Emergence of *Salmonella* Typhimurium definitive type 104 (DT104) as an important cause of salmonellosis in horses in Ontario

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Abstract — *Salmonella* Typhimurium definitive type 104 (DT104) has emerged as a common cause of salmonellosis in humans and cattle, yet previous reports involving horses are sparse. This study reports the emergence of DT104 as an important pathogen in horses in Ontario. The first clinical case of DT104 infection at the Ontario Veterinary College was identified in 1997. Seventeen cases of DT104-associated salmonellosis were identified between 1997 and 2000. In 2000, 12 of 13 cases of salmonellosis were due to DT104. Salmonellosis in horses due to DT104 is of concern, since the organism is multiresistant to antibiotics and poses increased zoonotic risk. Phage type distribution of *Salmonella* isolates should be monitored to determine whether DT104 will remain a prevalent equine pathogen.

Résumé — Émergence de *Salmonella* Typhimurium DT 104 comme cause importante de salmonellose chez les chevaux en Ontario. *Salmonella* Typhimurium DT 104 est apparue comme cause fréquente de salmonellose chez les humains et les bestiaux, bien que jusqu'à maintenant les rapports impliquant des chevaux soient rares. Cette étude rapporte l'émergence de DT 104 comme pathogène important chez les chevaux en Ontario. Le premier cas clinique d'infection à DT 104 à l'Ontario Veterinary College a été identifié en 1997. Dix-sept cas de salmonellose associés à DT 104 ont été identifiés entre les années 1997 et 2000. En l'an 2000, 12 cas sur 13 de salmonellose étaient attribués à DT 104. La salmonellose équine causée par DT 104 est inquiétante puisque l'organisme est multirésistant aux antibiotiques et pose un risque de zoonose accru. La lysotypie des isolats de salmonelles devrait être surveillée afin de déterminer si le DT 104 continuera à être un pathogène équin courant.

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Introduction

Salmonellosis is a recognized cause of enteric disease in a number of species, including horses (1–3), and is caused by a variety of strains of *Salmonella* spp. All salmonellae are classified as *S. enterica* or *S. bongori* (4). *Salmonella enterica* isolates can be further separated into 6 subgroups or subspecies, including subspecies *enterica,* which accounts for most cases of human and domestic animal disease (1). Based on antigenic properties, namely somatic and flagellar antigens, subspecies can be divided into a number of serovars or serotypes. Currently, 1435 serotypes of *S. enterica* subsp. *enterica* are recognized (4). The most frequently isolated serotypes in horses include *S.* Typhimurium, *S.* Anatum, *S.* Newport, *S.* Krefeld, and *S.* Agona (1). Serotypes can be further divided into phage types.

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Salmonella enterica subspecies *enterica* serotype Typhimurium definitive type 104, also known as *S.* Typhimurium DT 104 (DT104), has emerged internationally as an important cause of enteric disease in humans and domestic animals (5–7). *Salmonella* Typhimurium DT104 is currently recognized as a prevalent *S.* Typhimurium strain worldwide and is increasing in prevalence (8). Cattle are considered to be the main reservoir (5,7,9), but DT104 strains are frequently isolated from pigs (7) and increasingly from other species, including poultry, sheep, horses, goats, cats, and dogs (10–13). The organism has also been isolated from mice, elk, squirrels, raccoons, wild birds, and pet birds (5,11,14). In Canada, the percentage of DT104 isolates among *S.* Typhimurium isolates from cattle rose from 19.2% in 1994 to 76.7% in 1997 (15). Asymptomatic carriage by cattle has been reported (16,17).

The incidence of DT104 infection in humans has increased dramatically over the last decade, especially in Europe and North America, and there is a clear association between DT104 infection of farm animals or foods of animal origin and humans (5,14,18,19). Rural residence and contact with livestock have been associated with an increased risk of DT104 infection (5,9,14,20,21). Transmission between animals and humans may occur more frequently with DT104 than with other *Salmonella* spp. (22). A study by Calvert et al

(5) reported that 37.8% of humans infected with DT104 had regular contact with cattle through their employment compared with 9.4% of people infected with other *Salmonella*. It has been suggested that infection with DT104 may be associated with a higher morbidity and mortality than is infection with other *Salmonella* phagetypes (21), and children may be at additional risk for DT104 versus non-DT104 salmonellosis (5).

