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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Bacterial infertility and ascending uterine infections with respect to pneumovagina and urovagina in cows

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Abstract

Ascending bacterial infections of the reproductive tracts are considered the most important causes of infertility in the cows. The aim of this study was to determine the types of bacteria causing endometritis in cows with pneumo-/urovagina and to compare them with bacterial flora of the cows with endometritis resulted from other reasons. The study was planned under two groups (Group 1: samples from cows with pneumo-/urovagina in the southern region of Marmara in Turkey. Group 2 (control): samples from cows with infertility due to other reasons in the Hessen region of Germany. In group 1, 101 dairy cows with pneumo-/urovagina revealed that 8 were negative on bacterial culture (7.9 %) in 101 uterine swabs. Bacteria was detected in the other samples (92.1%) including 41.8% *Escherichia coli*, 14.4% *Streptococcus bovis* I, 14.4% *Streptococcus bovis* II, 5.9% *Streptococcus equines* and 3.9% *Bacillus licheniformis.* The remaining 19.6% were 18 different bacterial isolates. In Group 2, the samples obtained from 142 cows were cultured, of which 140 samples (98.5%) were positive bacteriologically. Out of 22.1% was *Escherichia coli*, 15.3% *α-haemolytica Streptococci*, 14.3% *γ-Streptococcus*, 12.1% *Truperella pyogenes*, 4.2% *Streptococcus uberis*, 3.6% *Staphylococcus aureus*, 0.7% *Staphylococcus heamolyticus* and *β-Streptococcus* each, and the remaining 27% were 16 different bacterial isolates. As a conclusion, depending on the group, despite differences between endometrial swabs isolated from cows with infertility problems, *Escherichia coli* was the most dominant agent. The samples in Group 2 might explain why the ratio of *Escherichia coli* isolates was lower in Germany. This could be valuable data for diagnosis and treatment of the cows having clinical signs of pneumo-/urovagina.

Keywords: Cow; infertility; pneumovagina; urovagina; endometritis

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Introduction

Mammary infections, reproductive problems and foot diseases cause the most economic losses in the dairy cows. The most important reproductive problem in cows is infertility (Mukasa-Mugerwa 1989; Alacam 2002; Kaufmann et al., 2010; Lu et al., 2011). Ascending bacterial infections of the reproductive tracts are considered the most important causes of infertility in cows. The major reason for infertility in cows is resulted from endometritis (Mukasa-Mugerwa, 1989; Lu et al., 2011).

Endometritis is associated with bacterial infections (Sheldon, 2007; Potter et al., 2010). Pathogen bacteria introduced in the uterus during natural breeding, artificial insemination, in parturition, during examination and as a result of failure of physical barriers to infection such as pneumovagina and urovagina.

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Pneumovagina and urovagina occur due to congenital and/or acquired perineal malconformation, perineal atrophy, rectovaginal laceration in various degrees and ongoing fistula following dystocia and predispose cows to endometritis and other uterine diseases (Seyrek Intaş et al., 2010).

The aim of the presenting study was to determine the types of bacteria causing endometritis in dairy cows with pneumo-/urovagina and to compare them to bacterial flora of cows with endometritis resulted from other reasons.

Materials and Methods

This study was planned under two groups and performed between 2007-2009 years. Group 1 consisted of bacterial swap samples taken from 101 Holstein and Brown Swiss cows diagnosed with pneumo-/urovagina in the southern region of Marmara in Turkey. Group 2 (control) included samples from cows with infertility due to the other reasons in the Hessen region of Germany). Group 1 samples were examined in Bacteriology Laboratory of Uludağ University, Faculty of Medicine. Group 2 samples were examined in Bacteriological Diagnostic Laboratory, the Institute for Hygiene and Infectious Disease of Animals, Justus Liebig University, Giessen.

Microbiological analysis of the swabs samples

Initially, thioglycollate agar were incubated at 37°C for 24 hours and cultured in bloody agar (Merck 110886) and EMB agar (Eosin-Methylenblue-Lactose-Saccharose) (Merck 101347). Then, they were incubated at 37°C for 24 hours again. According to colony morphology and Gram color features, identified colonies were assessed. In the identification of the bacteria, their direct cultures were performed using BBL Crystal (Becton-Dickinson, Sparks, USA) Gram-Positive and Gram-Negative ID system kits and its computer program in Turkey and with traditional bacteriologic techniques by bloody agar (Merck 110886, 10% defibrinated sheep blood), Gassner-Agar (Merck 101282) and serum bouillon in Giessen. Cultured discs were incubated for 24 hours at 37°C in an aerobic incubator with 10% CO_2. Cultures were assessed; unimportant up to 1-4 colony, + up to 5-50 colony, ++ up to 50-200 colony, +++ (strong) up to 200 colony. After 48 hours, sub-cultured discs were obtained from described colonies. Microorganisms were identified complying with the biochemical tests and API system procedures (BioMérieux, Milan–Italy), and bacterial agents of endometritis were determined.

Statistically, collected data in both groups were compared and then they were analyzed with Pearson Chi-square and the Pearson Correlation Analysis tests.

Results

Out of 101 endometrial uterine swabs, 8 (7.9%) were negative but 93 (92.1%) were positive in Group 1. In 37 samples (36.6%), one bacterial species and in 56 swabs (55.4%) two or more bacterial species were isolated. In 64 swabs, a potentially pathogenic Gram-negative bacterium was *E. coli* (41.8 %).

Bacterial species isolated are summarized in Table 1. Significant correlations were determined between bacteria in endometrial swabs of cows with pneumo-/urovagina ($P \leq 0.001$). Gram negative pathogens were more commonly isolated bacteria. Both infection rates were 92.1%, and bacterial infections rate originated from fecal contamination (49.6%) were high in cows with pneumo-/urovagina ($P \leq 0.001$).

