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Antibiogram and pathogen isolation from vaginal discharge in dairy cows with metritis

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ABSTRACT:

The fertility success of dairy cows is greatly associated with their uterine health status in the postpartum period. However, the functioning of the uterus in cows is frequently put at risk due to contamination of the uterine lumen by pathogenic bacteria. The most common bacterial species causing uterine infections are *Escherichia (E) coli*, *Trueperella (T) pyogenes*, *Streptococcus spp*, *Fusobacterium (F) necrophorum*, *Bacteroides* and *Prevotella*. One of the most prevalent postpartum uterus diseases is defined as metritis. The objective of this study was to evaluate the bacterial isolation and antibacterial susceptibility resulting from vaginal discharges in dairy cows with metritis. The study was carried out on 15 multiparous (between 3 to 6 ages) Holstein dairy cows with metritis. Metritis was characterized by fever (>39.5°C), fetid watery red-brown vaginal discharge and systemic signs of diseases in the first 21 days postpartum. The vaginal swabs for microbiological analysis and the antimicrobial susceptibility test were taken under aseptic conditions from the cows. As a result, four different types of bacteria were isolated including *E. coli* (35.29%), *T. pyogenes* (29.41%), *Streptococcus spp* (17.65%) and *Staphylococcus (S) aureus* (17.65%). Then, 13 antimicrobial agents were used in the antibiogram test. It was found that the most effective antimicrobial agents were florfenicol, seftiofur, oxytetracycline, linkomycin and penicillin. Also, it was determined that some bacteria species have resistance against antimicrobial agents. In conclusion, it was concluded that the effects of antimicrobial agents and metritis agents changed from cow to cow. So, it was thought that uterine infections causing infertility can be restricted by the isolation of pathogen and the choice of appropriate antimicrobial agents.

Metritisli sütçü ineklerde vaginal akıntıdan etken izolasyonu ve antibiyogram

ÖZET:

Sütçü ineklerde fertilité başarısı çoğunlukla postpartum dönemde uterus sağlığı ile ilişkilidir. Ancak, ineklerde uterusun normal işleyişi sıklıkla patojen bakterilerin uterus lumenini kontamine etmesiyle riske atılır. Uterus enfeksiyonlarına neden olan en yaygın bakteri türleri *Escherichia (E) coli*, *Trueperella (T) pyogenes*, *Streptococcus spp*, *Fusobacterium (F) necrophorum*, *Bacteroides* ve *Prevotella* dir. En sık karşılaşılan uterus hastalıklarından biri metritis'tir. Bu çalışmanın amacı, metritisli ineklerin vaginal akıntılarında bakteri izolasyonu ve bu bakterilere karşı etkili antibiyotikleri tespit etmektir. Sunulan çalışmada 3 ile 6 yaş arasında 15 adet metritisli multipar Holstein sütçü inek kullanıldı. Metritis, doğumdan sonraki ilk 21 gün içinde ateş (> 39.5 ° C), sulu kırmızı-kahverengi ve kokulu uterus akıntısı ve sistemik hastalık belirtileri ile karakterize edildi. Metritisli ineklerden mikrobiyolojik analiz ve antimikrobiyal hassasiyet testi için streil şartlarda vajinal svab alındı. Analizlerde, *E. coli* (%35.29), *T. pyogenes* (%29.41), *Streptococcus spp* (%17.65) ve *Staphylococcus (S) aureus* (%17.65) dahil olmak üzere dört farklı bakteri türü izole edildi. Antiogram testinde 13 antimikrobiyal ajan kullanıldı ve en etkili antimikrobiyal ajanların florfenikol, seftiofur, oksitetrasiklin, linkomisin ve penisilin olduğu tespit edildi. Ayrıca bazı bakteri türlerinin antimikrobiyal ajanlara karşı direnç gösterdiği belirlendi. Sonuç olarak, metritis etkenlerinin ve antimikrobiyal ajanların etkilerinin inekten ineğe değiştiği görüldü. Bu nedenle, infertiliteye neden olan uterus enfeksiyonlarının etken izolasyonu ve doğru antibiyotiklerin seçimi ile kısıtlanabileceği düşünüldü.