One of the major concerns about the emergence of DT104 is the prevalence of antimicrobial resistance. When DT104 was first isolated in Canada in 1970, it was sensitive to all tested antimicrobials (20); however, by 1989, DT104 isolates resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) had been identified (20). Isolates are designated based on their resistance patterns, so these isolates are referred to as resistance type (R-type) ACSSuT. By 1997, R-type ACSSuT represented 63% of DT104 isolates in humans in Canada (20). It is unclear whether multiple drug resistance developed in Canadian DT104 strains, or whether there was international clonal spread of a multiresistant strain. In a survey of *Salmonella* isolates submitted to Office International des Épizooties (OIE) Reference Laboratory for Salmonellosis, Health Canada, in Guelph, Ontario, from 1994–1997, 85.6% of DT104 strains were R-type ACSSuT (15). Similarly, the prevalence of R-type ACSSuT isolates in the United States increased from 4% to 43% from 1989 to 1994 (11). Fiorentino et al (8) reported that R-type ACSSuT DT104 isolates accounted for 28% of *S.* Typhimurium isolates and 7% of all *Salmonella* isolates in the United States. In the United Kingdom, the situation is of greater concern with multiple-resistant DT104 being the second most prevalent *Salmonella* species isolated from humans (23).

Salmonellosis is uncommon in horses at the Ontario Veterinary College Veterinary Teaching Hospital (OVC-VTH), accounting for less than 10% of cases of equine colitis (Weese, unpublished data). Despite the low prevalence at this hospital, salmonellosis is of particular concern due to the risk of nosocomial outbreaks and zoonotic transmission. Numerous nosocomial outbreaks involving a number of *Salmonella* serotypes have been reported in equine hospitals (2). Consequently, a *Salmonella* surveillance program has been ongoing at the OVC-VTH for more than 10 y. In this program, *Salmonella* isolates are serotyped, phage typed, and tested for antimicrobial sensitivity. This report presents the distribution of *Salmonella* spp. isolates from horses at the Ontario Veterinary College from 1997 to 2000.

Materials and methods

Results from the bacteriological culture of fecal samples from horses hospitalized at the Ontario Veterinary College Large Animal Clinic were prospectively monitored as part of an ongoing salmonellosis surveillance program. Submission of fecal samples for *Salmonella* culture was at the discretion of the attending clinician; however, fecal samples are routinely submitted from all horses with diarrhea, as well as from those with nonspecific gastrointestinal disorders and fever of unknown origin; typically, 5 fecal samples are collected daily. Fecal samples were cultured by the University of Guelph Animal Health Laboratory (AHL) using direct inoculation and enrichment techniques. Briefly, fecal samples were inoculated directly onto MacConkey's agar and Hectoen enteric agar and incubated at 37°C for 48 h. Tetrathionate broth was also inoculated and incubated at 42°C for 24 h. The broth was then inoculated onto brilliant green agar. Inoculated plates were examined at 24 and 48 h postinoculation. Bacteriological identification and antimicrobial sensitivity testing were performed by the AHL, while serotyping and phage typing were performed by the Health Canada, OIE Reference Laboratory for Salmonellosis in Guelph. Case information, including historical and clinical findings, were obtained from the medical records.

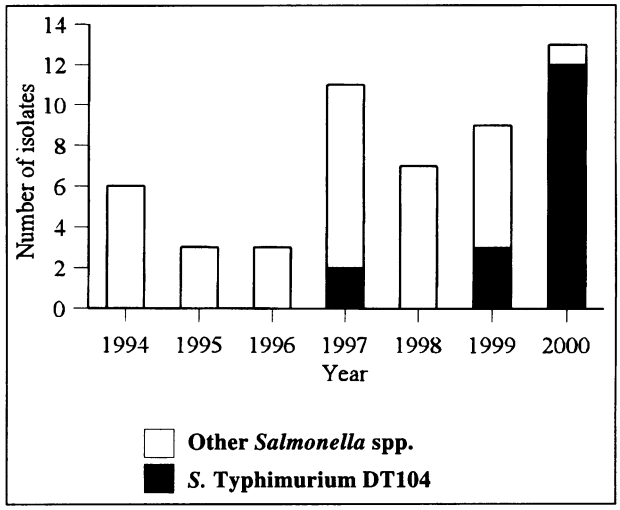

Figure 1. Distribution of *Salmonella* spp. isolates from horses at the Ontario Veterinary College from 1997 to 2000.

