

## RESEARCH ON REPRODUCTIVE PERFORMANCE OF MARES IN SERBIA USING BACTERIOLOGICAL EXAMINATION

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**Abstract:** The research was conducted on 19 stud farms in Serbia, on 80 mares used for breeding, with and without reproductive disorders. During the two years period (from 2009 to 2010) double guarded uterine swabs from 80 mares, aged between 3 and 22 years were collected. Mares belonged to the different breeds: Thoroughbred, Standardbred, Lipizzaner and mixed breeds. It was determined, that bacterial infection of genital organs was found in 24 mares in the examined population, and the bacterial species *Streptococcus zooepidemicus* was diagnosed in the 11 samples from cervical swabs. In the 5 samples, *Escherichia coli* was isolated, while *Staphylococcus epidermidis* and *Pasteurella multocida* were present in the 2 samples each, while the other causes and simultaneous isolation of two bacterial species are much less present. These species are: *Bacillus* spp. plus *Escherichia coli*; *Streptococcus zooepidemicus* plus *Klebsiella pneumoniae* and *Escherichia coli* plus *Streptococcus zooepidemicus*. In one swab we determined *Arcanobacter pyogenes*. In this examination, according to available data after natural mating, we found conception level of 43,10%, which is similar with previous reports in our country.

**Key words:** mare, bacteria, genital tract, fertility

### Introduction

The ability to maintain a uterine environment compatible with embryonic and fetal life is essential for reproductive efficiency in equids. However, the uterine environment is easily disturbed by an inflammatory process following bacterial invasion, which can occur during breeding, genital examination, and parturition (Asbury and Lyle, 1993). Bacterial uterine infections inflict major losses on the equine breeding industry, occurring in 25–60% of barren mares. Losses can appear

as failure to conceive, early fetal losses, mid-gestational abortion, placentitis, birth of a septic neonate, post-partum metritis, or delays in re-breeding (McKinnon and Voss, 1993). Bacterial infection of the genital tract of the mare has been recognized as an important cause of infertility (Hughes and Loy, 1975). Organisms isolated include the commensals *Streptococcus zooepidemicus* and *Escherichia coli*, the more pathogenic *Klebsiella pneumoniae*, especially capsule types 1, 2 and 5, also *Pseudomonas aeruginosa* (Shin et al., 1979) and *Taylorella equigenitalis* (Timoney, 1996). *Streptococcus zooepidemicus* is the pathogen most commonly identified in endometrial swabs from mares with endometritis (Timoney et al., 1986). In some mares, the bacteria become established and uterine infection develops in which the sources of uterine contamination include coitus, parturition, and reproductive examination (Card, 1997).

There are some data indicating that age, parity, and barren years may have important bearing on the breeding prognosis of the mare (Ricketts and Alonso, 1991). In general, loss of resistance to infection is associated with advancing age and multiparity, factors that are frequently associated with increasing value of mares (Asbury and Lyle, 1993).

Only limited information on the reproductive performance of mares in the Balkan countries, especially in Serbia, is available. Generally, in Serbia the number of horses has been decreasing, and today there are approximately 18.000 horses (Urošević et al., 2010). According to Trailović et al. (1996) out of 500 mares of Yugoslavian Standard breed, they were foal annually in average 50%. Horse breeding is not well organized (Petrujkic et al. 2000) and data on the fertility of broodmares are not available. Moreover, breeding soundness examination is not performed as a routine in mares with fertility problems. Radjenovic et al. (2000) confirms the above mentioned facts, stating that the fertility in mares of (Thoroughbred, Standard breed) is relatively low besides the good feeding conditions and care. This is mainly because of anovulation, late ovulation, and also because of limited breeding season in the mare; as a consequence, the large number of mares are repeatedly bred for a couple of years. The aim of the present study was to determine the most common bacterial species in uterine samples from mares with and without fertility problems in horse studs in Serbia.

## Materials and Methods

Eighty mares were examined and selected on the basis of some criteria (history of recent genital discharge and/or abortion and/or failing to conceive after repeated services), rectal palpation, and ultrasound examination of genital tract. From all mares in the study, 44 samples of genital swabs were taken from 5 horse studs, 31 from 12 private horse owners and 5 from 2 equestrian ranges (horse clubs). As mentioned earlier, we analyzed in our study bacteriological culture

results of 80 endometrial swab samples, but geographically arranged from the following municipalities: Belgrade (14 samples), Pozarevac and Novi Sad (11 samples each), Becej (10 samples), Subotica, Bajmok and Backa Topola (6 samples each), Novi Knezevac (4 samples), Pancevo, Sremska Mitrovica and Sombor (3 samples each), Apatin, Srbobran and Crvenka (1 sample each). Therefore, all mares in different reproductive status were examined from 2008 to 2010. Animals included were aged from 2 to 23 years (average 11,12 years). The dominating breeds of the included mares were Thoroughbred (41) and Standard breed (19). Other breeds were: Lipicaner and mixed breed (6 mares each), "Half" Thoroughbred (4), Cold blood breed (2), Pony and Donkey (1 mares each).

