Rev. sci. tech. Off. int. Epiz., 2006, **25** (2), 555-569

Escherichia coli: on-farm contamination of animals

J.M. Fairbrother & É. Nadeau

The *Escherichia coli* Laboratory, Faculté de médecine vétérinaire, Université de Montréal, 3200 Sicotte, Saint-Hyacinthe (Québec) J2S 7C6, Canada

Summary

Escherichia coli is one of the main inhabitants of the intestinal tract of most mammalian species, including humans, and birds. Shiga toxin-producing *E. coli* (STEC), also called verotoxinogenic *E. coli*, usually do not cause disease in animals but may cause watery diarrhoea, haemorrhagic colitis, and/or haemolytic uraemic syndrome in humans. Zoonotic STEC include the O157:H7 strains and, with increasing frequency, certain non-O157 strains. The importance of non-O157 zoonotic strains is probably underestimated as they have been less well characterised and are more difficult to detect in samples than O157:H7. Another large subset of STEC strains has been isolated from animals but has not, at the present time, been associated with disease in animals or humans. Cattle and other ruminants are the most important reservoir of zoonotic STEC, which are transmitted to humans through the ingestion of foods or water contaminated with animal faeces, or through direct contact with the infected animals or their environment. The main sources of STEC infection of cattle on-farm are the drinking water, the feed, and the immediate environment of the animal. Risk factors that have been identified for infection of animals with O157 STEC include age, weaning, movement of the animals, season, feed composition, and the ability of the bacteria to persist in the environment. On-farm control of the zoonotic risk of human infection with STEC should primarily target the main source of contamination: the animal reservoir. Various strategies to reduce intestinal colonisation of cattle by zoonotic STEC have been tried with varying results, including vaccination, treatment with probiotics, such as direct-fed microbials or competitive exclusion, administration of bacteriophages, and modification of the diet.

Keywords

Escherichia coli – Haemolytic uraemic syndrome – Haemorrhagic colitis – Non-O157 strain – O157:H7 strain – Probiotic – Shiga toxin – Shiga toxin-producing Escherichia coli – Vaccination – Verotoxin – Verotoxinogenic Escherichia coli.

Introduction

Escherichia coli is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but a small proportion are an important cause of disease worldwide. These potentially harmful *E. coli* are classified into categories based on the production of virulence factors and on the clinical manifestations that they cause.

Pioneering work in the 1970s demonstrated that certain *E. coli* strains produced a toxin, which was initially called verotoxin because of its distinct effect on Vero cells (27). This family of toxins was subsequently also called Shiga-like toxins, and more recently Shiga toxins (Stx), because of the close relation to the Stx of *Shigella dysenteriae* type 1. The latter nomenclature is now more universally accepted and will be used throughout the present review. The category of *E. coli* strains producing this family of toxins is

referred to as both verotoxinogenic *E. coli* and Stx-producing *E. coli* (STEC). The latter designation will be used throughout this review.

STEC are commonly found in a wide range of farm and wild animal species and, for the most part, do not seem to cause disease in animals (Table I) (6). However, strains of one subset of STEC are responsible for oedema disease in pigs (i.e. oedema disease *E. coli* – EDEC), and another group of STEC (i.e. non-O157 enterohaemorrhagic *E. coli* – EHEC) cause dysentery in young calves. Certain STEC strains are also zoonotic. Infection in humans is mainly associated with the ingestion of foods contaminated with the zoonotic bacteria, and clinical signs include watery diarrhoea, haemorrhagic colitis (HC), and/or haemolytic uraemic syndrome (HUS). These strains were originally named enterohaemorrhagic *E. coli* because of the associated clinical signs. In this review, the authors will refer to them as zoonotic STEC, which the authors feel is a more logical designation and results in a less confusing classification system for the unfamiliar reader (Table I). Another large subset of STEC strains have been isolated from animals, but have not as yet been associated with disease in animals or humans.

The common feature of all STEC is the production of bacteriophage-encoded Stx. These toxins belong to one of two main families, each with several variants. *Escherichia coli* strains belonging to over 200 serotypes can express Stx, but within most serotypes both Stx-positive and Stx-negative strains can be found (43).

In 1983, STEC strains of serotype O157:H7 were definitively linked for the first time to several major outbreaks of HC and HUS in the United States of America (USA) and Canada. Zoonotic STEC-related disease has been observed worldwide, and in most industrialised countries O157:H7 remains the predominant serotype. In addition, an increasing association has been observed between certain zoonotic non-O157 STEC strains, most often of the serogroups O26, O103, and O111, and outbreaks or sporadic cases of HC and HUS. Cattle are the main reservoir for zoonotic STEC throughout the world. The advent of selective media and kits for the rapid identification of O157:H7 strains has permitted a more accurate assessment of the role of this serotype in human disease outbreaks and the transmission of the infection from animal reservoirs. However, a lack of similar tests for the rapid and easy identification of zoonotic non-O157 STEC and of other STEC, which are found in the intestinal tract of animals but have not yet been implicated in human infections, has impeded assessment of the geographical distribution of these strains, the mode of transmission to humans, and the prevalence of these strains in human outbreaks and in animal reservoirs. Also, because of the use in many laboratories of selective media for the specific detection of O157:H7, on which most non-O157 STEC are not readily identified, the prevalence of non-O157 STEC is probably underestimated.

Hazard identification and characterisation

Characteristics of zoonotic Shiga toxin-producing *Escherichia coli*

More than 60 of the 200 Stx-positive *E. coli* serotypes have been associated with HC or HUS in humans, the predominant serotype being O157:H7. The most common

Table I
Classification of Shiga toxin-producing *Escherichia coli* (STEC) found in animals

Type	STEC subsets: common designation	Common serotypes/ serogroups	Geographical distribution	Animal reservoir	Site of isolation in animals and derived products
Zoonotic	O157 EHEC	O157:H7	Worldwide, more common in industrialised countries	Cattle, sheep, goats, pigs [c]	Intestine, faeces, meat, milk, cheese
	Non-O157 EHEC	O26 [b], O111 [b], O103, O113, O145	Worldwide	Cattle, sheep, goats, pigs, chickens	Intestine, faeces, meat, milk, cheese
Potentially zoonotic [a]	None	O17, O56, O87, O108, O109, O130, O136, O149	Worldwide	Cattle, sheep, goats, pigs	Intestine, faeces, meat
Animal pathogenic	EDEC	O138, O139, O141	Worldwide	Pigs	Intestine

a) not as yet associated with disease in animals or humans; few data are available on the characterisation of the virulence factors associated with these strains. Source: website of MicroBioNet, serotypes of verotoxinogenic *E. coli* (http://www.microbionet.com.au/vtectable.htm)
b) strains of some serotypes also cause haemorrhagic enteritis in cattle
c) probably an accidental host
EDEC: oedema disease *E. coli* which causes oedema disease in pigs
EHEC: enterohaemorrhagic *E. coli*

non-O157:H7 serotypes associated with human disease include O26:H11, O103:H2, O111:NM, and O113:H21. STEC of many other serotypes have been found in animals, but they have not as yet been associated with disease in animals or humans (2) (Table I). The zoonotic potential of these strains is not yet known. The O157:H7 STEC and many of the zoonotic non-O157 STEC possess in their chromosome a large multi-gene pathogenicity island, called the locus for enterocyte effacement (LEE), which contains the genes that enable the bacteria to attach to the gut epithelium and efface the microvilli. However, it is now recognised that some of the zoonotic non-O157 strains, such as some of the O111 (42) and the O113 (49) strains, do not possess the LEE and, hence, adhere to and colonise the gut epithelium by means of other uncharacterised adhesins. The O157:H7 STEC and many of the zoonotic non-O157 strains possess a large plasmid that contains the genes for several possible adhesins and for an enterohaemolysin, which may be involved in causing disease. Sequencing of the entire O157:H7 genome and genome-based studies have facilitated the identification of several additional possible virulence factors that are also present on many zoonotic non-O157 STEC, although the role of these virulence factors in the development of disease has not yet been determined. The elucidation of the role of the various virulence factors in causing human disease could eventually enable laboratories to predict the zoonotic potential of a strain based on its virulence factor profile.