Table 1. Distribution of *Salmonella* spp. isolated from fecal samples taken from horses at the Ontario Veterinary College from 1997–2000

	1997	1998	1999	2000
S. Typhimurium DT104	2	0	3	12
S. Typhimurium other	2	1	3	0
S. Orion	2	1	0	0
S. Anatum	0	2	1	0
S. Newport	0	1	0	0
S. Thompson	1	1	0	0
S. Worthington	0	0	1	0
S. Infantis	0	0	1	0
S. Seftenberg	1	0	0	0
S. spp.	3	1	0	0
Untyped	0	0	0	1
n	11	7	9	13

Results

The first isolation of DT104 from horses at the OVC-VTH occurred in 1997, when 2 cases of DT104-associated salmonellosis were identified (Figure 1, Table 1). The following year, no cases were reported; however, in 1999, DT104 was isolated from 3 of 9 horses. A dramatic increase in the occurrence of DT104-associated salmonellosis occurred in 2000. In

Table 2. Clinical equine *S.* Typhimurium DT104 cases from the Ontario Veterinary College Veterinary Teaching Hospital from 1997 to 2000

Case	Breed	Age	Sex	Presenting complaint	Other diarrheic animals on farm[a]	Possibly nosocomial	Outcome
1	Cly	3 y	M	Castration	No	Yes	Survived
2	Stb	Mat	F	Laceration	No	Yes	Survived
3	TB	4 mo	M	Diarrhea	No	No	Survived
4	TB	7 mo	M	Diarrhea	No	No	Euthanized
5	TB	9 mo	F	Colic	No	No	Survived
6	Stb	5 y	F	Diarrhea	Yes	No	Survived
7	TB	20 y	F	Postoperative diarrhea	No	Yes	Euthanized
8	TB	4 mo	F	Diarrhea	No	No	Survived
9	Stb	17 d	F	Weak	Yes	No	Survived
10	App	4 mo	MC	Diarrhea	Yes	No	Survived
11	Mix	12 d	F	Diarrhea	Yes	No	Survived
12	Stb	1 y	M	Colic	Yes	No	Survived
13	QH	4 y	MC	Pyrexia	No	Yes	Survived
14[b]	Mix	2 mo	M	Diarrhea	Yes	No	Euthanized
15[b]	Mix	7 y	F	Accompanying foal	Yes	No	Survived
16	Stb	3 y	M	Diarrhea	No	No	Euthanized
17	TB	3 y	M	Septic hock	No	No	Euthanized

Cly — Clydesdale; Stb — standardbred; TB — Thoroughbred; App — Appaloosa; Mix — mixed breed; QH — quarterhorse; M — male; F — female; MC — castrated male
[a]Within the previous 12 mo
[b]Mare and foal

Table 3. Signalment and clinical information for horses from which *Salmonella* Typhimurium DT104 was isolated from 1997–2000 at the OVC-VTH

Case	Feces	Pyrexic	Depressed	Neutropenic	Colicky	Toxemic[a]
1	Diarrheic	Yes	Yes	Yes	No	No
2	Diarrheic	No	No	No	Yes	No
3	Diarrheic	No	Yes	No	No	Yes
4	Diarrheic	No	No	No	Yes	No
5	Normal	Yes	No	No	Yes	Yes
6	Diarrheic	No	Yes	Yes	No	Yes
7	Diarrheic	No	Yes	No	No	Yes
8	Diarrheic	Yes	Yes	Yes	Yes	Yes
9	Diarrheic	Yes	No	No	No	No
10	Diarrheic	Yes	Yes	Yes	Yes	Yes
11	Diarrheic	Yes	No	Yes	No	No
12	Normal	Yes	No	No	Yes	Yes
13	Soft	Yes	No	No	No	No
14[b]	Diarrheic	Yes	Yes	Yes	No	Yes
15[b]	Soft	No	No	No	No	No
16	Diarrheic	Yes	Yes	Yes	No	Yes
17	Normal	Yes	Yes	Yes	No	No

[a]Subjective assessment by attending clinicians
[b]Mare and foal

2000, *Salmonella* spp. were isolated from 13 horses at the OVC-VTH. Isolates from 12 horses (92%) were identified as *Salmonella* Typhimurium phage type DT104. The isolate from 1 horse was not phage typed. Interestingly, case 4 from 1999 was from the same farm as cases 14 and 15, which were admitted in 2000.

The age of DT104-infected horses ranged from 2 mo to 20 y (Table 2). Nine of the 17 (53%) DT104 cases were admitted with a presenting complaint of diarrhea. Other clinical and hematologic findings were inconsistent (Table 3). Five of the 17 (29%) affected horses in this group died or were euthanized, compared with 2/22 (9%) non DT104 cases (chi-squared test, $P = 0.082$). The untyped case in 2000 was not included in the analysis. The duration of fecal shedding of DT104 is unknown, as limited follow-up information was available.

All equine DT104 isolates from 2000 were resistant to ampicillin and tetracycline, but all were sensitive to

trimethoprim-sulfonamide and enrofloxacin. One of 17 isolates was resistant to gentamicin, 4 of 4 were resistant to sulfonamides and chloramphenicol but sensitive to neomycin and ceftiofur, and 1 of 8 was resistant to amikacin, but was sensitive to gentamicin.

