results between laboratories.

Epidemiological characterisation of pathogens causing human outbreaks of food-borne disease is essential for many reasons. The outbreak is often identified by increased registration of a specific pathogen (the Epi-type) during routine diagnostics and surveillance, and is further confirmed by detailed molecular characterisations that support the hypothesis of a common source of origin. Furthermore, identification of similar strains in historical specimen collections from monitoring programmes of food and food animal production can contribute to the generation of a hypothesis for the outbreak investigation. During the outbreak investigation, epidemiological characterisation constitutes the basis for the comparison of strains from human cases and potential sources, and finally, when the actual food source is found, constitutes the final demonstration of the infection source.

In the last decades more and more food-associated outbreaks have involved more than one country and even more than one continent, primarily due to the ever increasing globalisation of the food supply [1-3]. Identification and control of food-borne disease outbreaks of international significance can only be performed when professionals work together and agree

on common methods applicable for definitive characterisation of pathogens like, in our case, *Salmonella*.

Serotyping according to the Kauffmann-White scheme [4] has for more than 80 years been the primary characterisation of *Salmonella*. This method is widely applied all over the world and harmonised to a degree that allows results to be compared between laboratories and countries. Some serovars, e.g. *Salmonella* Enteritidis and *S. Typhimurium*, are, however, so dominant in especially Europe and the United States that further characterisation is needed for surveillance.

In Denmark, phage typing as described by the World Health Organization (WHO) Collaborative Centre for phage typing of *Salmonella* (Health Protection Agency (HPA), Colindale, United Kingdom) has been applied for surveillance of *S. Enteritidis* and *S. Typhimurium* in humans, food and food production animals. Phage typing has proven to be an important tool for strain characterisation and the results obtained have been used since the mid-90s in surveillance, source attribution and outbreak investigations [5,6]. Phage typing is, however, also a phenotypical method that depends very much on the experience of the individual laboratory and on support from the reference centre that coordinates the maintenance of phages and the updating of the system. Only when the phage typing method is harmonised and the performance in different laboratories is controlled, can the results be regarded as definitive and comparable between laboratories.

The challenges encountered when using phage typing have become clear in a number of outbreak investigations in Denmark. A year after a large outbreak of human salmonellosis in April 2008 [7], more than 1,300 cases have been registered, and despite intensive epidemiological and microbiological investigations it has not been possible to identify the infection source. The outbreak had been identified due to an increase in the number of laboratory registrations of human *S. Typhimurium* with a unique multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) type corresponding to phage type U292. The strains were

further characterised by pulsed-field gel electrophoresis (PFGE) which showed a unique *Xba*I-profile, confirming the epidemiological relationship between the human cases [8].

The strains from the outbreak described here were phage-typed at the National Food Institute as is routine for all *S. Typhimurium* strains from human cases, food items and food productions animals in Denmark. The lysis pattern obtained from the initial typing was characterised by strong reactions with phages 11 and 14, and weaker reactions with phages 26 and 35, although some variations in the extent of lysis were observed during the outbreak that did not influence the phage type. There was no reaction with the remaining routinely used phages. This lysis pattern was in acceptable agreement with phage type U292. In summer 2008 during the continued outbreak, representative outbreak strains were sent to the WHO Collaborative Centre for phage typing of *Salmonella*, where they were characterised as U276. The results obtained there were similar to those obtained at the National Food Institute, but the interpretation of the results was different. Identical strains from this outbreak were therefore assigned to two different phage types by two experienced laboratories. As the different interpretation was the result of weighing the interpretation of all lysis reactions against the others, it can be difficult to judge which is the 'right' type designation.

Even though phage typing during this outbreak has been a valuable tool for the national investigation, the disagreement between two laboratories puts into question its usefulness as a definitive typing system that would allow comparison and communication between laboratories and countries. Disagreement between laboratories has caused confusion in past outbreak investigations: In 2003, an outbreak of human salmonellosis was identified in Sweden, and Danish pork was pointed out as the most probable source of infection. The source of infection was identified as *S. Typhimurium* DT108, but because this *Salmonella* type had not been detected by the Danish surveillance of pigs and pork production, pork did not seem to be the likely source. However, based on further joint investigation by the two countries it emerged that the strain initially identified as *S. Typhimurium* DT108 in Sweden was known as *S. Typhimurium* DT170 in the Danish surveillance, and consequently Danish pork could not be ruled out as a probable source (unpublished results, Baggesen, 2003). Late in 2008, an outbreak of *S. Typhimurium* characterised by a specific MLVA-type was identified in Sweden, Norway and Denmark and traced back to Danish pork. Strains related to this outbreak was assigned to several phage types (U288 in Denmark, RDNC in Norway and U302 in Sweden), and only due to inclusion of MLVA results, these cases were recognised as a common outbreak [9].

