Article

Tyzzer's disease in foals: Retrospective studies from 1969 to 2010

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Abstract — Reports of 148 cases of Tyzzer's disease in foals in central Kentucky were analyzed to identify features of the disease and factors associated with it. The records indicate that Tyzzer's disease is a rapidly progressive, highly fatal hepatitis caused by *Clostridium piliforme.* Common clinical findings are lethargy, fever, anorexia, and icterus. Seizures, coma, and death may rapidly ensue. Laboratory findings are leukopenia, metabolic acidosis, hypoglycemia, and increased activity of hepatic enzymes. Diagnosis is primarily based on clinical signs and postmortem findings but a polymerase chain reaction (PCR) is now available to detect *C. piliforme* DNA in organs and feces. Disease occurred most frequently in foals between 9 and 30 days of age that were born in April to May and was associated with heavy rainfall in the spring and high protein and nitrogenous diets fed to nursing mares. The findings are consistent with the ingestion of *C. piliforme* in the feces of adult horses and overgrowth in the intestine of foals with a high level of nutrients in their intestine.

Résumé — Maladie de Tyzzer chez les poulains : études rétrospectives de 1969 à 2010. Des rapports de 148 cas de la maladie de Tyzzer chez les poulains dans le centre du Kentucky ont été analysés pour identifier les caractéristiques de la maladie et les facteurs qui y sont associés. Les dossiers indiquent que la maladie de Tyzzer est une hépatite rapidement progressive et hautement mortelle causée par *Clostridium piliforme*. Les résultats cliniques fréquents sont la léthargie, la fièvre, l'anorexie et l'ictère. Des crises d'épilepsie, le coma et la mort peuvent rapidement survenir. Les résultats de laboratoire sont la leucopénie, l'acidose métabolique, l'hypoglycémie et une activité accrue des enzymes hépatiques. Le diagnostic se base principalement sur les signes cliniques et les résultats post mortem, mais une réaction d'amplification en chaîne par la polymérase (ACP) est maintenant disponible pour détecter l'ADN de *C. piliforme* dans les organes et les fèces. La maladie se produit le plus fréquemment chez les poulains âgés d'entre 9 et 30 jours qui sont nés en avril et en mai et elle a été associée à des pluies abondantes au printemps et à des diètes à teneur élevée en protéines et en azote données aux juments allaitantes. Les résultats sont conformes avec l'ingestion de *C. piliforme* dans les fèces des chevaux adultes et à la prolifération dans l'intestin des poulains ayant un niveau élevé de nutriments dans leur intestin.

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Introduction

Tyzzer's disease was first described in mice in 1917 (1) and in laboratory rabbits in 1965 (2). As early as 1964 a fatal hepatitis consistent with Tyzzer's disease occurred on a Thoroughbred horse farm in central Kentucky. These cases and others on the same farm were first reported as Tyzzer's disease by Swerczek et al in 1973 (3). Other cases have subsequently been reported in foals elsewhere in the USA (4–15), England (16), Canada (17,18), New Zealand (19), Australia (20,21), South Africa (22), Japan (23), and Germany (24,25).

Tyzzer's disease frequently occurs in rodents, lagomorphs, and foals, and has been reported sporadically in other domestic and wild animals, including muskrats (26), cottontail rabbits (27), coyotes (28), dogs (29), snow leopards (30), lesser panda (31), gray fox (32), raccoon (33), cattle (34), marsupials (35), and white-tailed deer (36).

Tyzzer's disease is caused by a Gram-negative, pleomorphic, motile, endospore-bearing rod-shaped and flagellated obligate intracellular bacterium previously called *Bacillus piliformis.* Tyzzer originally named the unique organism *Bacillus piliformis* based on its morphology, but a new name, *Clostridium piliforme,* was proposed because the ribosomal nucleic acid sequences of this organism are closely related to clostridia (37). *Clostridium piliforme* may be common in the environment and, since it is difficult to culture, little knowledge has accumulated on epidemiology, pathogenesis and immunity.

The purpose of this study was to investigate predisposing factors for Tyzzer's disease in foals, and diagnosis and treatment

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of affected foals. These factors include age of foals, time of the year when they were born, rainfall, and diets fed to nursing mares.

