P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

Bacteriologic and Cytologic Examination Results of Mares with Pneumovagina in Bursa Region

Gulsen Goncagul*[1], Huban Gocmen[2], Sercan Koray Yendim[1], Kemal Yilmaz[1] and Kamil Seyrek Intas[3]

[1]Uludag University, Mennan Pasinli Vocational High School, Bursa, Turkey
[2]Near East University Faculty of Veterinary Medicine, Department of Microbiology, Nicosia, North Cyprus
[3]Uludag University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Bursa, Turkey
***Corresponding author:** goncagul@uludag.edu.tr

Article History: Received: May 24, 2016 Revised: June 21, 2016 Accepted: June 28, 2016

ABSTRACT

Pneumovagina is one of the most important causes for bacteria origin infectious infertility in mares. The aim of this study was to determine side effects of the pneumovagina on uterus bacteriology and cytology and its prevalence in Bursa region. A total of 156 mares were evaluated regarding to prevalence of pneumovagina, and bacteriologic and cytologic examination results. Group I had 11 mares with pneumovagina (n=11), and group II was control group including 23 mares (n=23). Bacteriologic culture and cytological examination were performed from samples taken by endometrial swabs in both groups. The prevalence of pneumovagina was 7.1% (n=11). Microbiologically, group I had 100% positive culture results. The rate of *Escherichia coli*, *Streptococcus equi* subsp. *zooepidemicus*, *Streptococcus equinus* and *Enterococcus faecium* were 40.0-15.0-10.0%, respectively. Group II had 30.4% (n=23) positive results. Seven of them were including 14.3% rate of *Escherichia coli*, 28.5% rate of yeasts, and 57.2% rate of 4 different microorganisms. There were intense neutrophils in cytological preparations of group I. Antimicrobial susceptibility was performed in both groups' microorganisms and penicillin, cefquinom, marbofloxacin tetracycline, erythromycin, florfenicol Ceftiofur amoxicillin, amoxicillin / clavulanic acid, enrofloxacin, gentamicin gentamicin colistin and Sulfamethoxazole/Trimethoprimwere selected as antibiotics for this test. Isolates were much more resistant to tetracycline, erythromycin, gentamicin, colistin and sulfamethoxazole/trimethoprim. As a conclusion, it has been emphasized that examination of endometrial bacteriology and cytology in mares with pneumovagina should be done to determinate early stage genital system infections.

Key words: Pneumovagina, Mare, Bacteriology, Cytology

INTRODUCTION

Bacterial infections of the genital canal are responsible to infertility in mares (Schliesser and Behtelsman, 1976; Asbury, 1986; Erdeger *et al.*, 1999; Zonturlu and Kacar, 2004; Neves *et al.*, 2007; Koca *et al.*, 2016). Pathogenic microorganisms introduce to genital canal during natural breeding, artificial insemination, during and after parturition, during gynecological examination and as a result of the failure in anatomo-physiological vaginal barrier (ie. pneumovagina) in mares. In addition, when uterine defense mechanism is not functional properly, the bacterial infections are seen in mares (Asbury *et al.*, 1982; Evans *et al.*, 1986; Nikolakopoulos and Watson, 1999; Patrick, 2008).

Perineal mal-conformation is associated with pneumovagina due to predispose factors such as deficiency of the one-way vulvovaginal "valve" mechanism and vestibule-sphincter weakness in the mares and it can cause to uterine infections and infertility. The pneumovagina also affects the body condition and estrous (etc. diestrous) in foaling (Easley, 1993; Pycock, 2001; Pascoe, 2007; Newcombe, 2011). It also leads to early stage embryonic dead, infections and infertility (LeBlanc *et al.*, 2007). Thus, spectrum of the vaginal, cervical and bacterial flora of the uterus is so crucial to determine the chronic infertility problems of the mare (Zonturlu and Kacar, 2004). Here, we aimed to determine prevalence and affectivity of the pneumovagina on uterus bacteriology and cytology in mares.

