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BACTERIAL INFECTION ASSOCIATED WITH REPRODUCTIVE FAILURE IN DAIRY COWS AND THEIR ANTIMICROBIAL SENSITIVITY IN KHARTOUM NORTH-SUDAN

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ABSTRACT

To investigate the risk factors and bacterial infections associated with reproductive failure in dairy cows. Vaginal swabs and blood samples were collected from 408 cows in all farms visited in Khartoum North, Khartoum State, Sudan**.** The bacteria isolated from these vaginal swabs were 232(39.1%) *Staphylococcus spp*, 162(27.2%) *Streptococcus spp*, 150(25.2%) *E. coli spp* and 50 (8.5%) *Pseudomonas spp*. The prevalence rate of brucellosis was (16.2%). The number of bacteria isolated from cows with reproductive problem was higher than randomly sampled cows. Brucellosis seroprevalence was higher in problematic cows 43(20.8%) compared to randomly selected cows 23(11.4%) this difference was found to be significant with $p< 0.05$, and the relative risk of problematic cow to get brucellosis was twice the randomly selected cow. The antimicrobial sensitivity of the isolated Gram positive bacteria, was Vancomycine, Gentamycine and Amoxyclave are considered and that of Gram negative bacteria were Ciprofloxacine, Ampcilline, Gentamycine and Cotrimoxazole.

KEYWORDS: Sudan, reproductive failure vaginal swabs, brucellosis.

INTRODUCTION

Microbial infection of the female genital tract is most common and of greatest economic importance in humans and cattle, (Ross, 2002). Bacterial infections of the endometrial that cause uterine disease are common in modern dairy cattle after parturition, and lead to decreased productivity and sub fertility (Sheldon *et al.*, 2009). The Brucellosis has been extensively studied, because it is a disease of major public health importance and one of the most common zoonotic diseases globally and it causes widespread economic losses, due to abortion, infertility and extended calving intervals, (Ariza *et al*., 2007**,** Nasir *et al*., 2005, Schelling *et al*., 2003 and Zinsstag *et al*., 2007).

Bacteria isolated from cows with endometritis were *Escherichia coli, Hemolytic streptococci* and other bacterial Species. From Cows with metritis Knudsen, *et al*, (2014) isolated *E. coli*, *Streptococcus spp*, *Staphylococcus spp*, *Pseudomonas spp* and other bacterial Species. Using traditional culture methods, Azawi, *et al*, (2008) in their study to determine bacteria causes of repeat breeding of cycling Iraqi buffalo cows at Nineveh province, reported that the most prevalent bacteria in uterine lumen were *E. coli*, *Archanobacterium pyogenes* and *Staphylococcus aureus*. Kather, *et al*, (2012), isolated and identified bacteria species colonizing the genital tract of heifers and multiparous cows during the estrus cycle. He reported that the *E. coli* was the most prevalent bacteria; followed by *S. aureus* and *proteus spp*. *Staphylococcus aureus* is the most common isolate of *Staphylococcus spp* and causes postpartum infection, (Sadig, 2010). In cattle, infectious causes of pyometra include *Campylobacter spp*, *Staphylococcus spp* and *Streptococcus spp* bacteria, as well as protozoa including *Trichomonas spp* and *Brucella spp*, (Foldi *et al*., 2006).

Residual tissue encountered in the case of a retained placenta represents a favorable medium for the growth of bacteria in the uterus and necrotic tissue delays uterine involution and the repair of the endometrium. Furthermore, residual tissues may result in the cervix being held open and an increase in bacterial contamination of the endometrium, (Kim and Kang, 2003). Several strains of *E.coli* were resistant to Oxytetracycline, this information suggests that parenteral Oxytetracycline is a poor treatment for postpartum metritis, (Okker *et al*., 2002; Risco, 2002). Sadig, (2010), reported Ampicillin and Gentamycin are the drugs of choices for treatment of postpartum bacterial infection.