Materials and methods

Complete case reports and epidemiological investigations on affected farms performed by the author from 1969 to 1986 were used to identify risk factors for Tyzzer's disease in foals. Horses from 76 cases of Tyzzer's disease from 1969 to 1986 were submitted to the Department of Veterinary Science, University of Kentucky for necropsy. Archives of cases that were submitted to the University of Kentucky during 1986 to 1993 were incomplete and these cases were not investigated. An additional 72 cases submitted to the Livestock Disease Diagnostic Laboratory, University of Kentucky from 1993 to 2010 were also investigated. Published reports on Tyzzer's disease in foals involving clinical pathologic, serologic, and molecular tests, and treatments for the disease were reviewed and included in this study. Data on the average number of Thoroughbred foals born each month were provided by the Thoroughbred Jockey Club, Lexington, Kentucky.

Results

From 1969 to 1986 Tyzzer's disease occurred on 44 horse farms, 4 of which experienced more than 1 case. One farm (A) had 24 cases, another (B) had 6 cases, a third farm (C) had 4 cases, and the fourth farm (D) had 2 cases. In 1970 no cases were reported, but from 1971 to 1985, 1 to 13 cases occurred each year. From 1993 to 2010, 72 cases occurred with an average of 4 cases reported per year.

Age of affected foals at the day of death ranged from 5 to 30 d. Most foals died between 9 and 30 d, with the average age being 20 days. Younger affected foals were often found dead, while older foals showed clinical signs 2 to 48 h before death. The gender of foals was equally distributed. Breeds affected were Thoroughbreds, Standardbreds, American Saddle horses, Tennessee Walking horses, and crossbred foals. The majority of foals were Thoroughbreds, but this reflected the horse population in the central Kentucky area.

Diets fed to nursing mares were commercial 14% or higher protein mixed grain ration in a textured grain mixture or complete pellet. Forage rations were primarily legume-grass mixtures, clover, and alfalfa hay. Pasture grasses were a mixture of orchard, timothy, fescue, bluegrass, and white and red clover. Farms with multiple cases of Tyzzer's disease fed their mares a higher protein diet than farms without any cases of the disease.

The average percentages of foals contracting Tyzzer's disease each month were as follows: February, 5%; March, 18%; April, 31%; May, 30%; and June, 16%. The average percentages of the North American registered Thoroughbred foal crop born each month are as follows: January, 7.8%; February, 17.8%; March, 27.7%; April, 29.5%; May, 15.5%; June, 1.4%; July and later, 0.2%. On average, 54% of foals are born in January, February, and March, yet only 23% of the cases of Tyzzer's disease occurred during these months. Also, 46% of foals were born in April, May and June, while 77% of Tyzzer's disease cases occurred during these months.

Clinical signs

Clinical signs were acute, and typically, previously healthy, robust, fast-growing foals between 1 and 4 wk of age had acute onset of clinical signs. Often, younger foals were found dead without having shown signs of disease. Generally, foals died 2 to 48 h after the onset of illness. Typical signs included weakness, lethargy, anorexia, dehydration, pyrexia, tachycardia, and icterus. Some foals had a yellowish semi-liquid diarrhea. Often, seizures, coma, and death rapidly ensued. A provisional diagnosis of Tyzzer's disease was made based on the clinical signs and age of the foal.

Clinicopathologic findings

The hematological and serum biochemical values were similar in all affected foals. Leukopenia, hypoglycemia, and elevated hepatic enzymes were common findings. When foals first showed clinical signs, the majority had a severe leukopenia with values between 2000 to 4000 white blood cells (wbc)/μL. Foals had marked hypoglycemia, metabolic acidosis, elevated serum fibrinogen, and elevated bilirubin. Aspartate aminotransferase (AST), alanine aminotransferase (SGPT), lactate dehydrogenase (LDH), and alkaline phosphatase were elevated. Similar hematologic and hepatic enzyme values in foals with Tyzzer's disease were reported by others (9,12).

