

SZENT ISTVÁN UNIVERSITY

Doctoral Training Programme in Veterinary Science

Infective abortions and reproductive disorders in mares

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1. The aim of the study

Only few data is present about the infective abortions and reproductive disorders of mares in Hungary. The aim of the study was to collect samples from possibly large amount of cases and to investigate several infectious agents and their role in abortions and reproductive disorders of mares.

2. Materials and methods

In the first part of the research in six healthy mares and 24 mares showing reproductive disorders swab samples were taken from the fossa clitoridis to isolate *Taylorella (T.) equigenitalis*, and from the uterus to isolate mycoplasmas, ureaplasmas and other aerobic bacteria. Swab samples were also taken from the uterus for *Chlamydia* antigen ELISA and *Chlamydia* PCR studies. The uterus of 27 mares was examined cytologically, and biopsy samples were taken from the endometrium for histological examinations and for immunohistochemical examinations aimed at the detection of chlamydiae.

In the second part of the research 92 aborted foals and 4 newborn foals were examined. Fetal membranes from 76 cases and the dam's sera of 65 cases were also investigated. Histological methods were used to detect the microscopical lesions and leptospire or fungi. Microbiological examinations were done from several organs, stomach and fetal membranes for isolation of aerobic bacteria. Smears from the stomach and fetal membrane were collected to detect chlamydiae and campylobacter. The serum samples of the mares were examined for *Chlamydia*, *Brucella*, *Leptospira*, *Salmonella abortusequi*, *equine herpesvirus 1 typ* (EHV-1) and *equine arteritis virus* (EAV). The fetal sera were examined for EHV-1 and EAV. Virus isolation was attempted also for these two viruses. Immunohistochemical examination was used to detect EAV, leptospire, chlamydiae, *Toxoplasma (T.) gondii*, *Neospora (N.) caninum* and in-situ hybridization for EHV-1 in fetal membranes.

2. Results

T. equigenitalis, mycoplasmas, ureaplasmas and chlamydiae could not be detected from any of the mares examined. Three mares free of reproductive disorders were found to have mild endometritis, while among 19 mares with reproductive problems 11, 7 and 1 had mild, moderate and severe endometritis, respectively. By cytological examination this could be confirmed in only 13 mares showing reproductive problems. In the remaining 9 cases inflammation of the uterine

mucosa could only be detected by histological examination of uterine biopsy samples. In 8 out of these 9 cases this inflammation was consistently mild. Fibrosis in the endometrium was found in 18 mares (67%), and it was accompanied by inflammation in all but two cases. In mares affected with reproductive problems, the endometrium was free from pathological changes in one case (category I), while it could be classified into category IIa, IIb and III in 6, 11 and 4 mares, respectively. The endometrium of two of the healthy mares without any reproductive problems proved to be free from pathological changes (category I), and three mares could be assigned to category IIa. Pneumovagina or abnormal position of the vulva was seen in only four out of the 30 mares. All of these mares had also endometritis. In two of these cases *Pseudomonas (P.) aeruginosa* and *Escherichia (E.) coli* were isolated from the uterus. In two other cases *Streptococcus (Str.) equi* subsp. *zooepidemicus* was isolated from the uterus accompanied by endometritis. No bacterium was isolated from other mares.

In the second part of the research the abortion cases were divided in four groups (table):

- I. Infectious abortions
- II. Possibly infectious abortions
- III. Non-infectious abortions
- IV. Abortion with unknown cause

Infectious abortions were diagnosed, when the infectious agent was demonstrated by isolation, special histological stains or immunohistochemistry and the typical histological lesions were also present. This category contained 6 cases of EAV abortion, 15 cases of EHV-1 abortion, 3 cases of *Leptospira* abortion, 9 abortion cases caused by other aerob bacterium and one by fungi. In 3 cases of the 6 EAV abortion the virus was not demonstrated, but the serological response of the fetus against the virus and the typical histological lesions were present. Twenty cases were placed in the category of the possibly infectious abortions. The histological lesions were suspicious for infection, but no infectious agents could be demonstrated in these cases. Eleven cases were placed in the category of non-infectious abortions and in 31 cases no causes of the abortion were found.

Chlamydiae were found in 71 % of the fetal membranes examined by immunohistochemistry. No typical histological lesions were found with the connection of this infection, even unequivocal reason for abortion other than *Chlamydia*-infection was found in 59 % of the cases. The *Chlamydia*-infection cases and the cases where the EAV-infection was demonstrated only by the fetal serology (14 cases) were not placed in to the group of the infectious abortions; only the presence of infection was stated in these cases.