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1. Introduction

Reproductive tract diseases have a detrimental effect on the fertility of dairy cows and the economic profitability of dairy farms (1). The fertility success of dairy cows is greatly associated with uterine health status in the postpartum period (2). Normal functioning of the uterus in cows is frequently put at risk due to the contamination of the uterine lumen by pathogenic bacteria which often causes uterine diseases in the early postpartum period. And these bacteria are an important cause of infertility (3). The uterine lumen is sterile before calving (4). However, nearly 100% of dairy cows in the early postpartum period suffer from bacterial contamination of the uterus (5). The most common types of bacteria species isolated in this period are *T. pyogenes* (1), *E. coli*, *Pseudomonas spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, *Pasteurella (P) multocida*, *Clostridium spp.*, *Fusobacterium spp.* and *Bacteroides spp.* (5). The uterus of a healthy cow efficiently eliminates the majority of these bacteria until the first 3 weeks after parturition (5). However, the elimination period is prolonged in cows with uterine health problems and immune function deficiencies (6). As a result of the inability to eliminate bacteria from the uterus, uterine infections occur (7). Also, it was stated that about half of the dairy cows suffer from uterine health problems in the first two months postpartum (6). One of most common postpartum uterus diseases is stated as metritis (7) that is defined as inflammation of the uterus. It results in systemic signs of diseases, including fever, as well as fetid watery red-brown uterine discharge (8). It occurs mostly in the first 21 days postpartum (3,9). The occurrence of metritis is largely associated with immune dysfunction in the early postpartum period (3, 6). Its incidence varies between 5-20% (10). Metritis has an adverse effect on reproductive performance because it causes delays in the involution of the uterus (11) and in the onset of normal ovarian activity as well as prolongs calving to pregnancy interval. Due to these reasons, conception and the pregnancy rate decrease (2). There are several diagnostic techniques such as transrectal uterine palpation, transrectal ultrasonography (7), vaginoscopic examination, culture of uterine fluids, uterine biopsy and uterine cytology (2) for uterine infections within metritis (3) and endometritis (2). The evaluation of vaginal discharge is the most effective and practical procedure for diagnosis (8). However, pathogen isolation from vaginal discharge and antibiogram are the most important points to combat metritis (12).

The objective of the study was to evaluate the pathogen isolations and antibacterial susceptibilities from vaginal discharges in dairy cows with metritis and then the scientific information obtained was intended to contribute to the treatment and prevention of metritis.

2. Material and Methods

The study was carried out on 15 multiparous (between 3 to 6 years old) Holstein dairy cows with metritis. The cows were managed in free-stall barns, fed according to their individual needs and produced above 6000 kg of milk per cow, per lactation. The cows were milked with an automatic milking system twice a day. Systematic vaccinations were done periodically as well as controlled health monitoring programmes. Metritis was described as uterine inflammation associated with bacterial infection. It was characterized by including fever ($>39.5^{\circ}\text{C}$) (13), fetid watery red-brown uterine discharge and systemic signs of diseases (8) in the first 21 days postpartum (3). The examination of cows was performed by transrectal ultrasonography, rectal palpation, rectal temperature measurement, and the state of the vaginal discharge was visually examined. Vaginal discharge was assessed using the Metricheck™ device (Simcro Tech, Hamilton, New Zealand). After cows were diagnosed with metritis, the vulva and perineum were thoroughly washed and disinfected with an antiseptic solution (0.05% ben-zalkonium chloride) and were subsequently dried with a paper towel. After these processes, vaginal swabs were collected under aseptic condition from the cows. The samples were placed in the cool box with ice and were transferred to the laboratory for microbiological analysis and an antimicrobial susceptibility test.

Bacterial isolation and identification from the samples was carried out with standard procedures (14). The antibacterial susceptibility was determined by the Kirby-Bauer disk diffusion susceptibility test (14) for the isolated

strains against 13 different antibiotics (florfenicol, ceftiofur, oxytetracycline, linkomycin, cloksacillin, amoxicillin, gentamycin, chloramphenicol, spectinomycin, penicillin, ampicillin, sulbactam and cefoperazone).

3. Results

Four different types of bacteria as 3 gram positive (*Streptococcus spp*, *T. pyogenes*, *S. aureus*) and 1 gram negative (*E. coli*) were isolated from samples of vaginal discharge. The results were presented on Table 1. In this study, the most commonly isolated bacteria was *E. coli* (35.29%). The rate of isolated other bacteria species including *T. pyogenes*, *Streptococcus spp* and *S. aureus* were 29.41%, 17.65% and 17.65%, respectively. And also, in a cow *S. aureus* + *Streptococcus spp*, in 2 cows *S. aureus* + *E. coli* and in a cow *Streptococcus spp* + *T. pyogenes* was identified as a combined infection. Furthermore, any pathogen was isolated in two samples.

Table 1: Bacteria species and isolated rate from vaginal discharge of 15 cows with metritis at postpartum first 21 days.