Treatment of foals with Tyzzer's disease

On farms with confirmed cases of Tyzzer's disease, clinicians reported successful treatment with IV dextrose and broad spectrum antibiotics in a few cases. In the majority of cases, foals responded temporarily to the IV dextrose, but then rapidly deteriorated and succumbed to the disease. A presumptive case of Tyzzer's disease in a foal was successfully treated by intensive administration IV of dextrose, sodium bicarbonate, potassium chloride, penicillin, and sulphamethazole-trimethoprim (13). Successful treatment has also been reported with IV fluids, antimicrobial and anti-inflammatory drugs, and parenteral nutrition (15).

Necropsy findings

Postmortem examination of foals notably revealed a pronounced hepatomegaly with multifocal light-colored areas in the liver capsule and parenchyma. The hepatic lymph nodes, and often the mesenteric lymph nodes as well, were hyperplastic and edematous. Contents of the gastrointestinal tract were liquid or semi-solid and yellowish or normal. Ecchymotic and diffuse hemorrhages occurred on the serosal surface of the heart, diaphragm, and the small and large intestine.

Histopathologic lesions in the liver of affected foals consisted of numerous widespread multifocal areas of necrosis and hepatitis and were pathognomonic. Often necrotic foci coalesced. Hepatocytes in the center of the necrotic foci were destroyed and replaced by mixed mononuclear cells, neutrophils, and red blood cells. Hepatocytes at the periphery of the necrotic foci contained intracellular filamentous bacilli that were faintly stained with hematoxylin and eosin. Silver and Giema stains demonstrated more clearly the intracellular interlacing bundles of filamentous bacilli. To a lesser extent, microscopic inflammatory changes and

bacilli were present in the hepatic lymph nodes, in the mucosal cells of the small and large intestine and in focal inflammatory areas in myocardial cells.

Diagnosis and diagnostic tests

The diagnosis of Tyzzer's disease was made in all cases by observing the characteristic gross and microscopic lesions in the liver of foals at necropsy. In the early cases, when the disease was first confirmed as Tyzzer's disease (3), the bacterium was isolated in the yolk sac of developing chicken embryos. However, the causative agent, *C. piliforme,* is very difficult to culture from clinical or postmortem specimens. The agent can be cultivated in tissue culture (38), but this technique has not been attempted for the cultivation of the bacterium from foals.

A recently developed real-time polymerase chain reaction (RT-PCR) assay detects DNA from *C. piliforme* and is a feasible test for routine diagnosis. The PCR targeting 16S ribosomal ribonucleic acid (rRNA) genomic sequences can detect the organism in the feces of foals showing clinical signs and provides for early diagnosis and possible treatment. There is also a real-time *Taq*Man assay that detects *C. piliforme* gene sequences in liver tissue from affected foals (15).

Serologic tests can detect exposure to *C. piliforme* in horses (39). In laboratory animals serologic tests are used to detect *C. piliforme* antibodies, but are insufficient for a definitive diagnosis of an active *C. piliforme* infection (40,41). Serologic tests have been evaluated for the diagnosis of Tyzzer's disease in multiple studies in laboratory animals (42–44).

Serological tests in rodents and rabbits showed that seroprevalence to *C. piliforme* isolates was more widespread in rodents than was clinical disease (45–47). This suggests that asymptomatic infections in rodents and lagomorphs are widespread, and unless animals are compromised by stress factors or immunologic dysfunction, clinical signs of the disease may be absent. Immunosuppressive treatment with corticosteroids (48) and challenge without steroids (49) often result in asymptomatic infection in rodents.

Discussion

Tyzzer's disease is characterized primarily by severe hepatic lesions in young foals, with lesser involvement of the intestinal tract and rarely the myocardium. In contrast, gerbils infected with *C. piliforme* often exhibit diarrhea, and, although focal necrosis may be present in the liver and myocardium of rodents and lagomorphs, lesions also occur in the intestinal tract (50).

Clostridium piliforme is difficult to culture from animals with clinical signs or from tissues at necropsy. Other than the early work that first reported the discovery of Tyzzer's disease in foals (51,52), the bacterium has not been cultured from any of the subsequent reported cases in foals. The bacterium may be common in the environment, but since it is difficult to culture, little knowledge has accumulated on its epidemiology, pathogenesis, and immunity.