Cite This Article as: Goncagul G, H Gocmen, SK Yendim, K Yilmaz and KS Intas, 2016. Bacteriologic and cytologic examination results of mares with pneumovagina in Bursa Region. Inter J Vet Sci, 5(4): 295-298. www.ijvets.com

MATERIALS AND METHODS

Animals and Collections of the Samples

In this study, a total of 156 (age 2-20 years) mares were examined to determine the pneumovagina between October 2013 and April 2015. They were also evaluated by abnormal perineal conformation in two study groups (Group I: mares with pneumovagina n=11, and Group II: mares without pneumovagina (control) group n=23) in Bursa region.

A total of 34 uterine swabs were collected from mares in groups. Before sampling, external genital region of the mares was cleaned by an antiseptic solution and swabs were taken carefully with the help of Polanski speculum. All endometrial swabs were transported using Amies Agar Gel –With Charcoal transport swabs (COPAN ITALIA,114C.USE) to the microbiology laboratory.

Bacteriological and Mycological Isolations

The endometrial samples preserving in cold chain were inoculated on Columbia Agar with 5% sheep blood, Tryptic Soy Agar, Mac Conkey Agar, Eosin Methylene Blue Agar (EMB) as agars and Serum Broth, Brain Heart Infusion Broth as broths for general microbiological examination.These samples were also inoculated on Eugon Chocolate Agar (ECA) for isolation of Taylorella equigenitalis and Sabouraud Dekstroz Agar (SDA) for mycological isolation. For general bacteriological examination, all agars and broths were incubated both aerobically and anaerobically at 37ºC for 1-5 days; ECA was incubated in 5-10% CO_2 atmosphere at 37ºC for 5-7 days. For mycology examination, SDA was incubated at 25ºC for 7-15 days and they were controlled daily.

Identification

The bacterial identification was performed with routine methods (colony morphology, hemolysis characteristics, microscopy morphology, Gram stain and features of biochemical tests). In bacterial isolation, API systems (api 20Strep, api 20E, api Coryne, api Stahp; BioMerieux Industry, Italy) were utilized for identification of pure cultures.

Antibiotic Susceptibility Test

The bacteria isolates were used for antibiotic susceptibility testing by the Kirby-Bauer Disc Diffussion method. The antibiotics (Penicillin G (10 IU), Cefquinom (30 µg), Marbofloxacin (10 µg), Tetracycline (30µg), Erythromycin (30µg), Florfenicol (15µg), Ceftiofur (30µg), Amoxicillin / Clavulanic acid (30µg), Amoxicillin (25 µg), Enrofloxacin (5µg), Gentamicin (10µg), Colistin (50 µg), Sulfamethoxazole/Trimethoprim1: 19 (25µg) (Oxoid)) were selected according to bacteria strains complying to the direction of Clinical and Laboratory Standards Institute (CLSI):

Endometrial Cytology

Endometrial swabs were taken for microbiological and cytological examinations using double-guarded swabs introduced inside the uterus (Equi-Vet®, Kruuse, Marslev, Denmark). The samples were stained using Diff Quik (Hemacolor TM) after air drying and methanol fixation. The samples were examined under light microscope (Olympus®, U-DO3, Tokio, Japan).

RESULTS AND DISCUSSION

Eleven (n=11) pneumovagina (7.1%) in different degree were diagnosed. Particularly, *Escherichia coli*, *Streptococcus equi* subsp. *zooepidemicus* was the potential pathogens in mares with pneumovagina. Fecal origin bacteria were isolated more commonly in the samples taken from mares with pneumovagina (Table 1). This result was due to mechanical dysfunction of the genital tract resulted from pneumovagina, which led to bacterial location. Mycotic agents were more common in normal flora of mares. *Taylorella equigenitalis* is a responsible agent for contagious metritis in mares that this microorganism was not obtained in both groups (Duquesne *et al*., 2007). Macroscopic and microscopic evaluations of the samples revealed *Aspergillus fumigatu*s and *Aspergillus* spp in two and one cases of the control group, respectively. In both groups, isolated microorganisms (*Escherichia coli*, *Streptococcus equi subsp.zooepidemicus, Streptococcus intermedius, Streptococcus equinus, Enterococcus faecium, Gardnerella vaginalis and Corynebacterium glucuronolyticum*) were evaluated in terms of antibiotic susceptibility against to 13 microbial agents including penicillin, tetracycline, erythromycin, florfenicol, ceftiofur, amoxicillin, amoxicillin / clavulanic acid, enrofloxacin, gentamicin and cefquinome. Isolates had maximum resists to tetracycline, erythromycin, gentamicin, colistin and sulfamethoxazole/trimethoprim (Table 2). In cytologic smear prepared, neutrophil rate was 3.8% in group I and 37.5% in group II.

