Figure 1. Fetuses with different ages (A, B and C) and signs of septicemia and friable organs at necropsy (D, E and F)

the neonates could born infected showing septicemia and polyarthritis. *Salmonella* Abortusequi was isolated from abortions in 1948 for first time in Argentina. Between 1948 and 1994, abortion outbreaks, mares with reproductive pathologies and goals with septicemia and polyarthritis were described. Nowadays, the worldwide prevalence of paratitic abortion is low but in our country, the disease has been reported several times since 2011. The aims of this study were isolated and characterized *Salmonella* Abortusequi from equine fetuses. Six fetuses with different ages were processed in The Laboratory of Infectious Diseases (LEEI) of Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. The fetuses were from two farms of Buenos Aires province (25 de Mayo and Trenque Lauquen cities) where have been more than 40 aborts. Necropsy, bacteriological and histopathological studies were done. Samples of different organs, as stomach contents, liver, spleen, lungs, kidneys, jejunum, colon and cecum were cultured in Xylose Lysine Deoxycholate agar (XLD), blood agar and Selenito broth for 24 h at 37°C. Bacteria were identified by biochemical test and PCR. Antibiotic susceptibility of the strains was performed by the agar diffusion disk method as outlined by the National Committee for Clinical Laboratory Standards (CLSI) using chloramphenicol, florphenicol, tetracycline, ampicillin, cefotaxime, gentamicin, ciprofloxacin, enrofloxacin, streptomycin and trimethoprim/sulfamethoxazole. All the fetuses showed signs of septicemia and friable organs at necropsy but those lesions were more intense in younger animals. Autolysis was observed in most of organs and cell edema and fat damage were observed in liver by histological studies. Lactose and sulfidric negative colonies were isolated from all samples in XLD. Biochemical test were performed by *Salmonella* sp identification. PCR was carry on from Selenito broth and invA gen was detected in all cultures. The isolates were identified as *Salmonella* Abortusequi by White-Kaufman-Le Minor schema. All six isolates were sensible to the antibiotics used. Epidemiological characteristics, macroscopic and microscopic lesions, molecular detection and *Salmonella* Abortusequi in purity isolation from all samples allowed identified those *Salmonella* outbreaks. Large economical loses were generated by the large number of lost foals and treatment costs. Previously, infectious abortions were associated with viral etiology as Equine Herpesvirus-1. However, epidemiological data of the last years show an increasing relevance of *Salmonella* Abortusequi in reproduction problems and it could be considered as an emergent pathogen in Argentina. In conclusion, paratitic abortion should be suspected in any abortion and both viral and bacterial diagnosis always must be done in all cases to establish appropriate prophylactic and therapeutic measures. We propose to study the clonal relation between strains to understand the epidemiology of the disease in our country.

**056**

**Determination of whole-genome sequence of *Salmonella* Abortusequi**

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Equine paratypoid is an infectious disease specific to the family Equidae and caused by *Salmonella* Abortusequi. It is a designated notifiable disease in Japan. Abortion or multiple abscesses are major signs of infection, and the disease is diagnosed by detecting the etiological agent or by serodagnosis. However, these methods are problematic in terms of sensitivity or specificity: for example, cross-reactivity to *Salmonella* Typhimurium can occur in serodagnosis. In addition, conventional molecular epidemiological methods have insufficient resolution to discriminate the strains isolated in Japan. To search for a way of solving these problems, we determined the whole-genome sequence (WGS) of *Salmonella* Abortusequi L-2508, which was isolated in 1987 in Japan, was used as representative strain of *S. Abortusequi* to determine the complete genome sequence by using next-generation sequencing, optical genome mapping, and a gap-closing technique. The size of the genome of L-2508 was 4,738,978 bp; the GC content was around 52%; and 4710 ORFs were detected. A phylogenetic tree analysis based on core-genome single nucleotide polymorphisms (SNPs) revealed that *S. Abortusequi* was genetically close to *Salmonella* Choleraesuis and *Salmonella* Paratyphi C. There were 136 pseudogenes; this number was similar to that of *S. Choleraesuis* (host-adapted serotype) and was half as many as that of *S. Dublin* (host-restricted serotype). The WGS of L-2508 was compared with those of other serotypes of *Salmonella* spp. A region unique to *S. Abortusequi* that seemed to be derived from a bacteriophage was detected. Comparison of the presence of known virulence factors in *Salmonella* spp. between *S. Abortusequi* and *S. Typhimurium* detected no *S. Abortusequi*-specific gene. On the other hand, several pathogenic genes were observed only in *S. Typhimurium*. This result suggests that these *S. Typhimurium* genes might be useful for developing a serological method to discriminate infections with the two organisms. The draft whole-genome sequences of 24 strains isolated in various geographic areas and at various times were also determined. Phylogenetic tree analysis based on core-genome SNPs was performed on these strains and on L-2508. There were 1316 SNPs among the strains. A difference of about 1100 SNPs was observed between Japanese and foreign strains, whereas only a 61-SNP difference was observed among the Japanese strains. There were two lineages among the Japanese strains. Our results suggest that SNP-based molecular epidemiological methods might be useful for discrimination among Japanese endemic strains.