Carriage and excretion of *C. piliforme* are difficult to measure because it is not possible to grow the organism in cell-free media. The PCR can detect the presence of *C. piliforme* DNA, but not the quantity of the organisms in feces (15,53). Adult horses may be carrying *C. piliforme* in the gastrointestinal tract and passing it to susceptible young foals through feces. Although no information is available on the survival of the endospores on open pastures, endospores are resistant to heating up to 60°C for 30 min or to exposure to 70% ethanol, 3% cresol, 4% chlorhexidine, or 0.037% formaldehyde; they are sensitive to heating at 80°C for 15 min or exposure to 0.4% peracetic acid, 0.015% sodium hypochlorite, 1% iodophol, or 5% phenol (54).

Tyzzer's disease often occurs sporadically, but multiple cases occurred on some farms. The source of *C. piliforme* in the environment and the factors predisposing foals to Tyzzer's disease are open to speculation. Diarrhea and fecal contamination of bedding are involved in transmitting the organism in laboratory rodents, and coprophagous oral transmission of *C. piliforme* occurs in foals (51).

Adult horses are resistant to disease caused by *C. piliforme,* but the organism appears to proliferate in the gut, especially if adults are fed a nutrient-rich diet. Foals normally consume freshly deposited feces from their dams most often between the second and fifth week of age, and rarely after the fifth week of age (55). Interestingly, foals most often succumb to Tyzzer's disease between the second and fourth week of age, and Tyzzer's disease rarely occurs in foals after the fifth week of age. It is likely that the feces of nursing mares are a primary source of *C. piliforme* infection for young foals.

Nutrient-rich diets fed to nursing mares and coprophagia were suspected to be the primary predisposing factors for developing Tyzzer's disease in young foals. Since *C. piliforme* is commonly found in rodents and lagomorphs, it was suspected that nursing mares may be infected after being exposed to fetal contamination from wildlife. On horse farms where Tyzzer's disease was most common, nursing mares often grazed pastures where the waterways routinely flooded lowland pastures after heavy rainfall. The streams contained numerous muskrats, which are known to be carriers and infected with *C. piliforme* (26).

There have not been any studies in foals involving stress factors and immunosuppressive steroids, but they likely play a role in the pathogenesis of the disease. Stress-related factors and dietary factors, including high-protein diets fed to nursing mares seemingly are involved in predisposing suckling foals to Tyzzer's disease (56). Similarly, it has been shown that high-protein diets predispose laboratory animals to Tyzzer's disease (57).

The sporadic nature of Tyzzer's disease in foals reported in the literature has not revealed any predisposing factors which may increase the susceptibility of foals to the disease. However, numerous cases seen on Kentucky horse farms and a case-control study (14) designed to determine variables associated with increased risk of *C. piliforme* infection in foals on a California Thoroughbred breeding farm have identified some predisposing factors. In the California study, foals born between March 13 and April 13 were 7.2 times more likely to develop *C. piliforme* infections than foals born at other times of the year. A similarly high incidence of the disease occurred in foals born in the spring on Kentucky horse farms where 58% of the foals were affected in April and May. Also, foals from nonresident mares were 3.4 times more likely to develop *C. piliforme* infection. And, foals of mares less than 6 years of

age were 2.9 times more likely to develop *C. piliforme*-associated illness (14).

Amount of rainfall during the early spring dramatically affects growth of cool-season grasses. Furthermore, a spike in the protein and non-protein nitrogenous compounds, including nitrate, commonly occurs in the early spring pasture forages (58). If there is a sudden increase in nitrogenous compounds in the diet, there may be a change in the distribution of the normal bacterial gut flora which often leads to increased nitrate and ammonia levels in the hindgut (59). Farms with multiple cases of Tyzzer's disease also had foals with congenital musculoskeletal lesions. The cause of skeletal abnormalities is unknown, but Canadian researchers suggest that there may be an association with nitrate toxicity and hyperthyroidism (60,61).