The prevalence of bovine brucellosis in Khartoum state was studied by many workers, (Salman and Nasri, 2012), reported that the average prevalence rate of bovine brucellosis when using RBPT was 27.2% in Khartoum State, (Salman, *et al.*, 2014), recorded the highest

prevalence rate value was in Bahri, (47.2%) then Khartoum province (45.3%), then Omdurman province (9%). Angara *et al.,* (2016), reported the prevalence rate of bovine brucellosis in Khartoum State based on Rose Bengal Plate Test, to be 27.5%.

The objectives of this study were to detect the association between the isolated bacteria and reproductive problems in dairy cows and to detect the antibiotics sensitivity tests to the isolated bacteria.

MATERIALS AND METHODS

Study area: All farms were located in the region of Bahri North and East Nile localities in Khartoum State.

Study design: For the purpose of the study a stratified random samples was undertaken to include large dairy farms and small dairy units around Khartoum North. In all farms visited animals with known reproductive problems at the time of the survey were investigated and sampled and Five % of the animals were selected randomly to detect the presence of bacterial infection in Reproductive System.

Sample collection: A total of 408 vaginal swabs were collected from both cows with reproductive problems and others randomly selected. These samples were collected between Januarys to October 2018. The swabs were collected from cow's vagina; sterile cotton swabs were inserted deeply in the vagina and then rotated 3 to 5 times then pulled out gently, and placed in sterile container. A total of 207 swabs were collected from cows with reproductive problems and 201 swabs were randomly collected.

All collected samples were placed on ice bags in a thermos flask immediately after collection and transported to University of Bahri, College of Veterinary Medicine laboratory at Alkadaro, and were cultured within 2 hours of collection. The swabs were cultured on Blood agar, Nutrient agar and MacConkey agar then incubated at 37°C for 24-48 hours then culturing on selective media and biochemical tests identification of bacteria were conducted.

Blood samples

A total of 408 blood samples were collected from both randomly and with infertility problem cows, the blood samples were taken aseptically from milk vein using plain vacutainer. These samples were centrifuged (at 3000g for 20 min) for separation of serum, serum samples were screened using the Rose Bengal Plate Test (RBPT), ato Alton *et al.* (1988).

Antibiotic sensitivity test (Disc diffusion susceptibility tests)

The isolates were tested for their sensitivity to various chemotherapeutic agents by disc diffusion method. A plate of susceptibility testing agar uniformly inoculated with the test organism, Monica, (2006). Using a ruler on the underside of the plate to measure the diameter of each zone of inhibition in mm, resistant=0 mm, small inhibition=4-13mm, intermediate=14-18mm and sensitive= >18, David *et al.,* (2002).

Data management and analysis

The data obtained from questionnaire and result of samples were analyzed using Statistical Package for Social Sciences version 20 (SPSS). The association between risk factors, was analyzed using X2 (Chi-square) with $p<0.05$ considered as significant, relative risk values were also to determined.

RESULT

Five hundred ninety four isolates were obtained from the 408 vaginal swa, of which *Staphylococcus spp* were 232(39.1%), *Streptococcus spp* were 162 (27.3%), *E. coli spp* 150 (25.2%) and *pseudomonas spp* 50(8.4%)**.** The number of bacteria isolated from cows with reproductive problem were 355(59.7%) and that from randomly sampled cows were 239(40.3), table (1).

Table (1): The frequency of isolated bacteria in all sampled cows.