In human cases, identification of the zoonotic STEC, the suspected food sources of infection, and the potential animal reservoirs is based on the detection of strains producing one or more of the Stx. This has traditionally been accomplished by observation of the effects of the Stx produced by the bacteria, using time-consuming cell culture or immunological techniques. More and more laboratories are using highly sensitive and rapid molecular techniques, such as polymerase chain reaction (PCR), to detect the genes encoding the Stx: the original sample or a direct broth culture of the sample are used as the test material and the STEC colonies are isolated and identified by PCR. As many STEC have not yet been associated with disease in humans, detection of genes for virulence factors, in addition to identification of the Stx, will provide more information on the pathogenic potential of the strain, and serotyping will confirm the identification. It is important to keep in mind that the increasing reliance on molecular techniques used to initially screen for STEC may result in a failure to identify emerging STEC producing new variants of the Stx which are less closely related genetically. Hence, it will be important that at least some reference laboratories continue to screen for toxin production via observation of the biological effects of the toxin.

The use of selective growth media, which facilitates selective growth of the bacteria based on the characteristic ability of the strain to slowly ferment sorbitol, and of immunomagnetic separation techniques, which detect the O157 antigen, has permitted the rapid and sensitive detection of O157:H7. These methods are particularly useful in small laboratories that are not equipped to carry out molecular or tissue culture techniques. However, these techniques do not detect non-O157 STEC, which usually ferment sorbitol and do not contain the O157 antigen. Immunomagnetic separation techniques and selective media are being developed for non-O157 STEC, such as O26, O103, O111 and O145 (7, 23, 51), which will facilitate rapid identification of these serotypes. O157:H7 isolates have been further characterised and sub-typed by pulse-field gel electrophoresis. Standardisation of this technique by the Centers for Disease Control and Prevention (CDC) in the USA and the establishment of PulseNet, a database and network into which strain profiles are deposited by laboratories throughout the USA and Canada, permits, by cluster analysis, the rapid tracing of O157:H7 isolates to non-human sources and identification of common source outbreaks (16, 64).

How zoonotic Shiga toxin-producing *Escherichia coli* cause disease

Zoonotic STEC cause non-bloody to bloody diarrhoea in humans. Concurrent HUS may lead to acute kidney failure, especially in children and elderly patients. The steps in the development of disease are shown in Figure 1.

The clinical course and outcome of disease due to O157 and non-O157 STEC appears to be similar, but O157 STEC may be more frequently associated with HC. The risk of disease associated with O157:H7 can be high, even at doses of < 1,000 bacteria (66), although this may be related to a variety of factors, including acid resistance of the bacteria and, hence, the ability to survive in acidic foods, which varies greatly between isolates. The infectious dose for O111 strains appears to be similar (50) but is unknown for other non-O157 STEC.

The geographical distribution of zoonotic Shiga toxin-producing *Escherichia coli* infections

STEC infections occur worldwide but are most commonly reported in the USA and Canada (43). In the USA, it is estimated that every year O157 STEC infection causes 73,000 cases of illness and approximately 61 deaths, and that zoonotic non-O157 STEC infections are responsible for about half this number of cases and deaths (40). The estimates for zoonotic non-O157 STEC are considered to be less accurate because these cases are not routinely reported and few laboratories are capable of identifying non-O157 STEC strains. The number of reported outbreaks of O157 STEC infections began rising in 1993,

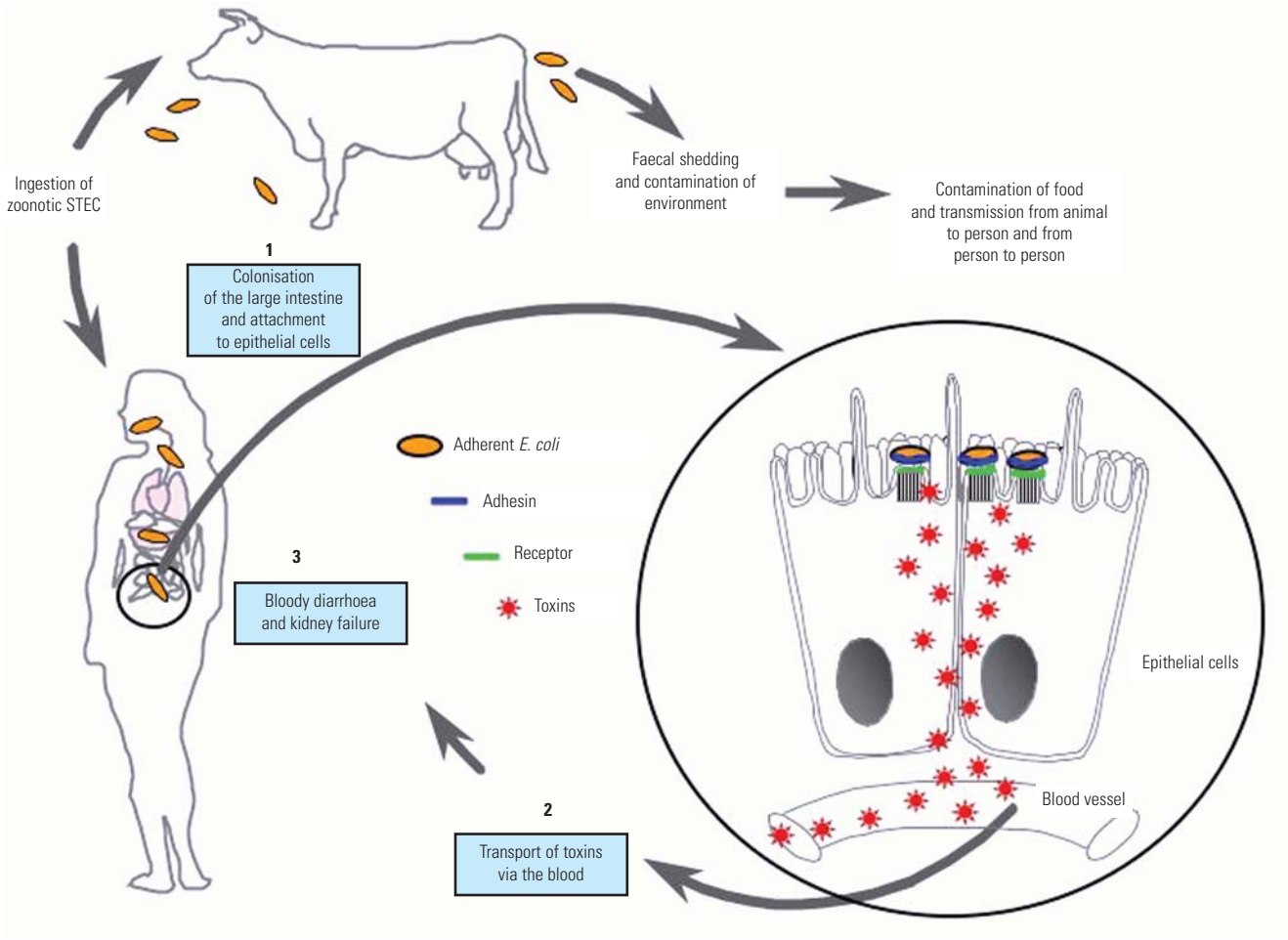

Fig. 1

How zoonotic Shiga toxin-producing *Escherichia coli* (STEC) cause bloody diarrhoea and haemolytic uraemic syndrome in humans

Zoonotic STEC principally colonise the large intestine (1). The adherent bacteria produce Shiga toxin which is transported across the epithelial cells and via the blood (2). This toxin acts on the endothelial cells of blood vessels and causes non-bloody to bloody diarrhoea and abdominal cramps (3)

Source: website of the *Escherichia coli* Laboratory (www.ecl-lab.ca)

peaked in 2000, and has subsequently decreased (55). On the other hand, the size of outbreaks steadily declined from 1982 to 2002. The increase in the number of outbreaks and the corresponding decrease in the size of the outbreaks is probably a result of greater public awareness of the association between STEC infections and illness, improved techniques for the detection and identification of O157:H7, increased testing for O157:H7 following its designation as a notifiable infection (and the subsequent requirement for the mandatory reporting of cases), and improved tracing of outbreaks due to the introduction of molecular subtyping and PulseNet. Most outbreaks occurred from May to November, and outbreaks appeared to be more common in the northern states of the USA and western provinces of Canada.