The increase in availability of nutrients may encourage the overgrowth of *C. piliforme* in the gut of the nursing mare and foal. Other similar peracute clostridial diseases in foals (62), and clostridial infections in herbivores in general, also often affect apparently healthy fast-growing young animals when the intestinal environment is altered by sudden changes in the diet that cause the overgrowth of clostridial organisms in the gut of affected animals (63). Since Tyzzer's disease is a peracute, highly fatal clostridial disease of foals, avoiding sudden changes in the diet of nursing mares may be helpful in controlling the disease in foals.

References

1. Tyzzer EE. A fatal disease of Japanese Waltzing mouse caused by a spore-bearing bacillus *Bacillus piliformis* N.S.P. J Med Res 1917;37:307–338.
2. Allen AM, Ganaway JR, Moore TD, Kinard RF. Tyzzer's disease syndrome in laboratory rabbits. Am J Path 1965;46:859–882.
3. Swerczek TW, Crowe MW, Prickett ME, Bryans JT. Focal bacterial hepatitis: Preliminary report. Mod Vet Pract 1973;54:66–67.
4. Hall WC, Van Kruiningen HJ. Tyzzer's disease in a horse. J Am Vet Med Assoc 1974;164:1187–1188.
5. Pulley LT, Shiveley JN. Tyzzer's disease in a foal. Light and electron microscopic observations. Vet Pathol 1974;11:203–211.
6. Harrington DD. Naturally-occurring Tyzzer's disease *(Bacillus piliformis* infection*)* in horse foals. Vet Rec 1975;96:59–63.
7. Harrington DD. *Bacillus piliformis* infection (Tyzzer's disease) in two foals. J Am Vet Med Assoc 1976;168:58–60.
8. Turk MA, Gallina AM, Ferryman LE. *Bacillus piliformis* infection (Tyzzer's disease) in foals in Northwestern United States: A retrospective study of 21 cases. J Am Vet Med Assoc 1981;178:279–281.
9. Brown CM, Ainsworth DM, Personett LA, Derksen FJ. Serum biochemical and haematological findings in two foals with focal bacterial hepatitis (Tyzzer's disease). Equine Vet J 1983;15:375–376.
10. Nold JB, Swanson T, Spraker TR. *Bacillus piliformis* (Tyzzer's disease) in a Colorado foal. J Am Vet Med Assoc 1984;185:306–307.
11. Scarratt WK, Saunders GK, Welker FH, Halpern NE, Cordes DO, Camp GM. *Bacillus piliformis* infection (Tyzzer's disease) in two Virginia foals. J Equine Vet Sci 1985;5:135–138.
12. Humber KA, Sweeney RW, Saik J, Hansen TO, Morris CF. Clinical and clinicopathological findings in two foals infected with *Bacillus piliformis.* J Am Vet Med Assoc 1988;193:1425–1428.
13. Peek SF, Byars TD, Rueve E. Neonatal hepatic failure in a Thoroughbred foal: Successful treatment of a case of presumptive Tyzzer's disease. Equine Vet J 1994;6:307–309.
14. Fosgate GT, Hird DW, Read DH, Walker RL. Risk factors for *Clostridium piliforme* infection in foals. J Am Vet Med Assoc 2002;220: 785–790.
15. Borchers A, Magdesian KG, Holland S, Pusterla N, Wilson WD. Successful treatment and polymerase chain reaction (PCR) confirmation of Tyzzer's disease in a foal and clinical and pathologic characteristics of 6 additional foals (1986–2005). J Vet Intern Med 2006;20:1212–1218.
16. Whitwell KE. Four cases of Tyzzer's disease in foals in England. Equine Vet J 1976;8:118–122.
17. Thomson GW, Wilson RW, Hall EA, Physick-Sheard P. Tyzzer's disease in the foal: Case reports and review. Can Vet J 1977;18:41–43.
18. St. Denis KA, Waddell-Parks N, Belanger M. Tyzzer's disease in an 11-day-old foal. Can Vet J 2000;41:491–492.
19. Dickinson LG. Tyzzer's disease in foals. New Zealand Vet J 1980;28:60.
20. Corrigan MJ, Pedrana RG, McKibbin AW. Tyzzer's disease in foals. Aust Vet J 1984;61:199–200.
21. Copland MD, Robartson CW, Fry J, Wilson G. Tyzzer's disease in a foal. Aust Vet J 1984;61:302–304.
22. Van der Lugt JJ, Coetzer JA, Jordaan P, Marlow CH. Tyzzer's disease in two foals. J S Afr Vet Assoc 1985;56:107–108.
