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High mortality in foals associated with *Salmonella enterica* subsp. *enterica* Abortusequi infection in Italy

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Abstract. *Salmonella enterica* subsp. *enterica* serovar Abortusequi is frequently reported as a cause of abortion in mares and neonatal septicemia and polyarthritis in Asian and African countries, but only sporadically in Europe and the United States. We report an outbreak of *S.* Abortusequi in foals in Italy, characterized by high mortality. In a herd of Murgese horses, 10 of 34 newborns died at birth and a further 7 died, after developing severe clinical signs, during the first 10 d of life. Tissue specimens from different organs of 2 dead foals, synovial fluids from 4 sick foals, and vaginal and rectal swabs from their dams were cultured. A total of 16 isolates, all as pure cultures, were obtained and identified as *Salmonella*. The isolates exhibited the same antimicrobial resistance pattern and the same sequence type, ST251, a type that has been associated with *S.* Abortusequi. Six of 16 isolates were serotyped and found to be *S.* Abortusequi 4,12:-:e,n,x. Equine practitioners should be aware of *S.* Abortusequi infection as a cause of neonatal mortality in foals.

Key words: Murgese foals; outbreak; *Salmonella* Abortusequi.

Salmonella enterica subsp. *enterica* serovar Abortusequi is a host-adapted serovar that is associated with abortion in mares and neonatal septicemia and polyarthritis.[3,15,17] Moreover, equine salmonellosis is often reported as a secondary bacterial infection associated with equid herpesvirus 1 (EHV-1; species *Equid alphaherpesvirus 1*) infection.[18,19] Although reported commonly from Asian and African countries, *S.* Abortusequi is only isolated sporadically in Europe, the United States, and Argentina.[3,9,12-14,16]

We describe herein a severe disease outbreak in foals, characterized by high mortality, which was caused by *S.* Abortusequi. The herd was located in Altamura, Apulia, Italy, and consisted of 72 Murgese horses (40 mares, 7 stallions, and 25 yearlings). From January to April 2016, 34 foals were born from mares without clinical signs. Ten of the 34 newborns died at birth. A further 7 foals developed severe clinical signs including fever up to 41°C, lethargy, bloody diarrhea, and lameness, and died during the first 10 d of life (overall mortality rate of 50%). An additional 4 foals of the same herd, 15–20 d of age, exhibited similar clinical signs. Abortion or clinical signs were not reported in the mares in the months before the disease outbreak.

Autopsies of 2 dead foals revealed diffuse hemorrhagic inflammation of the cecum and large colon, hepatization of the lungs, and necrotic lesions of the kidneys and liver. The lymph nodes were swollen and hemorrhagic. Tissue specimens from mediastinal lymph nodes, lung, kidney, liver, spleen, and colon of the 2 dead foals, and synovial fluids of 4 sick foals, were collected. Vaginal and rectal swabs from their dams ($n = 6$) were also included. The tissues were cultured on MacConkey agar and 5% sheep blood agar and incubated at 37°C for 24 and 48 h, respectively.[4] Gram-negative, lactose-negative organisms were cultured on triple sugar iron (Oxoid, Milan, Italy). All lactose-negative, H_2S-negative isolates were tested by means of a PCR assay targeting the *invA* gene.[11] Antimicrobial susceptibility testing was performed by the agar diffusion disk method,[5,6] testing the following antimicrobials: chloramphenicol (30 µg), doxycycline (30 µg), tetracycline (30 µg), ampicillin (10 µg), amoxicillin–clavulanic acid (20 µg of amoxicillin + 10 µg of clavulanic acid), cefuroxime (30 µg), ceftazidime (30 µg), colistin (10 µg), gentamicin (10 µg), ciprofloxacin (10 µg), enrofloxacin (5 µg), streptomycin (10 µg), sulfamethoxazole (50 µg), and trimethoprim–sulfamethoxazole (1.25 µg of trimethoprim + 23.75 µg of sulfamethoxazole). The discs were all obtained from a single source (Liofilchem, Teramo,