Zoonotic STEC is also an important cause of disease in many other countries, particularly Japan, Australia, Argentina and European countries (8, 21, 43, 45, 70). It appears that the prevalence of HUS is similar in Australia, Europe, and North America and that the association between HUS and *E. coli* infection is similar in the different parts of the world (70). The proportion of human cases in which zoonotic non-O157 STEC are involved varies from 20% to 70% among geographical regions (69). In Japan, between 1991 and 1995, more than 80% of *E. coli* isolates were identified as O157:H7. The other most important isolates identified were the non-O157 serotypes O26 and O111 (21). By 2004, O157:H7 isolations had decreased by about 50%, while O26 and O111 isolations had increased by 24% and 8.2%, respectively. Nevertheless, in 2004, O157:H7 was the predominant serotype among STEC isolates causing HUS in Japan. In Australia, from 2002 to 2004, O157 was the predominant isolate from zoonotic STEC infections, except for infections manifesting HUS (45). Little information is available regarding HUS-associated infections, and non-O157 O86 and O111 were the only two serotypes identified upon STEC isolation from these cases. Data compiled from different countries in Europe indicated that about 80% of STEC isolated from cases of diarrhoea were non-O157: the

most common isolates identified were O26, O91, O103, O111, O113, O128, and O145 (5). On the other hand, STEC isolated from HUS cases were mostly O157:H7. In Argentina, the frequency of HUS appears to be very high (36, 46). In this country, as well as in Chile and Uruguay, O157 STEC appeared to be less predominant in HUS than non-O157 STEC, using data collected since 2000 (little data is available for cases occurring before 2000).

There are fewer reports on zoonotic STEC infections from other countries. In the People's Republic of China, a national network for the detection of O157:H7 was set up in 1997 (71). Only a few sporadic cases of diarrhoea associated with O157 STEC have been identified. Involvement of non-O157 zoonotic STEC is less well-defined, but there is some indication that these infections may be more predominant than O157 STEC infections. In countries in Africa from which data have been reported, including Kenya, Nigeria, Côte d'Ivoire, and the Central African Republic, O157 STEC have been isolated from sporadic cases of diarrhoea and HUS, and have also been associated with some diarrhoeal disease outbreaks, especially in southern Africa (12, 54). Non-O157 STEC have also been associated with sporadic cases and outbreaks of diarrhoea in Nigeria (44). Conversely, STEC were not frequently involved in cases of diarrhoea in Uganda (26). STEC do not appear to be an important cause of diarrhoea in India, at least in Calcutta, where non-O157 STEC were isolated from only a small proportion of cases (28). Similarly, STEC do not seem to be an important cause of bloody or non-bloody diarrhoea in Bangkok, Thailand (34).

Exposure assessment

Humans are infected with zoonotic STEC mostly through the consumption of foods contaminated with faeces containing the bacteria (Fig. 2). A large amount of data are available on the mode of transmission of O157 STEC, particularly in the USA (55). Food has remained the predominant transmission route: the most important food

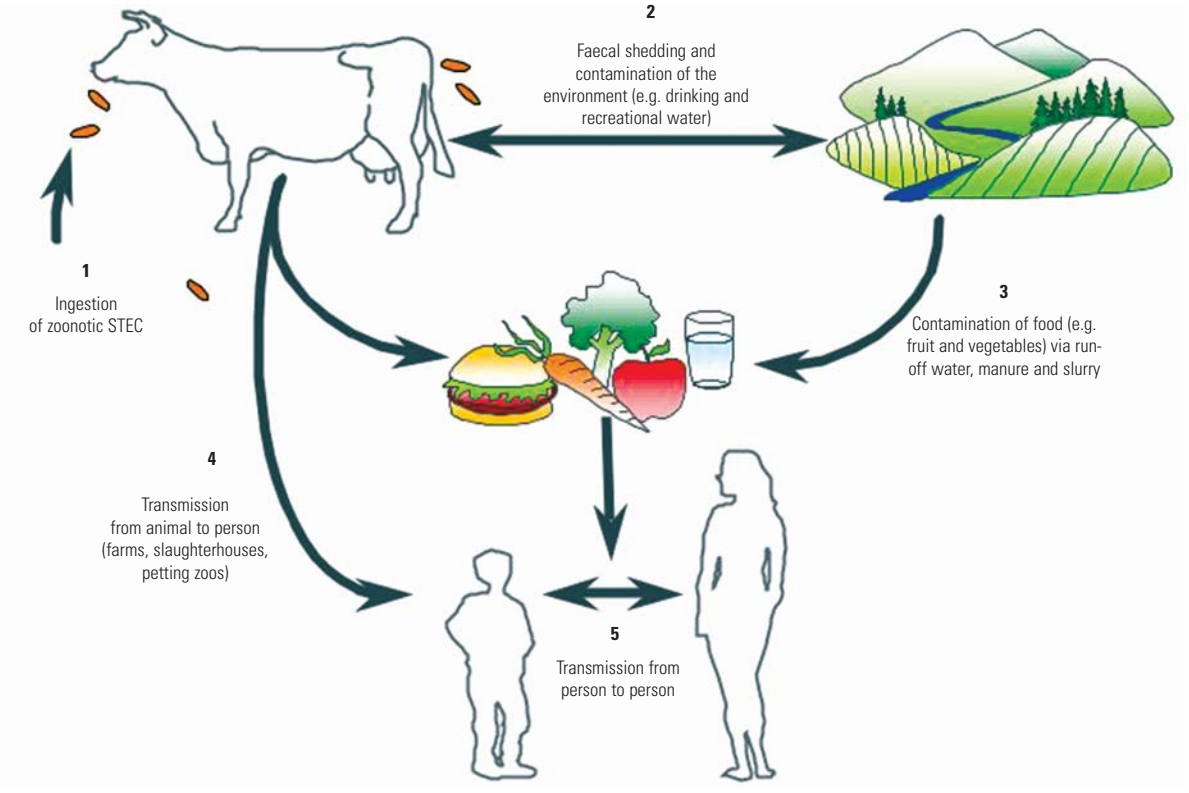

Fig. 2
How humans are exposed to zoonotic Shiga toxin-producing *Escherichia coli* (STEC)
Zoonotic STEC are ingested by cattle and other ruminants (1) and colonise the intestinal tract but do not cause any disease in these animals. The bacteria are shed in the faeces and contaminate the environment, including drinking and recreational water used by the human population (2). There may also be contamination of foods, such as fruits and fruit juices, vegetables, sprouts, and lettuce via run-off water, manure, or slurry (3). There may be contamination of milk during milking and of carcasses at slaughter such that bacteria will be mixed into ground beef. Persons working on farms or in slaughterhouses or visiting farms or petting zoos may also be infected with the bacteria through direct contact with animals (4). There may also be direct spread of bacteria from person to person (5)
Source: website of the *Escherichia coli* Laboratory (www.ecl-lab.ca)

sources being undercooked hamburgers and ground beef products. Raw milk and milk products, such as cheese curds, butter, and ice cream bars, have also been a source of infection. Since 1991, produce has been an increasingly important cause of outbreaks: high risk products include lettuce, unpasteurised apple cider and juice, salad, coleslaw, melons, and sprouts. Outbreaks of O157 STEC most commonly occurred in restaurants, often due to cross-contamination during food preparation. Person-to-person transmission via the faecal-oral route has been an important mode of transmission, particularly since the early 1990s, and occurs mostly in child day care centres, individual homes, communities, and schools. Waterborne outbreaks of O157 STEC associated with recreational waters, such as lakes, swimming pools, and contaminated drinking water, have been increasingly reported since the early 1990s. Outbreaks associated with contaminated water tend to be larger in size and have been attributed to local well, municipal, and spring water systems. Since 1996, outbreaks resulting from a new transmission mode have been recognised, i.e. direct contact between humans and cows or calves at farms, fairs, or petting zoos. For the most part, the modes of transmission in other industrialised countries appear to be similar to those observed in the USA. As more data become available from developing countries, other modes of transmission specific for the environmental, demographic, and farming conditions in these countries will certainly be elucidated. For instance, a large outbreak of bloody diarrhoea due to O157 STEC in southern Africa in 1992 was the result of a combination of carriage of O157 STEC by pastured cattle, cattle deaths due to drought, and ensuing heavy rains resulting in contamination of surface waters (12).

Exposure to *Escherichia coli* related to international food trade

There is a high potential for multinational food-borne outbreaks of illness following international trading of foods contaminated with zoonotic STEC, especially ground beef and beef products. This is well illustrated by the example of the 2004 outbreak of O157:H7 infections in humans in Japan following commercial distribution of contaminated ground beef that had been produced in the USA (30). Use of PulseNet protocols during the public health investigation by Japanese authorities enabled international comparison of isolates and facilitated detection of presumptively associated *E. coli* O157:H7 infections in the USA. The six-month lag between production of the beef products in the USA and sale of the products in Japan, with intervening cases in the USA, demonstrates the prolonged survival of O157:H7 STEC in frozen ground beef and the potential for outbreaks to occur over an extended time period and have a wide geographical distribution. PulseNet has now been established in several regions of the world. The use of standardised methods of molecular subtyping for O157:H7 and eventually for other non-O157 STEC will be invaluable in permitting the international collaboration necessary for investigation of these outbreaks.