Discussion

This is the first report describing *S.* Typhimurium DT 104 as a predominant cause of equine salmonellosis. While it cannot be ruled out that 2000 was an aberrant year with respect to phage type distribution, the increase in DT104 is consistent with the situation in cattle and humans, where DT104 is becoming a very common *S.* Typhimurium isolate (15). Equine colonization with this pathogen is not surprising, as it has been isolated from a diverse range of species.

Whenever there is an over-representation of an individual phage type isolated from horses at a referral hospital, nosocomial infection must be considered. Nosocomial infection with DT104 was only considered a *possibility* in 4 cases and was excluded in the other 13. This indicates that the emergence of *S.* Typhimurium DT104 was a community event and not a nosocomial epizootic.

The isolation of DT104 from diarrheic horses on the same farm over 2 consecutive years poses some interesting questions. Possible causes of this include prolonged shedding by asymptomatic carriers, transmission of the organism through a number of asymptomatic individuals over time, or reinfection with DT104 from an outside source. Asymptomatic carriage of *Salmonella* spp. is uncommon. A recent point prevalence study in the United States reported that the national prevalence of fecal shedding of *Salmonella* spp. in horses was only 0.8% (24). Prolonged subclinical carriage and shedding of DT104 has been observed in cattle and cats (19,25). It is possible that high infection pressure kept DT104 circulating through the horse population on this farm, with clinical disease occurring only when the proper combination of bacterial, environmental, and host factors was present.

Horse #12 passed normal feces throughout the period DT104 was shed. This demonstrates that diarrhea is not present in all cases of salmonellosis and emphasizes that salmonellosis should be considered in animals with less specific signs, such as fever of unknown origin, colic, and toxemia. It is unfortunate that information regarding the duration of shedding after discharge from hospital is unknown. This is an aspect of DT104 that must be examined, due to the risk of transmission to other horses and other species. In cattle, DT104 has been recovered from fecal samples of clinically normal cattle for up to 6 mo after outbreaks (17). Similarly, cats have been reported to shed DT104 for up to 12 wk (13). Interestingly, only 8/17 cases were neutropenic at the time salmonellosis was diagnosed.

Risk factors for the development of salmonellosis in horses include gastrointestinal disease, antimicrobial therapy, hospitalization, dietary changes, concurrent disease, and transportation (1,24,26,27). Specific risk factors for DT104 infection were not studied. Identification of DT104-specific risk factors would be very difficult, due to the low overall prevalence of salmonellosis in this region. The association of equine DT104 infection with proximity to livestock, such as cattle or pigs, should be investigated.

It has been suggested that, in humans, infection with DT104 is associated with higher morbidity and mortality than is infection with other salmonellae (21). There was a trend towards increased mortality for DT104 cases in this study; however, this was not statistically significant ($P = 0.082$). At this point, too few cases of equine DT104-associated salmonellosis have been studied to determine whether the morbidity and mortality associated with this type are different from those with other salmonellae. Calvert et al (5) reported that children might be at particular risk for DT104 infection, as they accounted for 57% of DT104 cases but only 28.4% of all *Salmonella* cases. In this study, 4/17 cases involved horses 4 mo of age or younger, and 6/17 that were less than 1 y of age; however, an increased relative risk cannot be determined at this point without proper epidemiological studies.

The high level of antimicrobial resistance and rapid emergence of resistance in DT104 isolates is of concern with respect to both treatment of affected animals and risks to in-contact individuals. Unfortunately, testing of sensitivity to chloramphenicol, streptomycin, and sulfonamides is not routinely performed on DT104 isolates at this institution, making it difficult to compare these isolates with multi-resistant (R-type ACSSuT) isolates reported elsewhere. Perhaps a standard antimicrobial susceptibility-testing regimen for veterinary isolates should be determined in order to more closely follow the emergence of multi-resistant strains. Further, since R-type ACSSuT isolates are most often DT104, antimicrobial testing can be used as a rapid screening tool for the presumptive identification of DT104 isolates (11).

At this point, it is impossible to predict whether DT104 will become a prevalent equine isolate. While this phage type accounted for 93% of equine *Salmonella* isolates in 2000 compared with only 19% of isolates from 1997 to 1999, it cannot be ruled out that 2000 was an aberrant year. Phage types can vary significantly from one year to another (3); however, the concerns regarding the phage type with respect to antimicrobial resistance and an apparent predilection for zoonotic transmission highlight the need for diligent surveillance.

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