Bacteria cultured in uterine samples belonged to 142 cows in Group 2. 140 samples (98.5%) were positive bacteriologically. 12 samples (8.5%) and 130 swabs (91.5%) resulted in isolation of one bacterial species and two or more bacterial species, respectively. 72 swabs were potentially pathogenic bacteria causing infertility including *T. pyogenes, S. uberis, E. coli,* β-*Streptococcus, S. heamolyticus* and *S. aureus*. Isolated bacterial species are given in Table 2.

Discussion

Peri- or post-parturient reproductive disorders are usually complex, and cows have predisposing factors for the development of uterine infections in this period. Acute clinical uterine disease (metritis) develops within a week of parturition in average 20–40% of cows. About 20% of the cows have a persistent clinical disease (endometritis) in 3rd weeks after calving (Sheldon et al., 2009). Moreover, it is well-known that ascending bacterial infections of genital tracts is one of the most important causes of the infertility in cows. Therefore, determination of the types of bacteria causing endometritis in cows with pneumo-/urovagina and comparison of the bacterial flora of the cows with endometritis resulted from the other reasons was aimed in this study.

Bacteria colonizing the genital mucosa have not been studied in a larger extend and bacteriological swabs are not used for routine gynecological examination in cows. If bacteria ascend from the vagina into the uterus, depending on a deficiency of the uterine clearance mechanisms of the cow, as well as the species and number of bacteria, endometritis may develop (Petit et al., 2009). It has been concluded in our previous study (Goncagul et al., 2012) that typical findings of the pneumovagina can be encountered following first parturition in cows, and this pathology leads to abdominal

Table 1: Micro-organism isolated from cows uterine swabs and their frequency of isolation in Group 1

Micro-organism	Number of times isolated	Frequency of isolation (%)
Escherichia coli	64	41.8
Streptococcus bovis I	22	14.4
Streptococcus bovis II	22	14.4
Streptococcus equinus	9	5.9
Bacillus licheniformis	6	3.9
Proteus mirabilis	4	2.6
Gemella morbilorum	5	3.3
Enterococcus hirae	5	3.3
Bacillus pumilus	2	1.3
Bacillus spp.	1	0.7
Bacillus subtilis	1	0.7
Comanas testosteroni	1	0.7
Citrobacter kosei	1	0.7
Streptococcus mitis	1	0.7
Arconobacterium heamolyticum	1	0.7
Aerococus viridans	1	0.7
Enterococus fergussonii.	1	0.7
Bacillus cereus	1	0.7
Staphylococcus haemolyticus	1	0.7
Enterococcus gallinarum	1	0.7
Enterococcus feacalis	1	0.7
Streptococcus uberis	1	0.7
Bacillus megaterium	1	0.7
TOTAL	153	100.0

Table 2: Micro-organism isolated from cows uterine swabs and their frequency of isolation in Group 2 (Control).

Micro-organism	Number of times isolated	Frequency of isolation (%)
Arcanobacter ium pyogenes	34	12.1
Escherichia coli	62	22.1
Streptococcus uberis	12	4.3
ß Streptococcus	2	0.7
Staphylococcus aureus	10	3.7
Escherichia coli haemolytic	4	1.4
Moraxella (*Branhamella*)	2	0.7
Coliform bacteria	4	1.4
γ Streptococcus	40	14.3
α *Streptococcus*	42	15.0
Bacillus spp.	24	8.6
Proteus *spp.*	6	2.1
Corynebacterium spp.	10	3.7
Morganella morganii	2	0.7
Pasteurella multocida	4	1.4
Staphylococcus epidemicus	4	1.4
Clostridium perfringens	2	0.7
Pseudomonas aeroginosa	2	0.7
Stenotrophomonas maltophilia	2	0.7
Staphylococcus haemolyticus	2	0.7
Enterococcus spp.	4	1.4
Erwinia spp	2	0.7
Micrococcus *spp.*	2	0.7
Klebsiella spp.	2	0.7
TOTAL	280	100.0

negative pressure and air suction to the vagina, and results in the fecal contamination of the genital tracts, particularly the uterus.

The qualitative and quantitative bacterial contamination to the uterus depends on the balance between bacterial contamination and the defense mechanisms of the cows (Sheldon and Dobson, 2004). Uterine contaminations are non-specific and involve a wide range of ubiquitous bacteria. In the first 10 days after calving, mainly, *Streptococcus (Sc.) spp.*, *Staphylococcus (St.) spp.* and *Bacillus spp.* can be isolated from the uterus of the cows without puerperal endometritis. The pathogenic bacteria generally are associated with endometritis are *E. coli* and *Arcanobacterium pyogenes* (Königsson et al., 2001; Sheldon et al., 2009). Gram-negative facultative anaerobes, such as *E. coli* can be cultured from the uterus of the many cows following calving for 2 weeks (Petit et al., 2009), although cows eliminate these bacteria spontaneously (LeBlanc et al., 2002). Generally, *E. coli* is the most common early postpartum uterine pathogen and almost always leads to the other uterine pathogens (Dohmen et al., 2000). The results of both the groups in present study proved that cultured bacteria from the uterine swabs included high rate of fecal and potentially pathogenic bacteria. In addition, statistically, there was a significant difference between the groups in terms of pathogen bacteria originated from feacal samples ($P<0.001$).

Conclusions

In conclusion, high rate of pathogenic bacteria resulted in pneumo-/urovagina in cows. Therefore, in case of pneumo-/urovagina, ascending infections and its clinical aspects can be the main cause of infertility in cows.

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