Animal reservoirs

STEC are found in the intestinal tract and are shed in the faeces of a wide variety of animal species, including cattle, sheep, goats, pigs, water buffalo, and wild ruminant species (6). Ruminants are the most important reservoir of the zoonotic STEC (Fig. 2), which are transmitted to humans through the ingestion of food or water contaminated with animal faeces or through contact with the infected animals or their environment.

O157 Shiga toxin-producing *Escherichia coli*

O157 STEC mostly originate from cattle. Faecal shedding appears to occur for longer periods and the number of bacteria shed in the faeces is greater in young calves and at weaning, compared to adult cattle (10). The amount of faecal shedding is the greatest in both young and adult cattle during the summer (19). O157 STEC are found in dairy cattle and in both pastured and feedlot beef cattle. The prevalence of infection in individual animals is low (often less than 1%), although the herd prevalence may be higher and is often between 10% and 20% in the USA (18). A similar prevalence has been observed in studies carried out in various European countries (6), although the rate of positive animals was as high as 17% in Italy. O157 STEC has been found in cattle in many other countries, including Japan (29), Korea (24), the People's Republic of China (73, 74), Argentina (41), and Brazil (22). On the other hand, O157 STEC were not found in cattle in studies conducted in India (28) and Thailand (47), where cases of diarrhoea in humans attributed to zoonotic STEC do not appear to be frequent. In the few reports from Africa, O157 STEC have been found in beef meat products in Botswana (38) and in cattle associated with a major outbreak of diarrhoea in humans in southern Africa in 1992, but have not been detected in cattle in Uganda (26).

O157 STEC are also isolated sporadically from non-ruminant species on farms, such as rabbits, pigs, horses, and dogs. It is not clear if these species are hosts of O157 STEC or if they become accidentally colonised due to contact with infected ruminants. Pigs are not considered a major source of O157 STEC (the prevalence rate is usually very low), although reports from certain countries,

such as Chile (56) and the People's Republic of China (71, 74), demonstrate a much higher prevalence of up to 10%, which could reflect different farming and slaughter practices and could represent an important hazard in countries where the consumption of pork is high.

Non-O157 Shiga toxin-producing *Escherichia coli*

Non-O157 STEC has been found in the animal population worldwide, including in Africa and the People's Republic of China (2, 6, 26, 35, 52). Non-O157 STEC are mostly associated with cattle but have also been isolated from sheep, goats, pigs, and chickens. The prevalence rate varies considerably, depending on the technique used for sampling and detection, but is usually between 10% and 20% and may be as high as 80% to 90%. The prevalence rate of zoonotic non-O157 STEC is often difficult to assess because in many studies detection is based only on the presence of the Stx, and the serotype of the isolate and presence of other virulence factors are not determined by the investigators. In studies in which serotyping has been performed, zoonotic STEC belonging to serogroups such as O26, O103, O111, and O145 have been found in the USA, the United Kingdom, Canada, Europe, Australia, Argentina, Hong Kong and Japan in different animal species: cattle were the most common species in which these serotypes were detected (1, 52) (Table I). When determined, prevalence rates for STEC of these serotypes were usually around 1% to 2%. However, this prevalence may be underestimated compared to that observed for O157 STEC cases in which techniques to concentrate the sample in order to detect the bacteria are often used. Prevalence rates for O26 and O103 STEC were 94% and 51%, respectively, in calves examined over a period of five months in Scotland using an immunomagnetic separation technique (51). Testing for certain virulence factors, which provides a means of more accurately assessing the prevalence of zoonotic STEC in animal populations and, hence, the potential hazard to human health, has been carried out in some studies and has demonstrated much lower prevalence rates of zoonotic STEC than those for total STEC (22, 29, 48, 57, 61). This approach will become more valuable as virulence factor profiles of these strains are identified and rapid high-throughput tests become available.

Sources of infection in animals

The main sources of STEC infection in cattle are drinking water, feed, and the environment of the animal (Fig. 3).

The environment may be contaminated by cattle carrying the bacteria as well as by production animals of other species (e.g. sheep, goats, or pigs), by companion animals (e.g. dogs, cats, or horses), by wild animal species (e.g. deer), or by insects (e.g. flies). Infection may also occur through direct contact with other cattle or animals of other species.

Run-off of water from dairies or from pastures where cattle carrying zoonotic STEC have been grazing can contaminate surface drinking waters, such as rivers, ponds, lakes, and ground water supplying wells and springs. Pastures where slurry or manure originating from cattle carrying zoonotic STEC has been spread as fertiliser may also be a source of contamination. Contamination of drinking troughs may originate from the water source or occur following faecal contamination or often, when troughs are covered, following oral contamination of the drinking water by cattle carrying STEC in their tonsils (62). O157 STEC can survive in water, faeces, or sediment from drinking troughs for several months (6, 60).

Contamination of feeds, such as grain pellets, soybean meal, silage grasses, and grass hay, may occur at the source of the feed (i.e. in the crop fields) following run-off of contaminated water, spreading of manure and slurries as fertiliser or via wild bird or mammalian faeces. Feeds may also be contaminated during transport by truck to a feed mill (11). Poor silage management may permit the survival of STEC found on faecally-contaminated grasses: proper silage processing normally eliminates STEC (13). Contamination of feed troughs may occur through saliva or following defecation in the troughs by cattle, wildlife, rodents, birds, or insects, such as flies.

Contamination of the environment of cattle, including pastures, feed and water troughs, and pen floors, is mostly a result of faecal contamination by the cattle living in the environment. Most importantly, it has been shown that some O157 STEC strains may persist for more than two years in a particular farm environment (62). The type of environment greatly influences the persistence of the bacteria. For instance, calves kept indoors in pens continued to shed O157 STEC for four months, whereas no shedding of O157 STEC was detected (over a period of six months) in calves on the same farm kept on pasture, possibly due to a reduced exposure of the pasture-raised calves to the bacteria (25). Poor husbandry will also affect STEC persistence. Cattle kept in feedlot pens with wet, muddy floors demonstrated a higher prevalence rate of shedding of O157 STEC than cattle raised in pens under normal conditions (63).

Risk factors for infection of animals

Risk factors that have been associated with the infection of animals with O157 STEC include age, weaning, movement

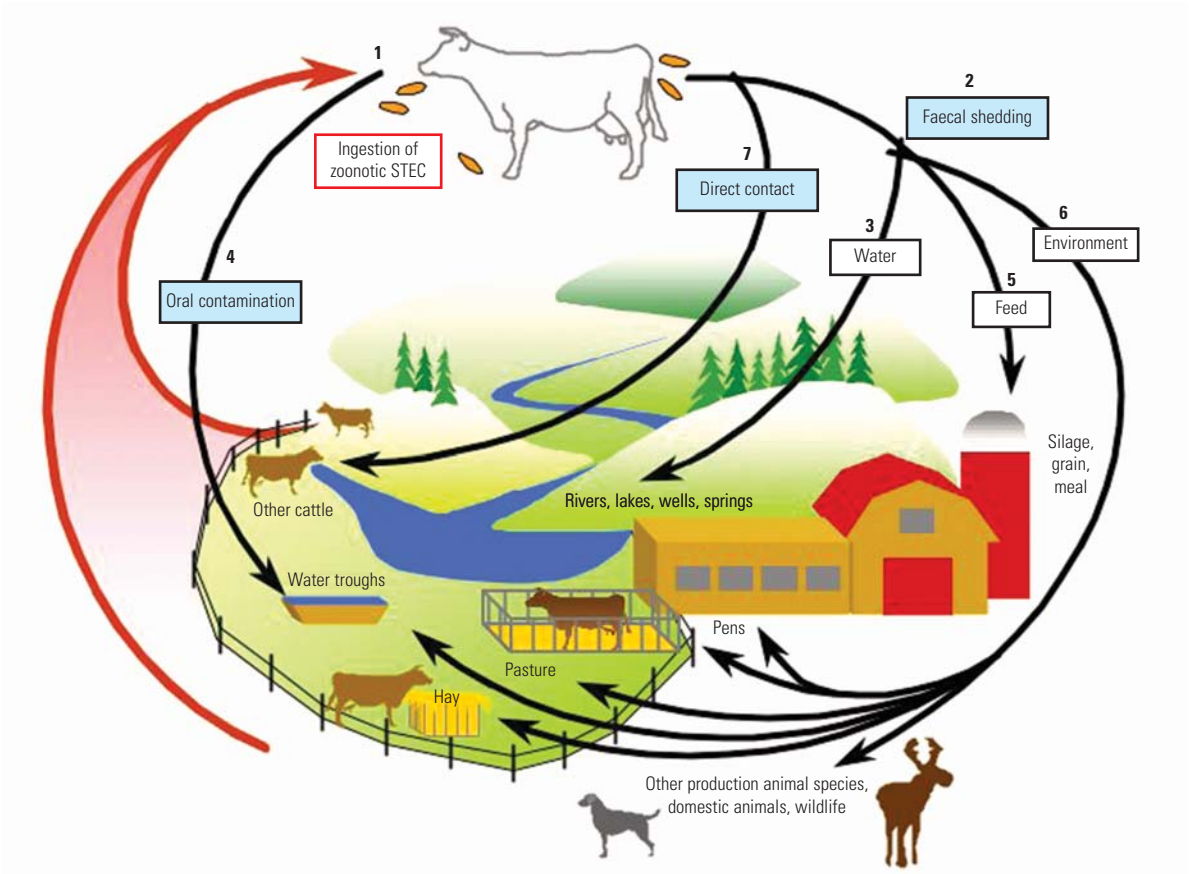

Fig. 3

Sources of zoonotic Shiga toxin-producing *Escherichia coli* (STEC) infection in farm animals