Trauma to the vulva during foaling and decreasing in the muscular tone with age may lead to a compromise of the vulvar seal and that situation predisposes organ to pneumovagina or “wind-sucking” problem. Sloping of the vulva secondary to recession of the anus and/or poor muscular tone to the labia of the vulva may predispose the mare to an ascending infection of the uterus (Caslick, 1937). As a disease, pneumovagina predisposes the genital tracts for bacterial infections (equine endometritis); thus, it is the most important factor for infertility (Senuver *et al.,* 1997).

Table 1: Cultured microorganisms’ rate (%) in groups.

Microorganisms	Group I	Group II
Escherichia coli	8 (40%)	1 (14.3%)
Streptococcus equi subsp.*zooepidemicus*	3 (15%)	-
Staphylococcus intermedius	1 (5%)	-
Streptococcus equinus	2 (10%)	-
Enterococcus faecium	2 (10%)	-
Bacillus circulans	1 (5%)	-
Bacillus licheniformis	1 (5%)	-
Bacillus brevis	1 (5%)	-
Gardnerella vaginalis	-	1 (14.3%)
Corynebacterium glucuronolyticum	-	1 (14.3%)
Aspergillus sp.	-	1 (14.3%)
Aspergillus fumigatus	1 (5%)	1 (14.3%)
Yeast	-	2 (28.5%)
Taylorella equigenitalis	-	-
TOTAL	20 (100%)	7 (100%)

Table 2: The bacteria strains and their antibiogram results isolated from mares in groups

Bacteria	Group no	R,I,S	Antibiotics												
			P	CEQ	MAR	TE	E	FF	EFT	AMC	AML	EN	GM	CO	SXT
Escherichia coli	I	R	100		12.5	12.5		12.5		37.5		37.5	87.5		12.5
		I		37.5			37.5			62.5	62.5	25		37.5	
		S		62.5	87.5	87.5	62.5	87.5	100		37.5	37.5	12.5	62.5	87.5
	II	R	100			100	100								
		I									100	100			
		S		100	100			100	100	100			100	100	100
Streptococcus equi subsp.*zooepidemicus*	I	R				66.6								66.6	
		I				33.3						100		33.3	33.3
		S	100	100	100		100	100	100	100	100		100		66.6
Staphylococcus intermedius	I	R	100			100	100						100	100	
		I		100	100					100					
		S						100	100		100	100			100
Streptococcus equinus	I	R										100	100	100	
		I			100										
		S	100	100		100	100	100	100	100	100				100
Enterococcus faecium	I	R			100	100	100								100
		I							100			100			
		S	100	100				100		100	100		100	100	
Gardnerella vaginalis	II	R											100		100
		I		100					100						
		S	100		100	100	100	100		100	100	100		100	
Corynebacterium glucuronolyticum	II	R													
		I													
		S	100	100	100	100	100	100	100	100	100	100	100	100	100

R: Resistant, I: Intermediate, S: Sensitive, P: Penicillin, CEQ: Cefquinome, MAR: Marbofloxacin, TE: Tetracycline, E: Erythromycin, FF: Florfenicol, EFT: Ceftiofur , AMC: Amoxicillin/Clavulanic Acid , AML: Amoxicillin, EN: Enrofloxacin, GM :Gentamicin, CO: Colistin , SXT : Sulfamethoxazole/Trimethoprim

Microorganisms most often isolated from mares with uterine infection include bacteria including *Streptococcus zooepidemicus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and various yeast species such as *Candida* or *Aspergillus* (LeBlanc, 2008; Asbury, 1986; Albihn *et al.,* 2003) The bacterium more commonly isolated during routine cultures of the uterus in mares is *Streptococcus equi* subspecies *zooepidemicus* (McCue *et al.,* 1991; Ricketts *et al.,* 1993). However, *Escherichia coli* is the primary bacteria of mares had uterus problem (LeBlanc *et al*., 2007). In this study, there was a similarity between the culture results of both groups.