Tablo 1: Postpartum ilk 21 günde 15 ineğin vaginal akıntısından izole edilen bakteri türleri ve oranları.

Bacterial identification	Number of isolates	Percentage (%)
<i>E. coli</i>	6	35.29
<i>Streptococcus spp</i>	3	17.65
<i>Trueperella pyogenes</i>	5	29.41
<i>S. aureus</i> ,	3	17.65
<i>Total</i>	17	100

It was found that whereas the most effective antimicrobial agents were florfenicol, ceftiofur, oxytetracycline, linkomycin and penicillin, the least effective antimicrobial agents were cloksacillin, amoxicillin, gentamycin. Also, chloramphenicol, spectinomycin, cefoperazone, ampicillin, sulbactam were found as moderately effective antimicrobial agents. Moreover, it was determined that the bacteria isolated from the vaginal discharge of cows with metritis could resistant to some antimicrobial agents. But, it was found that this situation changed from cow to cow.

4. Discussion and Conclusion

The bacterial contamination of the uterus is an inevitable condition after parturition in dairy cows. Aerobic and anaerobic bacterial existence in the uterus lumen during 2 weeks postpartum can be determined in at least 90% of dairy cows (4). Bacterial contamination does not always lead to uterine disease, nevertheless, up to 40% of dairy cows can be affected by uterine infections, because the uterine lumen in the postpartum period is suitable for the development of aerobic and anaerobic bacteria (15). Bacterial species which were commonly found in uterine lumen during 2-3 weeks postpartum are *T. Pyogenes* (1), *E. coli*, *Pseudomonas spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, *P. multocida*, *Clostridium spp.*, *Fusobacterium spp.*, and *Bacteroides spp.* (5). However, the uterine diseases are usually derived from *E. coli*, *T. pyogenes*, *F. necrophorum*, *Bacteroides* and *Prevotella* species (7). In our study, the bacteria isolated from the vaginal discharge of cows with clinical metritis were found to be *E. coli*, *Streptococcus spp.*, *T. Pyogenes* and *S. aureus*. The commonly reported bacterium types isolated from cows with metritis are *T. pyogenes* and *E. coli*. (16). In our study, the most commonly isolated bacteria were *E. coli* (35.29%) and *T. Pyogenes* (29.41%). Also, our study confirmed the work of Burfeind et al., who isolated 52.4% *E. coli* from uterine discharge of cows with metritis (17). Furthermore Sharma et al. determined that the most common isolated bacterium in cows with uterine infection is *E. coli* in their work (18). Recent studies have revealed the importance of bacterial isolation and antibiogram in the struggle against uterine infections (12, 18, 19) because the selection of antibiotics is very important for the treatment of metritis (12). Antibiotic selection based on pathogen isolation and antibiogram prevents the development of antimicrobial resistance in different bacterial species (12). Moges et al found that *S. aureus* have resistant to ampicillin, oxacillin and

vancomycin. Furthermore, in the same study, it was determined that *E. coli* have resistance to sulphamethaxazole (40%), polymixin (100%), tetracycline (100%), oxacillin (40%), gentamycin (40%) and cefoxitin (100%) (20). In the study, the most effective antimicrobial agents were florfenicol, ceftiofur, oxytetracycline, linkomycin and penicillin, against *E. coli*, *T. pyogenes*, *Streptococcus spp* and *S. aureus*. Yet, *E. coli* had resistance (100%) against all antimicrobial agents in the 3 samples. Also, *T. pyogenes* had resistance (100%) against amoxicillin, cloksacillin and gentamycin, in 2 samples. In our study, as a result of the obtained findings it was determined that both metritis agents and the types of antibiotics with which these agents are sensitive differed between cows. Consequently, importance of detection of bacterial species and antimicrobial susceptibility test against these bacteria was comprehended.

As a conclusion, significant majority of dairy cows are affected by uterine infections during the early postpartum period. The most important uterus infection is metritis, because it results in infertility and economic loss in dairy cows. Uterine diseases are usually associated with *E. coli*, *T. pyogenes*, *F. necrophorum*, *Bacteroides* and *Prevotella* species of bacteria. The most effective antimicrobial agents, however, demonstrate differences due to the resistance of bacteria to antibiotics. In our study, bacteria causing metritis was detected by agent isolation from vaginal discharge of cows with metritis. However, it was shown that the effects of antimicrobial agents changed from cow to cow. Therefore, it was thought that uterine infections can be restricted more effectively with the isolation of agents and selection of right antimicrobial agents.

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