From all mares included into the study, 14 mares were fertile and without reproductive disorders, and 9 mares were maiden. For other 57 mares included in the study, at least one fertility problem was noted: barren (failing to conceive after repeated services) in preceding season-one year barren (33), two years barren (8), four and three years barren (4 mares each), five years barren and abortion (2 mares each). The following medical problems were recorded only by one mare each: seven, eight, fifteen years barren and one resorption of embryo.

According reproductive status before investigation, we made different group of mares: 19 mares had one foal, 11 mares had 2 foals, 12 mares had 3 foals, and 23 mares had 4 foals or more. Beside that, 9 mares were maiden and 6 animals were no pregnant before (but breed in previous period).

Hence, we limited the results of this part of study to bacteriological sampling. For these samples, the external genitalia were carefully washed with soap and water and thereafter dried with paper. In order to minimize contamination of the sample by bacteria from the vagina and perineum the sampling was performed using a gloved hand in the vagina and double-guarded, occluded swabs enabling sampling from the uterus solely (Minitube GmbH, Tiefenbach, Germany). The swab was retracted into the sheath, removed from the uterus and placed into a transport medium. The uterine culture swabs were transported in Amies' modified media ("Becton Dickinson", Franklin Lakes, NJ, USA) at ambient temperature and cultured within 6 h. This medium has been widely used in World as an all-purpose transport medium for equine gynecological swabs (*Albihn, 2003*).

**Breeding soundness examination.** A careful examination of the genital organs, and endometrial sampling for bacteriology was performed. The uterus and ovaries of the mares were examined by rectal palpation together with transrectal ultrasound scanning (6 MHz linear transducer, Aquila Pro Vet, Pie Medical Imaging B.V., Maastricht, The Netherlands).

**Microbiology.** Endometrial cultures were plated on blood agar at the bacteriology laboratory of the Scientific Veterinary Institute Novi Sad within 6 h of sample procurement. All samples were smeared on the surface of a blood agar (with 5% toxin free ovine-blood), MacConkey agar and Sabouraud dextrose agar. After 24 h incubation in atmospheric air at 37 C, bacterial growth was evaluated

and identified. Agars with no growth were incubated another 24 h at 37 C and re-examined (Macintosh, 1981). A commercially available prepacked biochemical reaction system API strep<sup>R</sup> (Bio Mérieux, Vercieu, France) was used, if further identification of cultures was necessary. Only substantial growth (2 to 3 culture) in monoculture was recorded as positive growth.

**Statistical analysis.** Statistical analysis was performed using software S-Plus 16. A P-value  $\leq 0.05$  was considered significant. Information about results were evaluated considering the following parameters: “horse breed”, “age of mares”, “number of foals”, “length of barring period” and “results of bacterial isolation”. Differences between parameters were evaluated using the Chi<sup>2</sup>-analysis (X<sup>2</sup>).

## Results

The purpose of the present study was to provide an update on bacterial isolates in uteri of mares with and without fertility problems in 19 Stud farms in Serbia. Bacteriological examinations of the smears were positive in 24 (30%) of 80 mares. From these 24 positive swabs, the most frequently isolated bacteria were: *Streptococcus zooepidemicus* - 11 (45,83%) and *Escherichia coli* - 5 (20,83%). *Staphylococcus epidermidis* and *Pasteurella multocida* were found in 2 (8,33%) mare each. Bacterial swabs from 3 mares (4,16% each) yielded 2 species: *Bacillus* spp. plus *Escherichia coli*; *Streptococcus zooepidemicus* plus *Klebsiella pneumoniae* and *Escherichia coli* plus *Streptococcus zooepidemicus*. In one swab (4,16%) we determined *Arcanobacter pyogenes*.

