199
Faecal shedding and serological study of *Lawsonia intracellularis* from two horse farms in Argentina

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Lawsonia intracellularis (*Li*) is the causative agent of equine proliferative enteropathy (EPE), an endemic infection in pigs and an emerging concern in horses. EPE has been reported in North America, Europe, Australia and Brazil, and is more frequent in weaned foals. The antemortem diagnosis of EPE is based on the detection of *Li*–specific antibodies by serology and the molecular identification of DNA in feces by PCR. The aim of this work is to report an outbreak of EPE in two horse farms in Buenos Aires province. Serology tests were performed both in mares and weanlings (9-12 months) while PCR of faecal samples was performed only in weanlings. The DNA was extracted (ZR MiniPrep Zymo Research, USA) and the PCR was applied according to Jones et al. 1993. The indirect immunofluorescence was carried out according to Knittel et al. 1997. In farm one, sera from 32 mares and two foals with diarrhea and clinical signs were analyzed, while in farm two, sera from 14 mares and 15 foals were processed, where only one had chronic diarrhea and an antibiotic treatment. The results are shown in table 1. Total positive sera were 79% (50/63) and positive PCR in foals 12% (2/17). Besides, at least one animal was positive by PCR in each farm. The high percentage of seropositive animals may indicate previous exposure to *Li*. The seropositivity of mares implies subclinical infection, a situation of constant exposure or long term persistence of serum antibodies. In a foal with diarrhea and positive PCR may confirm the EPE. However, in a foal with diarrhea and negative PCR, the disease could not ruled out, because a negative result is expected in situations such as a prolonged course or antibiotic therapy. The detection of positive PCR animals without diarrhea, as observed in farm two, can be related to a subclinical infection, a common condition seen in swine. In conclusion, to our knowledge, this is the first description clinical and subclinical case of EPE with confirmed diagnosis by serology and fecal PCR in horses in Argentina. The EPE must be considered among the possible diagnoses in cases of enteritis in foals. Use of these tests may aid in detection of the disease allowing for early and specific treatment to improve the prognosis of affected horses.

Table 1
Serology and PCR of *Li* in horses.

Farm	Serology				PCR	
	Mares		Foals		Foals	
	-	+	-	+	-	+
1	8	24	0	2	1^{2}	1*
2	2	12	3	12	14^{1}	1^{1}

*Acute diarrhea; [1]Without diarrhea; [2]Chronic diarrhea + antibiotictherapy.

References

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Poster
122
Phylogenetic and structural analyses of equine rotaviruses: Three possible geographically related lineages

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Equine Group A Rotaviruses (RVA) are a major cause of neonatal diarrhea in foals under 4 months of age. Based on the VP7 and VP4 outer capsid proteins, RVA strains are classified into 27 G- and 37 P-genotypes, respectively. RVA G3P[12] and G14P[12] are epidemiologically the most important genotypes circulating in horses worldwide. An extended RVA classification system, which defines genotypes for all 11 genome segments, reveals that genomes of equine RVA G3P[12] and G14P[12] shown a highly conserved backbone with the exception of gene segments encoding the proteins VP7 (G3 and G14), VP6 (I2 and I6), and NSP4 (E2 and E12), which show to have at least two common variants in horses. Interestingly, within this genotype constellation, some striking geographic patterns can be seen occasionally. Despite each of the 11 gene segments can theoretically segregate independently during strain reassortment, a genetic linkage between VP6:VP7 equine RVA has been reported. Thus, viral protein interactions might drive the selection of strains that can replicate and spread in horses better. Besides, a geographical restricted genotype (E12) was also found. In this study we characterized the VP6, VP7, VP8 and NSP4 gene segments from a large field sample collection using phylogenetic and structural analyses. The data sets were constructed with all equine RVA sequences available in Genbank and, the phylogenetic analyzes were conducted in MEGA software, the molecular modeling was carried out in I-Tasser and the structures were analyzed with UCSF Chimera package. The phylogenetic analyses of VP7 showed that equine RVA form three different lineages (European, Asian and South American) for both, G3 and G14, genotypes. The same topology was observed for both genotypes (I2 and I6) in VP6. The analyses of VP8* also show the same pattern, clustering the strain in the same clusters but, the distance between them is not enough to defined lineages. Regarding the analyses of NSP4 gene segment, its show a monophyletic group in E12 genotypes (equine and other). The result obtained with the deduced amino acid (aa) sequences were in agreement with the ones described for nucleotides. The structural analyses revealed that the aa substitutions in VP7 are in the antigenic sites and in the VP7:VP6 interaction region. In accordance, most of the aa changes in VP6 are in the top of the protein involving antigenic and VP7:VP6 interaction sites. Interestingly, the structural analyses of VP8* showed a few aa differences in the antigenic sites and, all equine P[12] shown the same conserved aa sequence in VP8* pocket, only observed in P[12] genotype. Our study suggest the existence of geographically related equine RVA lineages and, describe the aa configuration of the VP8* pocket.