Zoonotic STEC are ingested by cattle and other ruminants (1) and colonise the intestinal tract but do not cause any disease in these animals. The bacteria are shed in the faeces (2). Contamination of drinking water from rivers, lakes, wells, and springs occurs following run-off of contaminated water from dairies and from pastures where cattle have been grazing or cattle manure has been spread (3). Contamination of water troughs may also originate from the saliva of cattle carrying the STEC in their tonsils (4). Contamination of feeds may occur at the source (i.e. in the crop fields) following run-off of contaminated water, spreading of manure and slurries as fertiliser, or via wild bird or mammalian faeces (5). Cattle faeces containing zoonotic STEC can also contaminate the immediate environment of the animals, including pastures, feed and water troughs, and pen floors (6). Other farm and wild animal species may be infected via the water, feed, or environment and, in turn, infect cattle via their faeces. Infection of cattle may also occur by direct contact with other cattle (7)

Source: website of the *Escherichia coli* Laboratory (www.ecl-lab.ca)

of animals, season, feed composition, and the ability of the bacteria to persist in the environment. Faecal shedding was higher in dairy calves at weaning than before weaning in studies conducted in the western USA and Denmark (15, 58) and was higher in weaned heifers than in calves or adults in a longitudinal study of cattle herds conducted in the northwestern USA (19). In the latter study, carried out over a period of more than one year, faecal shedding was highest in the summer months. In a risk-factor study of dairy herds in Denmark, calves up to two years old that had been moved to a new location within the previous two weeks had a higher risk of faecal shedding of O157 STEC (58). In a cross-sectional study of feedlot cattle close to their market date in the Midwestern USA (59), a positive association was observed between the heat index (combining heat and humidity) and levels of O157 STEC in feed sampled from feed bunks in feedlot pens. Interestingly, no difference in faecal shedding of O157 STEC was observed between cattle produced on conventional versus organic dairy farms in Switzerland (33).

Feed composition is also a possible risk factor for infection of animals with STEC. It has been shown that zoonotic O157 and non-O157 STEC survive in acid conditions and persist in rumen contents (3), which supports the proposal that a grain-rich diet may induce acid resistance of STEC in the rumen and permit the bacteria to survive in the abomasum, leading to increased faecal shedding. However, numerous field studies have demonstrated the opposite effect: hay-fed sheep (31) and cattle (20) shed O157 STEC for longer periods than grain-fed animals of the same

species. In another study, (17) no difference in faecal shedding of O157 STEC was observed between hay-fed and grain-fed cattle. On the other hand, in the aforementioned risk-factor study of dairy herds in Denmark (58), cows fed grain or molasses had a higher risk of shedding O157 STEC.

The type of grain used in a feed may also influence the risk of infection of cattle with STEC. For instance, in the cross-sectional study of feedlot cattle that were close to their market date in the Midwestern USA (59), a positive association was noted between the use of cottonseed meal and levels of O157 STEC in feed sampled from feed bunks in feedlot pens. It is not known if this effect is due to an ability of the cottonseed meal to enhance O157 STEC survival in the intestinal tract of the cattle or to contamination of the meal with the bacteria.

The ability of zoonotic STEC to survive and persist in faeces, manure, and soil in the environment can be considered as a risk factor for the infection of animals and humans. It has been shown that O157 STEC can survive for several months in water or sediment from drinking troughs. These bacteria can also survive for long periods in cattle faeces, particularly when the moisture content remains high (68), and in cattle or sheep manure piles and manure slurry (32). O157 STEC can survive in soil for long periods, particularly in the presence of manure, and during rainfall can be leached out of the soil and travel below the top layers of the soil for more than two months, increasing the probability of contamination of groundwater, which is recycled for crop irrigation, vegetable cleansing, or as drinking water for animals and the human population (14). Hence, run-off from manure piles contaminated with zoonotic STEC and using manure and slurries contaminated with zoonotic STEC as fertiliser on land used for animal grazing, crop or silage production for animal feed, or food crops for human consumption may result in persistent animal infection and a greater risk of human exposure.

Controlling the zoonotic risk at farm level

On-farm control of zoonotic STEC should primarily target the main source of contamination: the animal reservoir. Complete eradication of zoonotic STEC-positive farm animals would not be feasible due to the high prevalence rate of O157 STEC, the transient nature of the infection, and the difficulty in detecting low numbers of zoonotic STEC found in animal faeces (6). A more realistic aim would be to reduce intestinal colonisation of, and consequent faecal shedding by, animals. Such a measure would minimise STEC contamination of water sources used for human consumption and recreational activities; of food crops used for human consumption; and of meat, meat products, and milk. It would also minimise the possibility of infection of humans through direct contact with animals. At the same time, measures should be taken to limit the persistence of STEC in the farm environment.

Various strategies to reduce intestinal colonisation of cattle by zoonotic STEC have been attempted, including vaccination, treatment with probiotics (e.g. direct-fed microbials or competitive exclusion), administration of bacteriophages, and modification of the diet (4). There has been at least one report of field testing of a vaccine in cattle based on the virulence factors of O157 STEC. Vaccination resulted in decreased faecal shedding in experimentally infected cattle and in clinical trials in feedlot cattle, demonstrating the potential benefits of such an approach (53). Nevertheless, this approach still requires some optimisation as faecal shedding was not reduced after administration of the same vaccine to feedlot cattle in commercial operations (67). Another promising approach is feeding ruminants egg yolk antibodies purified from chickens immunised with specific virulence factors of zoonotic STEC. This approach resulted in a decrease in the duration and level of faecal shedding of O157 STEC in experimentally infected sheep (9).

Treatment with different probiotic strains has had variable effects on faecal shedding of STEC in cattle. Encouragingly, daily treatment of finisher beef cattle with direct-fed microbials, such as certain strains of *Lactobacillus acidophilus* (72), reduced faecal shedding of O157 STEC by over 50%. Treatment with a competitive exclusion probiotic containing *E. coli* strains reduced faecal shedding of both O157 and O111 but not O26 zoonotic STEC in weaned calves (65). Hence, these results suggest that a judicious choice of probiotic bacterial strains for the treatment of cattle could eventually permit a reduction in faecal shedding of not only O157 STEC but also a variety of zoonotic non-O157 STEC serotypes.

Antibacterial viruses, known as bacteriophages, that specifically target O157 STEC appear to be able to control the growth of these bacteria under laboratory conditions and have shown promising results in sheep; however, further work is necessary before the viruses can be considered a feasible approach for the control of STEC in cattle (4).

The application of epidemiological models to prevalence data on faecal shedding of O157 STEC in cattle in Scotland has demonstrated that only about 20% of the infections are responsible for 80% of the transmission to the cattle population (39). Hence, control strategies aimed at the 5% of animals in the population with high levels of intestinal carriage of the bacteria or interventions aimed at preventing high bacterial loads could very effectively

reduce the prevalence of O157 STEC. Such control measures could include testing and removal of high shedding individuals, vaccination or probiotic treatment.

The effect of changing abruptly from a grain diet to a hay diet on faecal shedding of O157 STEC has not been consistent (4). In general, there appeared to be a decrease in the intestinal *E. coli* population; however, the percent reduction in the bacterial population was not consistent between animals.