The largest number of mares (n=73) did not show any clinical sign of disorders of reproductive organs. For the seven remaining mares we diagnosed by ultrasonography: ovarian cyst by 3 mares, anatomical constriction of vagina, anatomical defect - cervix was short and with stenosis, persistent mating-induced endometritis “PMIE”, purulent discharge from vagina (one mare each). The total number of examined mares by the ultrasound was 38. Unfortunately, there was not possible to make this special diagnostic approach on all animals in our investigation. From these 7 mares with clinical signs, in 4 mares we found positive bacteriological sample: two mares with ovarian cyst, one with anatomical constriction of the vagina (*Streptococcus zooepidemicus*) and by one mare with purulent discharge from vagina it was determined *Escherichia coli* in uterine swab.

**Therapy and mating results.** Unfortunately, the same therapy could not be conducted in all mares in this investigation. To be more precise, three treatment methods were applied: uterine lavage saline infusion and/or uterine lavage with disinfectant; system therapy with antibiotic; uterine lavage with antibiotic solution

and preparations for oestrus induction. Also, it was possible to get complete data about pregnancy on 62 mares, and conception level of 43,10% has been achieved. After the testing and conducted medical treatment, in the overall conception rate of the mares in the study were not included three mares which died from colic or had abortion after colic (one mare).

No statistically significant relations between the different parameters evaluated and the age and history of the mares could be found.

## Discussion

*Streptococcus* and *E. coli* were isolated in the largest number (16) of bacteriological positive mares, what is in agreement with observations of *Waelchli et al* (1993). However, in the cited studies bacteria were found in similar relation - 36% of cases. Also, in the USA, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are relatively common isolates (*Dimock and Edwards, 1928*), but in the UK they are relatively rare (*Ricketts et al., 1992*). Of these organisms, *Streptococcus zooepidemicus* is by far the most common and accounts for around 66% of infections, what is in agreement with our results. This organism is part of the normal microflora of horse skin and is a common contaminant of the uterus after mating. Whether infection is established or not depends on the efficacy of the mare's uterine defence system. *Escherichia coli* is more frequently recovered from mares with anatomical defects of the perineal and vulvar region which predispose mares to pneumovagina and faecal contamination (*Le Blanc 1997*). The same author suggests that *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and yeasts are most commonly isolated from mares which had a previous history of intrauterine antibiosis or compromised uterine immune defence mechanisms. We didn't found such connection.

But, our results are consistent with previous studies in Hungary (*Szeredi et al., 2003*) demonstrated that the most common bacterial causes of uterine infections include *Streptococcus zooepidemicus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Bacteroides ureolyticus*.

The reports from Denmark (*Nielsen, 2005*) confirm this. In his research, the most common bacteria cultured was *Streptococcus zooepidemicus* (77%). In one investigation in Italy, *Frontoso et al. (2007)* reported the results of microorganisms isolated from uterine swabs between 1998–2004 and their frequency of isolation. The bacterial species most frequently isolated was *S. zooepidemicus* (31.7%), followed by *E. coli*, (19.6%), other *Streptococcus* spp., *S. aureus*, *Enterobacteriaceae*, *P. aeruginosa*, *Bacillus* spp., and *Staphylococcus* spp. were found at lower frequencies. No correlation was found between the type of

isolated bacterial species and mares with characteristic clinical signs or the age of the mares, which is similar to the parameters that we compare.

*Albihn (2003)* suggested something different. The key findings from his study of mares with a history of fertility problems were firstly: *E. coli* was the overall most frequently isolated bacterial species, while uterine pathogens such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were rare. Secondly: *Streptococcus zooepidemicus* was more frequently associated with clinical endometritis than with repeat breeding, whereas the opposite applied to *E. coli*.

The procedures for sample collection have improved and the use of a guarded swab to obtain samples from the endometrial surface for culture is now common practice (*Blanchard et al, 1981; Brook 1984*). This means that we have chosen the right solution, modern double-guarded swabs for our investigation.

A good example of the importance of bacteriological investigation is order in the central Kentucky Thoroughbred horse industry, where all barren and maiden mares on their first breeding are required to have a clean uterine culture before mating. On the majority of farms, all mares (barren, maiden, and foaling) are required to have a clean culture on subsequent matings. Only mares that have a signed clean culture certificate are allowed to be mated (*Riddle, 2005*). However, in horse industry in our country there are no such rules, and we think that something like that would be useful in our region.

Only in comparison with earlier reports in Serbia, a relative good level of conception has been achieved in our study (43,10%). *Petrujkic et al. (2000)* wrote about the reproductive status of mares in Serbia, the results of investigation in the region of Velika Plana. In the period from 1986 till 1995 the genital examination was conducted in 1665 mares, out of which only 21,5% conceived. According to *Trailovic et al. (1996)* out of 500 mares of Yugoslavian Standard breed, they were foal annually in average 50%.