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Italy). Clinical and Laboratory Standards Institute (CLSI) breakpoints[5] for bacteria associated with infections of humans were used for the interpretation of disk diffusion results for cefuroxime and ceftazidime, whereas veterinary CLSI breakpoints[6] were used for the interpretation of the remaining antimicrobials. Six of 16 isolates (obtained from lung and liver of 2 dead foals and synovial fluids of 2 sick foals) were serotyped at the National/OIE Reference Laboratory for Salmonella (Experimental Zooprophylactic Institute of Venice, IT) according to the Kaufmann–White scheme.[8,10] Strains were analyzed by multi-locus sequence typing (MLST).[1] The MLST sequence type was assigned through the EnteroBase website (https://goo.gl/3AfZP8). DNA and RNA were extracted from lung and liver of foals and from the vaginal swabs (QIAamp *cador* pathogen mini kit, Qiagen, Hilden, Germany) and tested by real-time PCR for EHV-1[7] and by reverse-transcription PCR for equine arteritis virus (EAV).[2]

From the synovial fluids of 4 sick foals, and from the organs of 2 dead foals, 16 isolates were obtained and identified as *Salmonella* spp. by PCR. The isolates exhibited the same antimicrobial resistance pattern (i.e., susceptible to chloramphenicol, tetracycline, ampicillin, amoxicillin–clavulanic acid, cefuroxime, ceftazidime, gentamicin, enrofloxacin, and trimethoprim–sulfamethoxazole, and resistant to doxycycline, colistin, ciprofloxacin, streptomycin, and sulfamethoxazole). Six representative strains were serotyped as *S.* Abortusequi 4,12:-:e,n,x. All of the isolates from the foals were characterized as sequence type (ST)251 (https://goo.gl/3AfZP8). All of the samples from the foals were negative to the virologic screening (EHV-1 and EAV), and no other bacteria were isolated. The rectal and vaginal samples obtained from the mares were negative in the virologic screening, and *Salmonella* spp. were not detected. After the etiologic diagnosis and on the basis of the antimicrobial resistance patterns of the isolates, 4 symptomatic foals were treated with trimethoprim–sulfamethoxazole (20 mg/kg once a day) and gentamicin (6 mg/kg once a day) via the intramuscular route for 10 d. Three foals recovered fully without permanent sequelae; one foal had permanent signs of polyarthritis.

Several microbes can be involved in the etiology of genital and neonatal disorders in equids.[17,18] Equine paratyphoid, caused by *S.* Abortusequi, is an infectious disease characterized by contagious abortions in most equids and is generally only reported sporadically in Europe.[12-14] In our study, pure cultures of *Salmonella* were isolated from all of the samples ($n = 16$) from the foals subjected to our laboratory investigations, with all isolates shown to be of the same sequence type and 6 confirmed as *S.* Abortusequi. No preliminary signs of abortion were reported, and the source of the outbreak could not be identified confidently. The rectal and vaginal samples of the relevant dams were negative, and it was not possible to test the other mares. However, the epidemiologic investigation indicated that a possible source of infection could have been 2 mares that had been introduced into the herd recently. Given that the horses lived and grazed freely, it is possible that one or more carriers had infected the other animals or the newborns at grazing or during delivery of some mares. Horses infected with *S.* Abortusequi may act as long-term carriers even after their recovery, and it has been suggested that recovered horses pose a risk as new sources of infection.[17] It is difficult to distinguish between infections acquired in utero and infections that are acquired neonatally; the long-term consequences for foals depend on the severity of the disease.[17]

The isolates obtained from the outbreak were typed as ST251, a clonal type previously described for isolates of *S.* Abortusequi from Croatia, Argentina, and the United States (https://goo.gl/3AfZP8). Disease caused by *S.* Abortusequi has been reported in Italy previously,[14] but the isolates were subjected to only serotyping and not genotyping, thus hindering a precise comparison. The Murgese horse is an autochthonous Italian breed, which is usually considered to be intrinsically resistant to most diseases, and vaccination was not a feature of herd management in our case. Equine practitioners and breeders should be aware of the risks posed by *S.* Abortusequi infection and employ appropriate preventive measures.

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