Manure piles and slurries are a potential source of zoonotic STEC contamination if manure-based fertilisers are used on food crops destined for human consumption or if fields and recreational waters become polluted by run-off water. Hence, reduction in the levels of STEC in the manure would be a logical strategy to reduce the risk of human infection. Composting has been shown to be very effective for the elimination of O157 STEC from manure and should be considered as a routine practice prior to spreading the manure (37).

Good management practices, such as routinely cleaning water troughs, chlorinating or ozonating the water supply, reducing the faecal contamination and humidification of cattle feeds, avoiding overcrowding and muddy pen floors in feedlots, and correctly preparing silage, will greatly contribute to minimising the spread and persistence of zoonotic STEC on the farm.

To control the risk of human infection through direct contact with farm animals, strict hygiene practices should be established, including controlling the movement of visitors to farms, restricting access to farm animals, making washing facilities readily available, providing a means of disinfection in case visitors come into contact with the animals, and segregating eating areas from areas where the animals are kept.

Conclusions

Great strides have been made in recent years in the detection, identification, and molecular characterisation of O157:H7 STEC, which has led to a more accurate assessment of the role of this serotype in human disease outbreaks and the transmission of infection from animal reservoirs. A major challenge will now be to better understand how these bacteria colonise the gut of the animal hosts. Such an understanding will permit the development of effective strategies to eliminate or greatly reduce the numbers of the bacteria in the animal reservoir.

In recent years, it has become apparent that certain non-O157 STEC can also cause human infections. Another challenge will be to more definitively identify and characterise these non-O157 STEC strains, which will allow a more thorough surveillance of the prevalence of the bacteria in animal populations, assessment of the importance of these bacterial species in human infections, and development of effective on-farm control strategies.

Acknowledgements

The authors thank Jacinthe Lachance for assistance in the preparation of the manuscript and for the design of the diagrams.

■

Escherichia coli : la contamination des animaux à la ferme

J.M. Fairbrother & É. Nadeau

Résumé

Escherichia coli est l'un des principaux microorganismes présents dans l'intestin de la plupart des espèces de mammifères, y compris les êtres humains, et des oiseaux. Les *E. coli* producteurs de Shiga-toxines (STEC), appelés aussi producteurs de verocytotoxine, ne sont généralement pas pathogènes pour l'animal, alors que chez l'homme l'infection se manifeste par une diarrhée aqueuse, une colite hémorragique et/ou un syndrome hémolytique et urémique (SHU). Si les souches O157:H7 sont le plus souvent incriminées lors des infections à STEC zoonotiques, il est de plus en plus fréquent de retrouver

d'autres souches. L'importance de souches autres que les O157:H7 a sans doute été sous-estimée, dans la mesure où leur caractérisation est moins aboutie que celle des O157:H7 et qu'elles sont plus difficiles à détecter dans les prélèvements. Un sous-type comprenant de nombreuses souches STEC a été isolé à partir de prélèvements animaux mais à ce jour il n'a été associé à aucune pathologie chez l'animal ni chez l'homme. Les bovins et les autres ruminants constituent le principal réservoir des STEC zoonotiques, qui sont transmis à l'homme par ingestion d'aliments ou d'eau contaminés par des matières fécales animales, ou par contact direct avec des animaux infectés ou avec leur environnement. Dans les exploitations, la contamination des bovins par des STEC se fait à travers l'eau, l'alimentation et l'environnement immédiat. Les facteurs de risque d'infection des animaux avec des STEC de sérotype O157 sont l'âge, les conditions de sevrage, les déplacements des animaux, la saison, la composition de la ration alimentaire, ainsi que la capacité de la bactérie à survivre dans l'environnement. Pour maîtriser le risque zoonotique de contamination par des STEC au niveau de l'exploitation, il convient de se concentrer sur la principale source de contamination, à savoir le réservoir animal. Plusieurs stratégies ont été tentées pour limiter les colonies de STEC zoonotiques dans l'intestin de bovins, avec des résultats variables : la vaccination, le recours aux probiotiques en administrant des agents microbiens dans l'alimentation ou en faisant intervenir le mécanisme d'exclusion compétitive, l'administration de bactériophages ou la modification de la ration alimentaire.

Mots-clés
Colite hémorragique – Escherichia coli – Escherichia coli producteur de Shiga-toxine – Escherichia coli producteur de verocytotoxine – Probiotique – Sérotype autre que O157 – Sérotype O157:H7 – Shiga-toxine – Syndrome hémolytique et urémique – Vaccination – Verotoxine.

Contaminación de animales por *Escherichia coli* en la finca

J.M. Fairbrother & É. Nadeau

Resumen
Escherichia coli es uno de los principales huéspedes del tracto intestinal de la mayoría de mamíferos, comprendidos los seres humanos, y las aves. Habitualmente, *E. coli* productora de toxina Shiga (STEC), también llamada *E. coli* verotoxigénica, no provoca enfermedades en los animales, pero puede producir diarrea acuosa, colitis hemorrágica o síndrome hemolítico ureico en los seres humanos. La STEC zoonótica comprende las cepas O157:H7 y, con una frecuencia cada vez mayor, otras cepas distintas. Probablemente se subestima la importancia de estas últimas dado que no han sido tan bien caracterizadas como las cepas O157:H7 y son más difíciles de detectar en las muestras. Se ha aislado en animales otro importante subconjunto de cepas de STEC, pero hasta el momento no se lo ha asociado con la aparición de enfermedades animales o humanas. Los bovinos y demás rumiantes son el principal reservorio de STEC zoonótica, que se transmite a los seres humanos por ingestión de alimentos o agua contaminados con heces animales, o por contacto directo con animales infectados o su entorno. Las principales fuentes de infección del ganado por STEC en las explotaciones son el agua de beber, los piensos y el entorno

inmediato de los animales. Los factores de riesgo de infección de animales por la cepa O157 de STEC identificados hasta la fecha comprenden la edad, el destete, los movimientos de animales, el celo, la composición de los piensos y la capacidad de la bacteria para resistir al entorno. En las explotaciones, el control del riesgo zoonótico de infección por STEC en los seres humanos debe concentrarse fundamentalmente en el reservorio animal, principal fuente de contagio. Se han probado, con mayor o menor éxito, distintas estrategias para reducir el establecimiento de colonias zoonóticas de STEC en los animales, comprendidas la vacunación, los tratamientos con probióticos, como la alimentación directa con microbianos y la exclusión competitiva, la administración de bacteriófagos y la modificación de la dieta.

Palabras clave
Cepa O157:H7 – Cepa distinta de la O157 – Colitis hemorrágica – Escherichia coli – Escherichia coli productora de toxina Shiga – Escherichia coli verotoxigénica – Probiótico – Síndrome hemolítico ureico – Toxina Shiga – Vacunación – Verotoxina.

References

1. Bettelheim K.A. (2003). – Non-O157 verotoxin-producing *Escherichia coli*: a problem, paradox, and paradigm. *Experim. Biol. Med.*, **228**, 333-344.

2. Beutin L., Geier D., Steinruck H., Zimmermann S. & Scheutz F. (1993). – Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. clin. Microbiol.*, **31** (9), 2483-2488.

3. Boukhors K., Pradel N., Girardeau J.P., Livrelli V., Ou Saïd A.M., Contrepois M. & Martin C. (2002). – Effect of diet on Shiga toxin-producing *Escherichia coli* (STEC) growth and survival in rumen and abomasum fluids. *Vet. Res.*, **33**, 405-412.

4. Callaway T.R., Anderson R.C., Edrington T.S., Genovese K.J., Bischoff K.M., Poole T.L., Jung Y.S., Harvey R.B. & Nisbet D.J. (2004). – What are we doing about *Escherichia coli* O157:H7 in cattle? *J. Anim. Sci.*, **82** (E. suppl.), 93-99.

5. Caprioli A. & Tozzi A.E. (1998). – Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe. *In Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains (J.B. Kaper & A.D. O'Brien, eds). ASM Press, Washington, DC, 38-48.

6. Caprioli A., Morabito S., Brugère H. & Oswald E. (2005). – Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.*, **36**, 289-311.

7. Catarame T.M., O'Hanlon K.A., Duffy G., Sheridan J.J., Blair I.S. & McDowell D.A. (2003). – Optimization of enrichment and plating procedures for the recovery of *Escherichia coli* O111 and O26 from minced beef. *J. appl. Microbiol.*, **95** (5), 949-957.

8. Combs B.G., Raupach J.C.A. & Kirk M.D. (2005). – Surveillance of Shiga toxigenic in Australia. *Communic. Dis. Intell.*, **29** (4), 366-369.