Should these and similar experiences disqualify phage typing as method for definitive strain characterisation

in relation to diagnostics and surveillance of *Salmonella* infections? Several authors have suggested that genotyping methods such as PFGE and MLVA, combined with harmonised computer-based evaluation of the typing results and electronic exchange of data, can fulfil the requirements for definitive typing [10,11].

There is no doubt that molecular typing methods, eventually whole genome sequencing, will replace phenotypic characterisation methods in the future. But is this time now? Compared with the genotyping methods, sero- and phage typing are cheap and less labour-intensive methods based on simple technology for which only limited equipment is needed. This opens an opportunity for screening a large number of *Salmonella* strains as part of human diagnostics and monitoring programmes in food and food production animals not only in the developed part of the world but also in developing countries. Nowadays, more and more countries contribute to the international food supply. A strengthening of the global *Salmonella* surveillance with improved characterisation of strains from humans, food and animals, and sharing of the results among professionals is essential for food safety.

The outbreaks described in this paper suggest that phage typing currently has limitations, which could become worse if it was to be implemented globally for *Salmonella* surveillance. Improved standardisation and quality assurance is essential in order to obtain a robust and harmonised method that allows comparison of results between laboratories. The phage typing system recommended by the WHO Collaborative Centre and applied in the studies described here was first described 1959 by Callow [12] and has since been extended. Today it utilises a comprehensive number of phages, which leads to a large number of different patterns and thereby phage types. Assignment of a lysis pattern to a specific phage type is based on interpretation of the individual lysis reaction and comparison to a standard scheme of lysis patterns and phage types, a procedure that leaves room for conflicting results.

In addition, experience from the WHO Global Salm-Surv programme (<http://www.who.int/salmsurv/en>) has proven that the capacity and quality of *Salmonella* serotyping can be enhanced through regular training of diagnostics staff and implementation of international external quality assurance systems [13]. It is likely that similar capacity-building and harmonisation efforts could improve phage typing.

We believe that phage typing can, for a while yet, play an important role in surveillance and control of the common *Salmonella* serotypes. However, this requires strengthened efforts to make the system available to more laboratories internationally, possibly a simplification of the system to enhance its robustness even though this may slightly compromise its discriminatory power, and finally improved external and internal quality assurance systems.

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Zoonoses in Europe: distribution and trends - the EFSA-ECDC Community Summary Report 2008

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On 28 January 2010 the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) launched their annual report on zoonoses and food-borne outbreaks for 2008. The report provides a comprehensive overview of zoonotic infections and disease outbreaks caused by consuming contaminated food. The number of reported human cases of the three most reported zoonotic infections, was lower in 2008 compared to 2007.

Campylobacteriosis was the most commonly reported zoonosis in the European Union (EU) for the last five years followed by salmonellosis and yersiniosis. The declining trend of salmonellosis continued, most likely as a result of the intensified control of *Salmonella* in animal populations, particularly in poultry, and better hygiene throughout the food chain.

The number of confirmed cases of listeriosis decreased by 11% in 2008 (1,381) compared to 2007 (1,554) in the EU. Foodstuffs that are considered the main source for human listeriosis in the EU include ready-to-eat (RTE) products (fish and meat), soft cheeses, salads and sandwiches. An EFSA-ECDC collaborative survey on *Listeria* in RTE products and in clinical cases of human listeriosis started in January 2010, the results of which will contribute to a better understanding about listeriosis in the EU.

Q-fever increased by 172% in 2008 (1,594) compared with 2007 (585). This was mainly due to several outbreaks in people entering areas with infected sheep and goats mainly in the Netherlands. In-depth investigations have been carried out in affected countries and it is suspected that the occurrence of Q-fever in humans and animals may be seriously underreported in Europe.

A total of 3,159 confirmed cases of Shiga-toxin/verotoxin producing *E. coli* (STEC/VTEC) were reported in 2008, representing an 8.7% increase from 2007 (2,905 cases). In animals VTEC was mainly isolated from cattle and, in lower proportion from small ruminants such

as sheep and goats. In food, VTEC was detected in a considerable proportion of cow milk samples.

The 2008 annual Community Summary Report describes the five-year trends, distribution and 2008 figures for zoonotic infections and agents in humans, animals and foodstuffs in the 27 EU Member States, the European Economic Area and Switzerland [1]. Information aimed at protecting human health is collected and analysed according to the Zoonoses Directive 2003/99/EC [2]. Assisted by the Zoonoses Collaboration Centre (ZCC) in Copenhagen, Denmark, EFSA and ECDC jointly analysed the data. The results of this report highlight the importance of close collaboration between public health specialists and veterinarians and the need for robust surveillance systems in order to detect trends in zoonoses in Europe.

The full version with data per country and annexes are available on EFSA's and ECDC's websites.

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