23. Shirakawa T, Maruyama K, Nakamura N, et al. Tyzzer's disease in a foal. Nippon Juigaku Zasshi 1989;51:444–446.
24. Paar M, Stockhofe-Zurwieden N, Pohlmeyer G, Gerhards H, Pohlenz J. Infection with *Bacillus piliformis* (Tyzzer's disease) in foals. Schweiz Arch Tierheilkd 1993;135:79–88.
25. Appel G, Burdinski K. Tyzzer's disease in a pony foal from Schleswig-Holstein Dtsch Tierarztl Wochenschr 1995;102:204–205.
26. Karstad L, Lusis P, Wright D. Tyzzer's disease in muskrats. J Wildl Dis 1971;7:96–99.
27. Ganaway JR, McReynolds RS, Allen AM. Tyzzer's in free-living cottontail rabbits *(Sylvilagus aoridanus)* in Maryland. J Wildl Dis 1976; 12:545–549.
28. Marler RJ, Cook JE. Tyzzer's disease in two coyotes. J Am Vet Med Assoc 1976;169:136–138.
29. Poonacha KB, Smith HL. Naturally occurring Tyzzer's disease as a complication of distemper and mycotic pneumonia in a dog. J Am Vet Med Assoc 1976;169:419–420.
30. Schmidt RE, Eisenbrandt DL, Hubbard GB. Tyzzer's disease in snow leopards. J Comp Pathol 1984;94:165–167.
31. Bonney CH, Schmidt RE. A mixed infection: Chaga's and Tyzzer's disease in a lesser panda. J Zoo Med 1975;6:4–7.
32. Stanley SM, Flatt RE, Daniels PY. Naturally occurring Tyzzer's disease in the grey fox. J Am Vet Med Assoc 1978;173:1173–1174.
33. Wojcinski ZW, Barker lK. Tyzzer's disease as a complication of canine distemper in a raccoon. J Wildl Dis 1986;22:55–59.
34. Webb DM, Harrington DD, Boehm PN. *Bacillus piliformis* infection (Tyzzer's disease) in a calf. J Am Vet Med Assoc 1987;191:431–434.
35. Canfield PJ, Hartley WJ. Tyzzer's disease *(Bacillus piliformis)* in Australian marsupials. J Comp Pathol 1991;105:167–173.
36. Brooks JW, Whary MT, Hattel AL, et al. *Clostridium piliforme* infection in two farm-raised white-tailed deer fawns *(Odocoileus virginianus)* and association with copper toxicosis. Vet Pathol 2006;43:765–768.
37. Duncan AJ, Carman RJ, Olsen, GJ, Wilson KH. Assignment of the agent of Tyzzer's disease to *Clostridium piliforme* comb. nov. on the basis of 16S rRNA sequence analysis. Int J Syst Bacteriol 1993;43:314–318.
38. Spencer TH, Ganaway JR, Waggie KS. Cultivation of *Bacillus piliformis* (Tyzzer) in mouse fibroblasts (3T3 cells). Vet Microbiol 1990;22: 291–297.
39. Hook RR, Riley LK, Franklin CL, Besch-Williford CL. Seroanalysis of Tyzzer's disease in horses: Implications that multiple strains can infect Equidae. Equine Vet J 1995;27:8–12.
40. Feldman SH, Kiavand A, Seidelin M, Reiske HR. Ribosomal RNA sequences of *Clostridium piliforme* isolated from rodent and rabbit: Re-examining the phylogeny of the Tyzzer's disease agent and development of a diagnostic polymerase chain reaction assay. J Am Assoc Lab Anim Sci 2006;45:65–73.
41. Pritt S, Henderson KS, Shek WR. Evaluation of available diagnostic methods for *Clostridium piliforme* in laboratory rabbits *(Oryctolagus cuniculus).* Lab Anim 2010;44:14–19.
42. Riley LK, Besch-Williford C, Waggie KS. Protein and antigenic heterogeneity among isolates of *Bacillus piliformis.* Infect Immun 1990;58:1010–1016.
43. Motzel SI, Riley LK. *Bacillus piliformis* flagellar antigens for serodiagnosis of Tyzzer's disease. J Clin Microbiol 1991;29:2566–2570.
44. Boivin GP, Hook RR, Riley LK. Antigenic diversity in flagellar epitopes among *Bacillus piliformis* isolates. J Med Microbiol 1993;38:177–182.
45. Hansen AK, Svendsen O, Mollegaard-Hansen KE. Epidemiological studies of *Bacillus piliformis* infection and Tyzzer's disease in laboratory rats. Z-Versiichstierkd 1990;33:163–169.
46. Goto K, Itoh T, Takakura A, Kunita S, Terada E, Kagiyama N. A serological survey on *Bacillus piliformis* infection in laboratory rabbits in Japan. Jikken-Dohiitsii 1991;40:231–233.
47. Boivin GP, Hook RR, Riley LK. Development of a monoclonal-antibody-based competitive ELISA for detection of *Bacillus piliformis*