9. Cook S.R., Bach S.J., Stevenson S.M.L., DeVinney R., Frohlich A.A., Fang L. & McAllister T.A. (2005). – Orally administered anti-*Escherichia coli* O157:H7 chicken egg yolk antibodies reduce fecal shedding of the pathogen by ruminants. *Can. J. anim. Sci.*, **85**, 291-299.

10. Cray W.C. Jr & Moon H.W. (1995). – Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. environ. Microbiol.*, **61** (4), 1586-1590.

11. Davis M.A., Hancock D.D., Rice D.H., Call D.R., DiGiacomo R., Samadpour M. & Besser T.E. (2003). – Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica*. *Vet. Microbiol.*, **95**, 199-210.

12. Effler P., Isaäcson M., Arntzen L., Heenan R., Canter P., Barrett T., Lee L., Mambo C., Levine W., Zaidi A. & Griffin P.M. (2001). – Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerg. infect. Dis.*, **7** (5), 812-819.

13. Fenlon D.R. & Wilson J. (2000). – Growth of *Escherichia coli* O157 in poorly fermented laboratory silage: a possible environmental dimension in the epidemiology of *E. coli* O157. *Lett. appl. Microbiol.*, **30** (2), 118-121.

14. Gagliardi J.V. & Karns J.S. (2000). – Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. *Appl. environ. Microbiol.*, **66** (3), 877-883.

15. Garber L.P., Wells S.J., Hancock D.D., Doyle M.P., Tuttle J., Shere J.A. & Zhao T. (1995). – Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. *J. Am. vet. med. Assoc.*, **207** (1), 46-49.

16. Gerner-Smidt P., Kincaid J., Kubota K., Hise K., Hunter S.B., Fair M.A., Norton D., Woo-Ming A., Kurzynski T., Sotir M.J., Head M., Holt K. & Swaminathan B. (2005). – Molecular surveillance of shiga toxigenic *Escherichia coli* O157 by PulseNet USA. *J. Food Protec.*, **68** (9), 1926-1931.

17. Grauke L.J., Wynia S.A., Sheng H.Q., Yoon J.W., Williams C.J., Hunt C.W. & Hovde C.J. (2003). – Acid resistance of *Escherichia coli* O157:H7 from the gastrointestinal tract of cattle fed hay or grain. *Vet. Microbiol.*, **95**, 211-225.

18. Hancock D.D., Besser T.E., Kinsel M.L., Tarr P.I., Rice D.H. & Paros M.G. (1994). – The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect.*, **113** (2), 199-207.

19. Hancock D.D., Besser T.E., Rice D.H., Herriott D.E. & Tarr P.I. (1997). – A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.*, **118** (2), 193-195.

20. Hovde C.J., Austin P.R., Cloud K.A., Williams C.J. & Hunt C.W. (1999). – Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. *Appl. environ. Microbiol.*, **65** (7), 3233-3235.

21. Infectious Disease Surveillance Center (2005). – Enterohemorrhagic *Escherichia coli* infection as of May 2005. *Infect. Agents Surv. Rep.*, **26** (6), 137-138.

22. Irino K., Kato M.A.M.F., Vaz T.M.I., Ramos I.I., Souza M.A.C., Cruz A.S., Gomes T.A.T., Vieira M.A.M. & Guth B.E.C. (2005). – Serotypes and virulence markers of Shiga toxin-producing *Escherichia coli* (STEC) isolated from dairy cattle in São Paulo State, Brazil. *Vet. Microbiol.*, **105**, 29-36.

23. Jenkins C., Pearce M.C., Smith A.W., Knight H.I., Shaw D.J., Cheasty T., Foster G., Gunn G.J., Dougan G., Smith H.R. & Frankel G. (2003). – Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques. *Lett. appl. Microbiol.*, **37** (3), 207-212.

24. Jo M.Y., Kim J.H., Lim J.H., Kang M.Y., Koh H.B., Park Y.H., Yoon D.Y., Chae J.S., Eo S.K. & Lee J.H. (2004). – Prevalence and characteristics of *Escherichia coli* O157 from major food animals in Korea. *Int. J. Food Microbiol.*, **95**, 41-49.

25. Jonsson M.E., Aspan A., Eriksson E. & Vagsholm I. (2001). – Persistence of verocytotoxin-producing *Escherichia coli* O157:H7 in calves kept on pasture and in calves kept indoors during the summer months in a Swedish dairy herd. *Int. J. Food Microbiol.*, **66** (1-2), 55-61.

26. Kaddu-Mulindw D.H., Aisu T., Gleier K., Zimmermann S. & Beutin L. (2001). – Occurrence of Shiga toxin-producing *Escherichia coli* in fecal samples from children with diarrhea and from healthy zebu cattle in Uganda. *Int. J. Food Microbiol.*, **66** (1-2), 95-101.

27. Karmali M.A. (1989). – Infection by verocytotoxin-producing *Escherichia coli*. *Clin. Microbiol. Rev.*, **2** (1), 15-38.

28. Khan A., Yamasaki S., Sato T., Ramamurthy T., Pal A., Datta S., Chowdhury N.R., Das S.C., Sikdar A., Tsukamoto T., Bhattacharya S.K., Takeda Y. & Nair G.B. (2002). – Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef, and humans, Calcutta, India. *Emerg. infect. Dis.*, **8** (1), 54-62.

29. Kobayashi H., Shimada J., Nakazawa M., Morozumi T., Pohjanvirta T., Pelkonen S. & Yamamoto K. (2001). – Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. *Appl. environ. Microbiol.*, **67** (1), 484-489.

30. Kudaka J., Asato R., Itokazu K., Nakamura M., Taira K., Kuniyosi H., Kinjo Y., Terajima J., Watanabe H., Kobayashi J., Swaminathan B., Braden C.R. & Dunn J.R. (2005). – *Escherichia coli* O157:H7 infections associated with ground beef from a US military installation – Okinawa, Japan, February 2004. *MMWR*, **54** (02), 40-42.

31. Kudva I.T., Hunt C.W., Williams C.J., Nance U.M. & Hovde C.J. (1997). – Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. *Appl. environ. Microbiol.*, **63** (10), 3878-3886.

32. Kudva I.T., Blanch K. & Hovde C.J. (1998). – Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl. environ. Microbiol.*, **64** (9), 3166-3174.

33. Kuhnert P., Dubosson C.R., Roesch M., Homfeld E., Doherr M.G. & Blum J.W. (2005). – Prevalence and risk-factor analysis of Shiga toxigenic *Escherichia coli* in faecal samples of organically and conventionally farmed dairy cattle. *Vet. Microbiol.*, **109**, 37-45.

34. Leelaporn A., Phengmak M., Eampoklap B., Manatsathit S., Tritilanunt S., Siritantikorn S., Nagayama K., Iida T., Niyasom C. & Komolpit P. (2003). – Shiga toxin- and enterotoxin-producing *Escherichia coli* isolated from subjects with bloody and nonbloody diarrhea in Bangkok, Thailand. *Diagn. Microbiol. infect. Dis.*, **46**, 173-180.

35. Leung P.H., Yam W.C., Ng W.W. & Peiris J.S. (2001). – The prevalence and characterization of verotoxin-producing *Escherichia coli* isolated from cattle and pigs in an abattoir in Hong Kong. *Epidemiol. Infect.*, **126** (2), 173-179.

36. Lopez E.L., Contrini M.M. & De Rosa M.F. (1998). – Epidemiology of Shiga toxin-producing *Escherichia coli* in South America. *In Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains (J.B. Kaper & A.D. O'Brien, eds). ASM Press, Washington, DC, 30-37.

37. Lung A.J., Lin C.M., Kim J.M., Marshall M.R., Nordstedt R., Thompson N.P. & Wei C.I. (2001). – Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. *J. Food Protec.*, **64** (9), 1309-1314.

38. Magwira C.A., Gashe B.A. & Collison E.K. (2005). – Prevalence and antibiotic resistance profiles of *Escherichia coli* O157:H7 in beef products from retail outlets in Gaborone, Botswana. *J. Food Protec.*, **68** (2), 403-406.

39. Matthews L., Low J.C., Gally D.L., Pearce M.C., Mellor D.J., Heesterbeek J.A.P., Chase-Topping M., Naylor S.W., Shaw D.J., Reid S.W.J., Gunn G.J. & Woolhouse M.E.J. (2006). – Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *PNAS*, **103** (3), 547-552.

40. Mead P.S., Slutsker L., Dietz V., McCaig L.F., Bresee J.S., Shapiro C., Griffin P.M. & Tauxe R.V. (1999). – Food-related illness and death in the United States. *Emerg. infect. Dis.*, **5** (5), 607-625.

