Bacterial flora isolated from genital tract of cows submitted for artificial insemination in Balad district

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Background: It is accepted that bovine genital infections particularly non-specific infections account for large number of pregnancy failure in cows after artificial insemination.

Objectives: To Isolate and identify bacteria species colonize the genital tract of heifers and multiparous cows during the estrus cycle.

Abstract:

This study was conducted at the Veterinary Clinic for Artificial Insemination and Genital Diseases in Balad district for the period from 1st. January/2011 to 30th. June/2011. Vaginal aspirates were collected from vaginal fornix of cows during the estrus cycle attending the clinic for artificial insemination using frozen semen. A total of 90 cows were included, 60 of which were multiparous and 30 cows were heifers. The aspirate materials were streaked on Blood agar and MacConkey agar plates. Bacterial isolation and identification was based on standard bacteriological criteria.

The study revealed that S. aureus was the most frequently isolated from heifers (36.7%), followed by E.coli (30.0%) and proteus spp. (6.7%). Whereas, 8 (26.6%) were culture negative. In multiparous cows, the highest bacterial isolates were E. coli (38.3%), and S. aureus (20.0%). Mixed bacterial growth was found in 40% of multiparous cows and 5 (8.3%) were culture negative. Multiparous cows with five previous deliveries had highest isolation rate (32.7%).

المستنبتات البكتيرية المعزولة من القناة التناسلية للأبقار المقدمة للتلقيح الاصطناعي في منطقة بلد شمالي العراق

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الخلاصة:

أجريت هذه الدراسة في العيادة الطبية البيطرية المتخصصة بالتلقيح الاصطناعي والأمراض التناسلية وللفترة من 1 كانون الثاني 2011 ولغاية 30 مايس 2011. كان عدد الأبقار المشمولة بهذه

الدراسة والتي جلبت لغرض التلقيح الاصطناعي 90 بقرة، بلغ عدد الأبقار المتعددة الولادات 60 بقرة من مجموع الأبقار في حين كان عدد الأبقار الاباكير 30 بقرة . جمعت الإفرازات المهبلية من القبو المهبلي لأبقار الدراسة قبل إجراء عملية التلقيح الاصطناعي لها، ومن ثم زرعت على الوسط ألزرعي المتكون من مادة الدم المثخنة وعلى وسط الماكونكي. تم العزل والتوصيف البكتيري وفقا للمعايير البكتريولوجية القياسية.

أوضحت الدراسة أن بكتريا *Staphylococcus aureus* كانت أكثر العتر البكتيرية المعزولة من القناة التناسلية للأبقار الاباكير وبنسبة (36.7%)، تلتها بكتريا *Escherichia coli* (30.0%) و بكتريا *Proteus spp.* (6.7%). بينما بلغت الزروعات البكتريا السالبة 8 (26.6%).
كانت بكتريا *E.coli* أعلى العزلات البكتيرية في الأبقار المتعددة الولادات وبنسبة (38.3%) تلتها بكتيريا *S.aureus* (20.0%)، في حين كانت نسبة النموات البكتيرية المختلطة في هذه المجموعة من الأبقار(40%)، بينما كانت العزلات البكتريا السالبة 5 (8.3). كان للأبقار المتعددة الولادات ذات الخمسة ولادات أعلى نسبة عزل بكتيري حيث بلغت (32.7 %).

Introduction:

The economy of dairy cows is largely depending on pregnancy rate after insemination. One of the major constraints of profitable dairy cows is low conception rate (1,2). It has been reported that probability of conception was lowered in cows with uterine and foot health problems (33.9%), in multiparous cows (34.1%), and in cows in the summer (29.1%), but no interactions with mastitis were detected (3). However, it is accepted that bovine genital infections particularly non-specific infections account for large number of pregnancy failure in cows (4). Bacterial infection is the most important among the various causes of the subfertility (5,6). Such conditions may cause cervicitis or endometritis to various degrees that in turn may lead to embryonic death and repeat breeding problems (7,8). These infections affect the fertility by altering the uterine environment resulting in impairment of sperm transport, sperm death and hostile environment to the subsequent development and maintenance of the conceptus leading to their death (2,9,10).

(11) in their study to determine the non-specific aerobic and anaerobic bacteria causes of endometritis causing repeat breeding of cycling Iraqi buffalo cows at Nineveh province, reported that the most prevalent bacteria in uterine lumen were *E. coli* (23%), *Archanobacterium pyogenes* (13%) and *Staphylococcus aureus* (10%). The present study was conducted to identify bacterial flora colonize genital tract of dairy cows.

Materials and methods:

Specimens included in this study were collected at the veterinary clinic for Artificial Insemination and Genital Diseases in Balad district for the period from 1st. January/2011 to 30th. June/2011. Vaginal aspirates were collected from vaginal fornix of cows during the estrus cycle

attending the clinic for artificial insemination using frozen semen. A total of 90 cows were enrolled in this study, 60 of which were multiparous and the another 30 cows were (heifers). The multiparous cows include those with one, two, three, four and five previous pregnancies.

About 3-5 milliliters of High vaginal aspirate was collected using vaginal speculum and sterile catheter by expert veterinarians. The aspirate was poured in sterile test tubes containing sterile Nutrient broth as transport media. The test tubes were placed in cool box and transport to the microbiology laboratory as soon as possible. In the laboratory, the test tubes were left for 1 hour in the incubator at 37 ^{0}C, then each aspirate material was streaked on Blood agar and MacConkey agar plates using sterile bacteriological loop. The plates were incubated at 37 ^{0}C for overnight. Bacterial identification was based on colonial morphology, Gram's stained smear, and biochemical reactions according to the methods described by (4).