But the newer report from *Đermanović et al. (2010)* from one English thoroughbred horse stud in Serbia has different research results. It was determined the number of colts per a mare since the first conception up to the year 2009, when the data for this research were taken. In 2009, out of 51 mares, 33 ones had normal conception and gestation, and gave birth to healthy offspring. Since the breeding period up to these days, the mares included in the research, (33) of various age had total of 124 colts of both sexes (3.76 colts per a mare).

Although our data about fertility rate of the mares in investigation are not complete, they are differ from reports of other authors who come from the countries with developed horse industry. *Katila et al. (2010)* investigated the change in foaling rates through the years, and it was studied both from year to year and in longer time periods (15 years) in Finland. The foaling rate in the whole material was 68% in the Finnhorse (the Finnish cold blood) and 71% in the Standardbred. *Nath et al. (2010)* reported results from Australia. Records from seven Thoroughbred and four Standardbred studs in north-east Victoria were

reviewed. The overall early pregnancy rate per cycle was 68.8% for Thoroughbred mares and for Standardbred mares, 68.3%. *Bosh et al. (2009)* made a cohort study of 1011 mares on 13 farms in central Kentucky (USA), which was followed during the 2004 mating and 2005 foaling season. Per season pregnancy rates on days 15 and 40 post mating and live foal rate were 92.1, 89.3 and 78.3%, respectively.

## Conclusion

After our research study, we have some useful data about bacterial infection in breeding mare population in Serbia especially according reproductive status. Because, animals in our study were broodmares from 14 different municipalities geographically located in a radius of 300 km in the northern and north-eastern part of Serbia. For our conditions in horse industry and veterinary practice in Serbia, the collection of an endometrial swab sample for bacterial culture is non-invasive and simple. And, this technique with double guarded swabs could be used also in field practice in Serbia, especially because it has been used so far only sporadically by a few veterinarians (personal communication). The usage of this technique would certainly demand additional training and more experience of veterinarians and bacteriologic laboratories in this region.

As a conclusion, we can underline that in spite of pathological problems of genital organs, prognosis and success of the treatment depend on a veterinarian intervention as well as improvements of management of mares which is in the compliance with the recommendation of *Samper and Tibary (2006)*, to prevent the spreading of any disease to susceptible populations through breeding, correct identification of infected animals as well as the implementation of appropriate managerial procedures is critical. Besides this, one of the measures for the improvement of mares' fertility in Serbia, we recommend a regular taking of endometrial swab sample for bacterial culture prior to the breeding season.

Further study will probably be necessary to make a investigation on bigger group in breeding horse population in Serbia, but also in other Neighboring Countries, having in mind, that exist relative big import-export activities with horses, and there are no sufficient dates in relation to the infections disease. By that, on the first place is "CEM" (*Taylorella equigenitalis*), which has not been proved so far in horse population in Serbia, and unfortunately there are no legal regulations which would control this infectious disease (*Urosevic et al., 2008*).

## Ispitivanje reproduktivnog statusa kobila u Srbiji pomoću bakterioloških pregleda

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### Rezime

Istraživanje smo sproveli na 80 priplodnih kobila sa i bez reproduktivnih poremećaja, koje se nalaze u 19 zapata u Srbiji. Tokom dvogodišnjeg perioda (2009-2010) prikupili smo 80 uzoraka sa endometrijuma kobila, starih između 3 i 22 godine, korišćenjem dvostruko-zaštićenih briseva. Kobile su pripadale različitim rasama: punokrvni engleski, kasači, Lipicaneri i mešanih rasa. Kod 24 ispitivane kobile su nađene bakterije u brisevima genitalnog trakta. Od toga, kod 11 grla nađena je bakterija *Streptococcus zooepidemicus*, dok je u 5 uzoraka izolovana *Escherichia coli*. *Staphylococcus epidermidis* i *Pasteurella multocida* su utvrđene kod po dve kobile. Ostali uzročnici i istovremena izolacija dve vrste bakterija bili su manje zastupljeni: *Bacillus* spp. i *Escherichia coli*; *Streptococcus zooepidemicus* i *Klebsiella pneumoniae*; *Escherichia coli* i *Streptococcus zooepidemicus*. U jednom uzorku brisa je dijagnostikovana *Arcanobacter pyogenes*. Na osnovu dostupnih podataka, u ovom istraživanju je nakon prirodnog pripusta konstatovan nivo koncepcije od 43,10%, što je slično ranijim izveštajima u našoj zemlji.

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