Table: Occurrence of the abortions compared with the data from abroad and from Hungary.

The cause of the abortion	Our results		Data from abroad (%)	Data from Hungary (%)
	Number of cases	(%)		
<i>I. Infectious abortions</i>	34	36	22-34	36,7
EHV-1 abortion	15	16	3-25	10-32,2
EVA abortion	6	6	0	0,2
<i>Leptospira</i> abortion	3	3	2,2-35	No data
Abortion caused by other aerob bacterium	9	10	3,2-17,8	4 – 4,4
Abortion caused by fungi	1	1	1,7-6,3	2
<i>II. Possibly infectious abortions</i>	20	21	3,1-25	No data
<i>III. Non- infectious abortions</i>	11	11	31-58	No data
Twins	2	2	4-7	No data
Excessively twisted umbilical cord	2	2	2,5-4,5	No data
Congenital malformations	1	1	2-3	No data
Prolonged foaling	1	1	19,5	No data
Trauma	2	2	1,7	No data
Severe calcification in the fetus and fetal membrane	2	2	No data	No data
Metabolic disease of the mare	1	1	No data	No data
<i>IV. Abortion with unknown cause</i>	31	32	17-33 %	No data
Total	96	100		

4. Conclusions and new results

According to the current state of knowledge, the individual susceptibility of mares is the most important factor in the development of endometritis. In these animals, the development of endometritis can often be attributed to non-infectious agents, such as spermatozoa, air, urine or glandular secretions accumulated in the uterine cavity. This was strengthened by our results, because no infectious agents were found in 82 % of the endometritis cases, and aerob, facultative pathogen bacteria were only demonstrated in the remaining 18 %. Anaerobe bacteria were demonstrated in endometritis cases by some researchers. We didn't examine the occurrence of these bacteria. It is possible that anaerobe bacteria were responsible for the endometritis in some of the cases. The susceptibility of the cytological examination was low, because only 59 % of the endometritis cases were demonstrated by this method.

To the best of our knowledge, this is the first reported work in which uterine infection of mares caused by *Chlamydia*, *Mycoplasma*, *Ureaplasma* species and other microaerophilic and aerobic bacteria was studied in parallel. Despite the relatively low number of samples examined, it appears that infection by *Chlamydia*, *Mycoplasma*, *Ureaplasma* species and *T. equigenitalis* does not play a role in the reproductive disorders of mares in Hungary. Like in other country with intensive horse breeding, it would be recommended also in Hungary to examine the uterus biopsy of each mare with reproductive disorders. It would be possible to find the appropriate therapy and the breeding management after taking the first, and to check the successfulness of the therapy after a second biopsy.

In the second part of the research we examined as first the causes of equine abortion by investigating almost 100 cases in Hungary. The abortion cases were placed in 4 groups. The occurrence of these groups was almost identical with the data in the literature from abroad. The occurrence of the infectious abortions was the same compared to the few earlier data in Hungary. The number of abortions caused by bacteria increased a little bit, while the EHV-1 abortions decreased on the half compared to the data came from the 60-70th years. A new agent, the EAV became an important infection, responsible for equine abortions; 17,6 % of the infectious abortion cases was caused by this virus infection.

We were the first, who reported about the occurrence of EHV-1 antigen in the trophoblasts of fetal membranes from spontaneous cases of equine abortion. We demonstrated that the virus might reach the placenta directly via endometrial infarcts or by infected, circulating, maternal leucocytes. We supposed that the endothelial cells and the circulating leucocytes play an important role in the pathogenesis of the infection in the fetuses. The distribution of virus appeared to be related to areas of vacuolar degeneration and desquamation of chorionic epithelium, mild lympho-histiocytic vasculitis and placentitis, and increased metabolic activity of mesenchymal cells in the villi of the fetal membranes. A clinically important finding, that the abortion rarely (6,7 %) accompanied by colic and retention of the fetal membranes. In natural circumstances the EHV-1 abortion occurs probably after the infection of the fetus, because no case was found where only the fetal membrane was infected.