Many authors (LeBlanc, 2008; Asbury, 1986; Albihn *et al.,* 2003) have reported *Streptococcus zooepidemicus* and *E. coli* in uterus infections of mares. *Escherichia coli* is a normal residence of gastrointestinal tract in most mammals (Maddox *et al*., 2015). We determined that *Escherichia coli* were the dominant agent in mares with pneumovagina. Its rate was 40.0%. The following microorganisms were *Streptococcus equi subsp. zooepidemicus* (15%), *Streptococcus equinus* (10%), *Enterococcus faecium* (10%). In addition, 2 yeast and 3 funguses were isolated in both groups.

It is stated that genital manipulations, wrong and irregular antibiotic usage are the cause of fungal infections (Blue, 1983). Amaral *et al.* (2007) have been reported penicillium (35.4%), aspergillus (20.3%) and candida (13.9%) in cultures of the uterus samples. In presented study, the isolation rate of Aspergillus fumigatus and Aspergillus sp was 33.6%, and isolated yeast rate was 28.5%.

Treatment regimen in bacterial infections resulted from pneumovagina should be planned complying the antibiotic susceptibility results. The main cause of the untreatable infectious infertility is irregular antibiotic usage without antibiotic susceptibility, which leads to antibiotic resistant bacteria (Cohen, 1992). Therefore, in presented study, antibiotic susceptibility was planned in both groups.

In a study performed on infertile mares (Frontoso, 2008), different antibiotics (amoxicillin clavulanic acid, enrofloxacin, erythromycin, gentamicin, rifampicin, sulfamethoxazole/trimethoprim, kanamycin and ampicillin) are used for the purpose of antibiotic susceptibility test. Here, 13 antibiotics were selected for antibiotic susceptibility test given in Table 2. Microorganisms had different sensibility rate. In both groups, antibiotic susceptibility results of *Escherichia coli* were 87.5% for marbofloxacin, florfenicol, ceftiofur and sulfamethoxazole/trimethoprim, and Streptococcus equi subsp. zooepidemicus was 66.6% sensible to tetracycline and colistin.

Neutrophil chemotaxis induced by bacteria is usually observed in the uterine lumen infections, and neutrophil migration aggravates the inflammation (Tibary *et al.,* 2014). The cytological smear evaluation in both groups revealed neutrophilia in group II with the rate of 37.5%.

As a conclusion, pneumovagina should be considered as a common problem in infertile mares. This condition characterized by continuous or intermittent air-sucking into the vagina can result in infection and chronic inflammation of the vagina and uterus in mares.

Acknowledgement

This study was supported by the Scientific Research Project Centre of Uludag University (KUAP (MPMYO)-2013/40.

.

REFERENCES

Albihn A, Baverud V and Magnusson U, 2003. Uterine microbiology and anti-microbial susceptibility in isolated bacteria from mares with fertility problem. Acta Vet Scand, 44: 121-129.

Amaral MG, Pimentel CA, Meireles M, Fiala SM, Schramm R and Xavier EG, 2007. Equine endometritis. Fungi and bacteria. Arch Zootec, 56: 875-884.

Asbury AC, 1986. Endometritis in the mare. In: Morrow DA, editor. Current therapy in theriogenology. 2nd edition. Philadelphia: WB Sounders, 718-722.

Asbury AC, Schultz KT, Klesius PH, Foster GW and Washburn SM, 1982. Factors affecting phagocytosis of bacteria by neutrophils in the mare's uterus. J. Reprod. Fertil, 32: 151–159 (Suppl.).

Blue MG, 1983. Mycotic invasion of the mare's uterus. Vet Rec, 113: 131-133.

Caslick EA 1937. The vulva and the vulvo-vaginal orifice and its relationship to genital health of the Thoroughbred mare. Cornell Vet, 27: 178–187.

Cohen ML, 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science, 257:1050-1055.

Duquesne F, Pronost S, Laugier C and Petry S, 2007. Identification of Taylorella equigenitalis responsible for contagious equine metritis in equine genital swabs by direct polymerase chain reaction. Res Vet Sci, 82: 47-49.