isolate-specific antibodies in laboratory animals. Lab Ani Sci 1994; 44:153–158.
48. Itoh T, Kagiyama N. Differences in susceptibility to peroral inoculation with *Bacillus piliformis* spores in rats and mice. Jikken Dohutsu 1990;39:425–428.
49. Motzel SI, Riley LK. Subclinical infection and transmission of Tyzzer's disease in rats. Lab Anim Sci 1992;42:439–443.
50. Licois D. La Maldie de Tyzzer. Ann Rech Vet 1986;17:363–386.
51. Swerczek TW. Experimental focal bacterial hepatitis in foals (Tyzzer's disease). Proceedings 55th Conference of Research Workers in Animal Diseases, Chicago, Illinois. 1975; p 13.
52. Swerczek TW. Multifocal hepatic necrosis and hepatitis in foals caused by *Bacillus piliformis* (Tyzzer's disease). Vet Ann 1976;17:130–132.
53. Furukawa T, Furumoto K, Fujieda M, Okada E. Detection by PCR of the Tyzzer's disease organism *(Clostridium piliforme)* in feces. Exp Anim 2002;51:513–516.
54. Itoh T, Ebukuro M, Kagiyama N. Inactivation of *Bacillus piliformis* spores by heat and certain chemical disinfectants. Jikken Dohulsu 1987;36:239–244.
55. Francis-Smith K, Wood-Gush DGM. Coprophagia as seen in the Thoroughbred foals. Equine Vet J 1977;9:155–157.
56. Kahn CM. Tyzzer's disease. In: The Merck Veterinary Manual. 10th ed. Hoboken, New Jersey: John Wiley and Sons, 2010.
57. Maejima K, Fujiwara K, Takagaki Y, Naiki M, Kurashina H, Tajima Y. Dietetic effects on experimental Tyzzer's disease. Jpn J Exp Med 1965; 35:1–10.
58. Taylor JR. Theory of ammonia toxicity as the mechanism of abortion in the mare reproductive loss syndrome. J Eq Vet Sci 2002;22:237–239.
59. Wooton JF, Argenzio RA. Nitrogen utilization within equine large intestine. Am J Physiol 1975;229:1029–1067.
60. McLaughlin BG, Doege CE. Congenital musculoskeletal lesions and hyperplastic goitre in foals. Can Vet J 1981;22:130–133.
61. Allen AL, Townsend HG, Doige CE, Fretz PB. A case-control study of the congenital hypothyroidism and dysmaturity syndrome of foals. Can Vet J 1996;37:349–358.
62. Swerczek TW. Toxicoinfectious botulism in foals and adult horses. J Am Vet Med Assoc 1980;176:217–220.
63. Uzal FA, Songer JG. Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. J Vet Diagn Invest 2008;20:253–265.