**050**

**Diagnostic epidemiology of nocardioform placentitis and abortion in Kentucky, 1991-2015**

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Equine nocardioform placentitis & abortion (NPA) first recognized in Kentucky in the 1980’s, is caused by a nocardioform actinomycete bacteria. *Crossiella equi* was first identified in equine placental
tissue in 2002. In 2003, three species of *Amycolatopsis* and in 2009 two species of *Streptomyces* were identified in association with NPA cases. *Amycolatopsis* spp. (48.7%) and *Crossiella equi* (28.9%) were the most prominent nocardioforms identified in a recent abortion outbreak. This infection leads to late abortions, stillbirths and premature foaling. Premature foals sometimes die shortly after birth. The mode of transmission of this syndrome is not known.

To date, nocardioform *Actinomycetes* have only been isolated from placental tissue. Most reported cases are from central Kentucky (Figure 1) but cases have also been diagnosed in Florida, Italy, and South Africa. In the 2010-2011 equine reproductive season, our Lexington laboratory diagnosed 118 cases of NPA by culture and PCR. Due to the high incidence of NPA that season, a farm-level study was conducted to identify possible risk factors for NPA. A total of 148 horse farms were included in a survey (98 affected, 50 unaffected). In total, 8075 mares were at risk on all farms (Figure 1) but cases have also been diagnosed in Florida, Italy, and South Africa. In the 2010-2011 equine reproductive season, our Lexington laboratory diagnosed 118 cases of NPA by culture and PCR. Due to the high incidence of NPA that season, a farm-level study was conducted to identify possible risk factors for NPA. A total of 148 horse farms were included in a survey (98 affected, 50 unaffected). In total, 8075 mares were at risk on all farms (Figure 1) but cases have also been diagnosed in Florida, Italy, and South Africa.

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**Figure 1.** Twenty-five years of NPA cases (1133) in Kentucky.

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Contagious equine metritis (CEM) is a venereally transmissible disease of equids. The etiologic bacterium, *Taylorella equigenitalis*, can cause widespread short-term infertility and very rarely, abortion in mares. A frequent sequel to exposure of stallions and mares to *T. equigenitalis* is establishment of a carrier state that is often long-term in stallions. Aims of this study are twofold: 1) to estimate frequency of the carrier state in stallions and mares by states testing the greatest number of imported horses for CEM; and 2) to establish the basis of determination of persistence of *T. equigenitalis* in individual carrier animals. Test subjects were stallions and mares imported between 1997 and 2014. Mares and stallions underwent post-entry quarantine and testing for CEM in accordance with USDA prescribed protocols. Findings of the study confirmed that there was a continuing risk of reintroduction of CEM into the USA from known CEM-affected countries. Over the 17-year study period, 38 stallions and mares were confirmed carriers of *T. equigenitalis*. Twenty-seven were stallions and 11 were mares. Some 24 of the carrier stallions and all 11 of the carrier mares were identified by the six selected states. There were two instances, both involving stallions, where the post-entry testing protocol failed to detect the carrier state prior to the stallion’s release from quarantine. Only eight of the 27 carrier stallions were detected by culturing a single set of swabs.

Detection of the carrier state in 18 stallions was only achieved by test breeding. The preponderance (80%) of *T. equigenitalis* strains isolated either from stallions or mares were streptococin sensitive. The overall positive rate for stallions was 0.98% (24 of 2,457 tested), whereas the corresponding rate for mares was 0.07% (11 out of 15,732). Test breeding as opposed to sole use of culture was a highly reliable but not totally foolproof means of identifying the carrier stallion. A fully validated, less costly, more rapid, and logistically less challenging in vitro test is sorely needed for detection of the carrier state, especially in the stallion.

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**References**


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**011**

Contagious Equine Metritis: Efficacy of US post-entry testing protocols for identifying carrier stallions and mares

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**060**

Sensitivity of qPCR for screening cryopreserved semen from *Taylorella equigenitalis*-carrier stallions

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The risk for disease transmission via cryopreserved semen contaminated with *Taylorella equigenitalis* remains largely