Results:

Table (1) showed that 22(73.4%) of specimens collected from heifers were yielded bacterial growth, while 8(26.6%) of the specimens were negative. The rate of different bacterial types isolated from heifers cows were as follows; *S. aureus* (36.7%), followed by *E.coli* (30.0%) and proteus spp (6.7%).

Table (1): Isolation rate of bacterial species from heifers cows.

Type of bacterial isolate	No.	%
S. aureus	11	36.7
E. coli	9	30.0
Proteus spp.	2	6.7
No growth	8	26.6
Total	30	100

Regarding the multiparous cows, the results in table (2) showed that 55 (91.7%) of the specimens yielded bacterial growth, 4(6.7%) of them revealed mixed growth (*E. coli* and proteus spp.). E. coli was the most prevalent bacteria with an isolation rate (38.3%), followed by *S. aureus* (20.0%) and proteus spp. (10.0%). Other bacteria in order and frequency were pseudomonas spp., Klebsiella spp., Bacillus spp., with an isolation rates 5.0%, 5.0% and 6.7% respectively.

Table (2): Isolation rate of bacterial species from multiparous cows.

Type of bacterial isolate	No.	%
E. coli	23	38.3
S. aureus	12	20.0
Proteus spp.	6	10.0
Pseudomonas spp.	3	5.0
Klebsiella Spp.	3	5.0
Bacillus subtalis	4	6.7
Mixed growth	4	6.7
No growth	5	8.3
Total	60	100

The distribution of the type of bacterial isolates according to the number of previous deliveries was shown in table (3). Among the total 55 bacterial isolates, *E. coli* was appeared to be the most frequently isolated bacteria from all cows included in the study regardless the number of previous deliveries. However, the highest number of isolates was recovered from multiparous cows.

Table (3): Frequency of bacterial isolates according to number of previous pregnancies.

Type of bacterial isolate	No. of previous pregnancies				
	1	2	3	4	5
	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)
E. coli	3 (60.0)	5 (62.5)	2 (16.7)	6 (50.0)	7 (38.8)
S. aureus	0	1 (12.5)	3 (25.0)	2 (16.7)	6 (33.3)
Proteus spp.	1 (20.0)	0	2 (16.3)	1 (8.3)	2 (11.1)
Pseudomonas spp.	0	0	1 (8.3)	1 (8.3)	1 (5.5)
Klebsiella spp.	0	1 (12.5)	1 (8.3)	0	1 (5.5)
Bacillus spp.	1 (20.0)	0	2 (16.7)	1 (8.3)	0
Mixed growth*	0	1 (12.5)	1 (8.3)	1 (8.3)	1 (5.5)
Total	5 (9.1)	8 (14.5)	12 (21.8)	12 (21.8)	18 (32.7)

- Mixed growth includes *E.coli* and proteus spp.

Discussion:

Factors that affect the rate of success of artificial insemination in cows are diverse. Genital tract colonization or infection by aerobic or anaerobic bacteria or even the presence of mycoflora undoubtedly constitutes one of these factors (4,7,8,12). In the present study, the

vaginal fornix area of 73.3% of heifers and 91.7% of multiparous cows was found to be colonized by different aerobic bacteria. In heifers, *S. aureus* was found the most prevalent bacteria, while *E. coli* was found to be the highly prevalent bacteria among multiparous cows. These results were consistent with that of(9) who found that bacteria was detected in uterine specimens from 62.2% of repeat breeding cows compared to 28.6% from normal fertile cows, and the most common bacterial isolates was staphylococcus spp. The difference in the rate of isolation certainly related to the site of specimen collection.

In the present study, the isolation rate was higher than that reported by (13) who found that 53.9% of swabs collected from the vaginal fornix of multiparous cows were culture positive, while 46.1% were negative. The increment in the isolation rate obtained in the present study (73.4%) in heifers and (91.7%) in multiparous cows may be attributed to primitive unhygienic housing and breeding of cows other factors may include sample size, techniques of sample collection and culturing. On the contrary, the present results were consistent with that of(14) who reported that out of 383 vaginal cervix swabs collected from post-puerperal cows, 88.3% were positive. In 3.4% of swabs pathogenic gram-positive and in 16.7% pathogenic gram-negative microorganisms were found. The percentage of positive vaginal swabs did not differ between pregnant and non-pregnant animals. Our results were also concordant with previous Iraqi study found that *E. coli* was the most prevalent bacterial flora among multiparous cows (11). These variations are not surprising, since sanitation, environmental conditions, breeding system and the general condition of animals play role in the colonization rate of vagina by bacteria (15). On the other hand, the current results are in agreement that as the number of previous delivaries increase, the rate of bacterial colonization in the vagina may increase (6,7,8).

Bacteria on the vaginal and cervical mucosa in cattle involve a wide range of species. Although the bacteria recovered in the present study cannot be considered as pathogenic, since the specimens were collected from cows attending for artificial insemination and had no complain. However, these bacteria may become so whenever a suitable environment is become available. Therefore, to increase the rate of success of artificial insemination in dairy cows particularly among multiparous cows, carful investigation of the vaginal mucous for bacterial colonization and management may be helpful.

Conclusion: High colonizing rate of genital tract of heifers and multiparous cows was found to be due to different bacterial species.

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