41. Meichtri L., Miliwebsky E., Gioffré A., Chinen I., Baschkier A., Chillemi G., Guth B.E.C., Masana M.O., Cataldi A., Rodríguez H.R. & Rivas M. (2004). – Shiga toxin-producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. *Int. J. Food Microbiol.*, **96**, 189-198.

42. Morabito S., Karch H., Mariani-Kurkdjian P., Schmidt H., Minelli F., Bingen E. & Caprioli A. (1998). – Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. *J. clin. Microbiol.*, **36** (3), 840-842.

43. Nataro J.P. & Kaper J.B. (1998). – Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.*, **11** (1), 142-201.

44. Okeke I.N., Ojo O., Lamikanra A. & Kaper J.B. (2003). – Etiology of acute diarrhea in adults in Southwestern Nigeria. *J. clin. Microbiol.*, **41** (10), 4525-4530.

45. OzFoodNet Working Group (2005). – Reported foodborne illness and gastroenteritis in Australia: annual report of the OzFoodNet network, 2004. *Communic. Dis. Intell.*, **29** (2), 164-190.

46. Pan American Health Organization (PAHO) (2001). – Executive Summary. 4th Meeting of the Emerging Disease Surveillance Network of the Southern Cone, Paraguay. PAHO, Washington, DC.

47. Panutdaporn N., Chongsa-nguan M., Nair G.B., Ramamurthy T., Yamasaki S., Chaisri U., Tongtawe P., Eampokalarp B., Tapchaisri P., Sakolvaree Y., Kurazono H., Thein W.B., Hayashi H., Takeda Y. & Chaicumpa W. (2004). – Genotypes and phenotypes of Shiga toxin-producing *Escherichia coli* isolated from healthy cattle in Thailand. *J. Infection*, **48**, 149-160.

48. Parma A.E., Sanz M.E., Blanco J.E., Blanco J., Viñas M.R., Blanco M., Padola N.L. & Etcheverría A.I. (2000). – Virulence genotypes and serotypes of verotoxigenic *Escherichia coli* isolated from cattle and foods in Argentina. *Eur. J. Epidemiol.*, **16**, 757-762.

49. Paton A.W., Woodrow M.C., Doyle R.M., Lanser J.A. & Paton J.C. (1999). – Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking eae responsible for a cluster of cases of hemolytic-uremic syndrome. *J. clin. Microbiol.*, **37** (10), 3357-3361.

50. Paton J.C. & Paton A.W. (1998). – Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.*, **11** (3), 450-479.

51. Pearce M.C., Jenkins C., Vali L., Smith A.W., Knight H.I., Cheasty T., Smith H.R., Gunn G.J., Woolhouse M.E.J., Amyes S.G.B. & Frankel G. (2004). – Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. *Appl. environ. Microbiol.*, **70** (3), 1708-1716.

52. Pearce M.C., Evans J., McKendrick I.J., Smith A.W., Knight H.I., Mellor D.J., Woolhouse M.E.J., Gunn G.J. & Low J.C. (2006). – Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111, and O145 shed by cattle in Scotland. *Appl. environ. Microbiol.*, **72** (1), 653-659.

53. Potter A.A., Klashinsky S., Li Y., Frey E., Townsend H., Roganc D., Erickson G., Hinkley S., Klopfenstein T., Moxley R.A., Smith D.R. & Finlay B.B. (2004). – Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine*, **22**, 362-369.

54. Raji M.A., Jiwa S.F., Minga M.U. & Gwakisa P.S. (2003). – *Escherichia coli* O157:H7 reservoir, transmission, diagnosis and the African situation: a review. *East Afr. med. J.*, **80** (5), 271-276.

55. Rangel J.M., Sparling P.H., Crowe C., Griffin P.M. & Swerdlow D.L. (2005). – Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. infect. Dis.*, **11** (4), 603-609.

56. Rios M., Prado V., Trucksis M., Arellano C., Borie C., Alexandre M., Fica A. & Levine M.M. (1999). – Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products. *J. clin. Microbiol.*, **37** (3), 778-781.

57. Rogerie F., Marecat A., Gambade S., Dupond F., Beaubois P. & Lange M. (2001). – Characterization of Shiga toxin-producing *E. coli* and O157 serotype *E. coli* isolated in France from healthy domestic cattle. *Int. J. Food Microbiol.*, **63**, 217-223.

58. Rugbjerg H., Nielsen E.M. & Andersen J.S. (2003). – Risk factors associated with faecal shedding of verocytotoxin-producing *Escherichia coli* O157 in eight known-infected Danish dairy herds. *Prev. vet. Med.*, **58**, 101-113.

59. Sargeant J.M., Sanderson M.W., Griffin D.D. & Smith R.A. (2004). – Factors associated with the presence of *Escherichia coli* O157 in feedlot cattle water and feed in the Midwestern USA. *Prev. vet. Med.*, **66**, 207-237.

60. Scott L., McGee P., Sheridan J.J., Earley B. & Leonard N. (2006). – A comparison of the survival in feces and water of *Escherichia coli* O157:H7 grown under laboratory conditions or obtained from cattle feces. *J. Food Protec.*, **69** (1), 6-11.

61. Shaw D.J., Jenkins C., Pearce M.C., Cheasty T., Gunn G.J., Dougan G., Smith H.R., Woolhouse M.E.J. & Frankel G. (2004). – Shedding patterns of verocytotoxin-producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm. *Appl. environ. Microbiol.*, **70** (12), 7456-7465.

62. Shere J.A., Bartlett K.J. & Kaspar C.W. (1998). – Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Appl. environ. Microbiol.*, **64** (4), 1390-1399.

63. Smith D., Blackford M., Younts S., Moxley R., Gray J., Hungerford L., Milton T. & Klopfenstein T. (2001). – Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. *J. Food Protec.*, **64** (12), 1899-1903.

64. Swaminathan B., Barrett T.J., Hunter S.B., Tauxe R.V. & CDC PulseNet Task Force (2001). – PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerg. infect. Dis.*, **7** (3), 382-389.

65. Tkalcic S., Zhao T., Harmon B.G., Doyle M.P., Brown C.A. & Zhao P. (2003). – Fecal shedding of enterohemorrhagic *Escherichia coli* in weaned calves following treatment with probiotic *Escherichia coli*. *J. Food Protec.*, **66** (7), 1184-1189.

66. United Kingdom Advisory Committee on the Microbiological Safety of Food (1995). – Report on verocytotoxin-producing *Escherichia coli*. Her Majesty's Stationery Office, London.

67. Van Donkersgoed J., Hancock D., Rogan D. & Potter A.A. (2005). – *Escherichia coli* O157:H7 vaccine field trial in 9 feedlots in Alberta and Saskatchewan. *Can. vet. J.*, **46**, 724-728.

68. Wang G., Zhao T. & Doyle M.P. (1996). – Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Appl. environ. Microbiol.*, **62** (7), 2567-2570.

69. World Health Organization (WHO) (1998). – Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific Working Group Meeting. WHO/CSR/APH/98.8. WHO, Geneva.

70. World Health Organization & Food and Agriculture Organization (2002). – Risk profile for enterohemorrhagic *E. coli* including the identification of the commodities of concern, including sprouts, ground beef and pork. Codex Committee of Food Hygiene CX/FH 03/5-Add 4. Food and Agriculture Organization of the United Nations, Rome.

71. Xu J.G., Cheng B.K. & Jing H.Q. (1999). – *Escherichia coli* O157:H7 and Shiga-like-toxin-producing *Escherichia coli* in China. *World. J. Gastroenterol.*, **5** (3), 191-194.

72. Younts-Dahl S.M., Osborn G.D., Galyean M.L., Rivera J.D., Loneragan G.H. & Brashears M.M. (2005). – Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. *J. Food Protec.*, **68** (1), 6-10.

73. Zheng H., Jing H., Wang H., Xia S., Hu W., Cui S., Bi Z., Yang J., Pang B., Zhao G., Zhang J., Li H. & Xu J. (2005). – *stx2vha* is the dominant genotype of Shiga toxin-producing *Escherichia coli* O157:H7 isolated from patients and domestic animals of three regions of China. *Microbiol. Immunol.*, **49** (12), 1019-1026.

74. Zhou Z., Nishikawa Y., Zhu P., Hong S., Hase A., Cheasty T., Smith H.R., Zheng M. & Haruki K. (2002). – Isolation and characterization of Shiga toxin-producing *Escherichia coli* O157:H7 from beef, pork and cattle fecal samples in Changchun, China. *J. vet. med. Sci.*, **64** (11), 1041-1044.