Easley J, 1993. External perineal conformation. In: McKinnon AO and Voss JL, editors. Equine reproduction. Philadelphia: Lea & Febiger, 21-24 .

Erdeğer J, Altay G, Akan M, Vural R, Dakman A and Çelebi M, 1999. Kısrakların genital organlarından izole edilen β hemolitik streptokok'ların identifikasyonu ve serogruplandırılması. Ankara Üniv Vet Fak Derg, 46: 69-76.

Evans MJ, Hamer JM, Gason LM, Graham CS, Asbury ACand Irvine CH, 1986. Clearance of bacteria and non-antigenic markers following intra-uterine inoculation into maiden mares: Effect of steroid hormone environment. Theriogenology, 26: 37-50.

Frontoso R, Carlo E, Pasolini MP,Meulen K, Pagnini U, Iovane G and Martino L, 2008. Retrospective study of bacterial isolates and their antimicrobial susceptibilities in equine uteri during fertility problems. Res Vet Sci, 84: 1-6.

Koca E, Özenç E, Şeker E, Sevimli A , Vural M R , Uçar M and Küplülü Ş 2016. Evaluation of the reproductive and metabolic problems encountered during two breeding seasons in barren Arabian mares. Turk J Vet Anim Sci, 40: 60-67.

LeBlanc MM, 2008 .When to refer an infertile mare to a theriogenologist. Theriogenology, 70: 421–429.

LeBlanc MM, Magsig J and Stromberg AJ, 2007. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. Theriogenology, 68: 403–412.

Maddox TW, Clegg PD, Williams NJ and Pinchbeck GL, 2015. Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance. Equine Vet J, 47: 756-65.

McCue PM, Hughes JP, Jang SS and Biberstein EL, 1991. Antimicrobial susceptibility patterns for aerobic bacterial isolates from the uterus of mares. Calif Vet, 45: 25–28.

Neves AP, Keller A, Trein CR, Möller G, Jobim MI, Castilho LF, Cardoso MR, Leibold W, Zerbe H, Klug E, Gregory RM and Mattos RC, 2007. Use of leukocytes as treatment for endometritis in mares experimentally infected with Streptococcus equi subsp. zooepidemicus. Anim Reprod Sci, 97: 314–322.

Newcombe JR, 2011.Why are mares with pneumovagina susceptible to bacterial endometritis? A personal opinion. J Equine Vet Sci, 31: 174-179.

Nikolakopoulos E and Watson ED, 1999. Uterine contractility is necessary for the clearance of intrauterine fluid but not bacteria after bacterial infusion in the mare. Theriogenology, 52: 413-423.

Pascoe RR, 2007. Vulvar conformation, In: Samper JC, Pycock JF and McKinnon AO, editors. Current therapy in equine reproduction. 1st edition. St Louis: Saunders Elsevier, 140-145.

Patrick M McCue, 2008. The Problem Mare: Management Philosophy, Diagnostic Procedures, and Therapeutic Options, J Equine Vet Sci, 28: 619-626.

Pycock J, 2001. Infertility in the mare. In: Noakes DE, Parkinson TJ and England GCW,editors. Arthur's veterinary reproduction and obstetrics. 9th edition. Philadelphia: WB Saunders, 577-608.

Ricketts SW, Young A and Medici EB, 1993. Uterine and clitoral cultures. In: McKinnon AO, editor. Equine reproduction. Philadelphia: Williams &Wilkins, 234–245.

Schliesser Tand Behtelsmann U, 1976. Bakteriologische Ergebnise bei der deckhygienischen Überwachung von Warmblutstuten in Hessen. Berliner und Münchener Tierärztliche Wochenschrift, 89: 93-95.

Tibary A, Pearson L K and Fite CL, 2014. Reproductive Tract Infections, In: Debra C. S and Maureen L, editors. Equine infectious diseases . 2nd edition. St. Louis, Saunders, 84–106.

Zonturlu A K and Kaçar C, 2004. Kısraklarda endometritisin tanı ve tedavi yöntemleri. Kafkas Univ Vet Fak Derg, 10: 131-134.