The macroscopical alterations were suspicious for the EHV-1 infection (petecchial haemorrhages on serous membranes, icterus, enlargement of the spleen and liver, small greyish-white herds in the liver, oedema in the lungs, prominent follicles of the spleen, 1-2 l of clear fluid in the body cavities). The typical histological lesions were always present in the lungs, liver or lymphoid organs: diffuse necrotizing or intraalveolar interstitial pneumonia, lympho-histiocytic portal hepatitis, multiple necroses in lungs, liver, spleen, thymus. The acidophilic intranuclear inclusionbodies, which is told to be diagnostic for EHV-1 abortion, were found only in the 28,7 %

of the virus-infected organ samples. The excessive karyorhexis occurred in the follicles in the spleen, seemed to be caused by fetal hypoxia preceding abortion, rather than from the direct effect of the virus.

The immunohistochemistry was more sensitive than virus isolation in the diagnostic of EHV-1 abortion. This method is recommended for the diagnostic work, because it is fast and relative cheap. The most appropriate organs for this examination are lungs, liver and lymphoid organs, but in 45 % of the cases alone the fetal membrane would be enough for the detection of the virus and for the diagnosis. For antigen retrieval we used as first microwave heating of the dewaxed sections in citrate buffer solution. With help of this method we could detect the virus in 20,6 % more samples than with the digestion by enzyme. The fetal serology seemed to be not appropriate method for the diagnostic of EHV-1 abortion. All EHV-1 abortion cases were negative with this method. Only 6,3 % of the mares were seropositive for EHV-1. This could be explained by the imperfect vaccination programmes or by the lack of vaccination.

The EAV infection is widely distributed (65 %) in Hungary according to the serological results. EAV abortion was diagnosed in 6 cases. The typical histological lesions were present in all cases (intraalveolar interstitial pneumonia, lympho-histiocytic vasculitis, perivasculitis and fibrinoid necrosis of the small muscular arteries in the lungs, spleen, heart). The histological lesions typical for EAV infection could be recognised only after a thorough examination of the sections compared to the EHV-1 abortion cases, where the lesions were more pronounced and easy to find. This is the first report on the detection of hyaline membrane in the lungs of EAV infected equine fetus and the first evidence of successful use of an nucleocapsid protein-specific monoclonal antibody in formaldehyde fixed and paraffin embedded tissue sections using horseradish peroxidase-labelled streptavidin-biotin method. The virus was detected in half of the cases with this method, while the virus isolation was negative in all cases. To examine the serum collected from the mare at the time of abortion and 2-3 weeks later was appropriate to diagnose the infection. The EAV-infection was demonstrated only by the fetal serology in 14 cases. Unequivocal reason for abortion other than EAV-infection was found in 5 of these cases. Further investigations are necessary to clear these cases.

Equine leptospiral abortion was described first time and was demonstrated immunohistochemically in Hungary. This method was much more sensitive than the silver staining. The method should be used in the routine diagnostic work. Beside the well-known, typical histological lesions, we found first time the proliferation of the bile duct in the liver of an aborted equine fetus. The serological examination of the dam's sera can be useful in the diagnostic, but it is not enough alone; it is always necessary to demonstrate the agent in the tissue samples.

Chlamydiae were demonstrated first time in the fetal membrane of equine fetuses using immunohistochemistry. The role of this infection was not clear in the abortion cases. It needs further investigations to determine the importance of the presence of chlamydiae in the placenta.

This is the first time to present the macroscopical and microscopical lesions caused by *Str. equinus*, *Str. equi* subsp. *zooepidemicus*, *Staphylococcus equorum*, *E. coli* and *P. aeruginosa* in equine fetus and fetal membranes in Hungary. According to the results, it is necessary to make not only bacteriological, but also histological examination from each abortion cases.

Equine abortion caused by fungi was described first time in Hungary. It was not possible to isolate the fungi, but according to their morphology in histological section, the agent was placed in the *Zygomycetes* class.

This is the first retrospective study for examination of the occurrence of *T. gondii* and *N. caninum* in equine fetal membrane. Although the organisms were demonstrated in few cases of equine abortions, we were unable to find the agents in any of the fetal membranes.

This is the first presentation about the occurrence of non-infectious equine abortions (like excessive umbilical torsion) in Hungary.

We couldn't find any explanation for the cause of abortion in 32 % of the abortions. The failure in feeding and breeding might have been the reason for abortion in these cases.

The occurrence of the different type of equine abortions in Hungary is similar to other countries. The cause of equine abortion can be recognised in about 70 % of the cases by the parallel using of macroscopical, microscopical and other laboratory methods.

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