Isolated Bacteria	Sampled cows		Total
	With infertility problems	Randomly	
Staphylococcus spp	136(58.6%)	96(41.4%)	232(100%)
Streptococcus spp	100(61.7%)	62(38.3%)	162(100%)
E.coli spp	86(57.3%)	64(42.7%)	150(100%)
pseudomonas spp	33(66.0%)	17(34.0%)	50 (100%)
Total	355(59.7%)	239(40.3%)	594(100%)

Out of 207 samples from problematic cows, 65.7% retained positive *Staphylococcus spp* isolate, while 34.3% out of 201 samples from the randomly selected cows revealed positive *Staphylococcus spp* isolates. This difference was found to be statistically significant, and the risk of revealing *staphylococcus spp* from the problematic cow is twice that of the randomly selected cow, with ($p \leq 0.05$), table (2). From the total of 232 *staphylococcus spp* isolates from vaginal swabs, 145(62.5%) were *Staphylococcus aureus* and 87 (37.5%), *Staphylococcus epidermis* figure (1).

Table (2): The association between *staphylococcus spp* and type of sampled cows.

Sampled cows	*Staphylococcus spp*		Total	Chi\ S	Sig	Risk value
	Positive	Negative				
With infertility problems	136(65.7%)	71(34.3%)	207(100%)	13.38	.000	2.09
Randomly	96(47.8%)	105(52.2%)	201(100%)			
Total	232(56.9%)	176(43.1%)	408(100%)			

Out of 207 vaginal swab samples from problematic cows (48.3 %) retained positive *Streptococcus spp* isolate, while (30.8%) out of 201 samples from the randomly selected cows revealed positive isolates. This difference was found to be statistically significant, and the risk of revealing *Streptococcus spp* from the problematic cow is two times that of the randomly selected cow with ($p \leq 0.05$), table (3). A total of 162 *streptococcus spp* were isolated from vaginal swabs, of which *Streptococcus pyogen were* 89 (54.9%), *Streptococcus Pnemonea* 40(24.7%) and *Streptococcus fecalis* were 33(20.4%), figure, (2).

Table (3): Association between *streptococcus spp* and type of sampled cows.

Sampled cows	*Streptococcus spp*		Total	Chi\ S	Sig	Risk value
	Positive	Negative				
With infertility problem	100(48.3%)	107(51.7%)	207(100%)	12.99	.000	2.10
Randomly	62(30.8%)	139(69.2%)	201(100%)			
Total	162(56.9%)	246(60.3%)	408(100%)			

Out of 207 vaginal swab samples from problematic cows 41.5% were positive for *Escherichia coli. spp,* while 31.8% out of 201 samples from the randomly selected cows revealed positive isolates. This difference was found to be statistically significant, and the risk of showing *Escherichia coli. spp* from the problematic cow was 1.5 times that of the randomly selected cow with ($p \leq 0.05$), table (4).

Table (4): The association between *Escherichia coli spp* and type of sampled cows.

Sampled cows	*E. coli spp*		Total	Chi\ S	Sig	Risk value
	Positive	Negative				
With infertility problem	86(41.5%)	121(58.5%)	207(100%)	4.13	0.042	1.52
Randomly	64(31.8%)	137(68.2%)	201(100%)			
Total	150(36.8%)	258(63.2%)	408(100%)			

***Pseudomonas spp* isolates;** Out of 207 vaginal swab samples from problematic cows 15.9% revealed positive *Pseudomonas aureginosa,* while 8.5% out of 201 samples from the randomly selected cows revealed positive isolates of *Pseudomonas aureginosa*. This difference was found to be statistically significant, and the risk of revealing *Pseudomonas aureginosa* from the problematic cow is two times that of the randomly selected cow with ($p \leq 0.05$), table (5).

Table (5): The association between *Pseudomonas aureginosa* and type of sampled cows.

Sampled cows	*Pseudomonas aureginosa*		Total	Chi\ S	Sig	Odd ratio
	Positive	Negative				
With infertility problem	33(15.9%)	174(84.1%)	207(100%)	5.312	.021	2.053
Randomly	17(8.5%)	184(91.5%)	201(100%)			
Total	50(12.3%)	358(87.7%)	408(100%)			

***Salmonella spp*:** No *salmonella* spp. was isolated from either vaginal swab samples or problematic cows in this study.

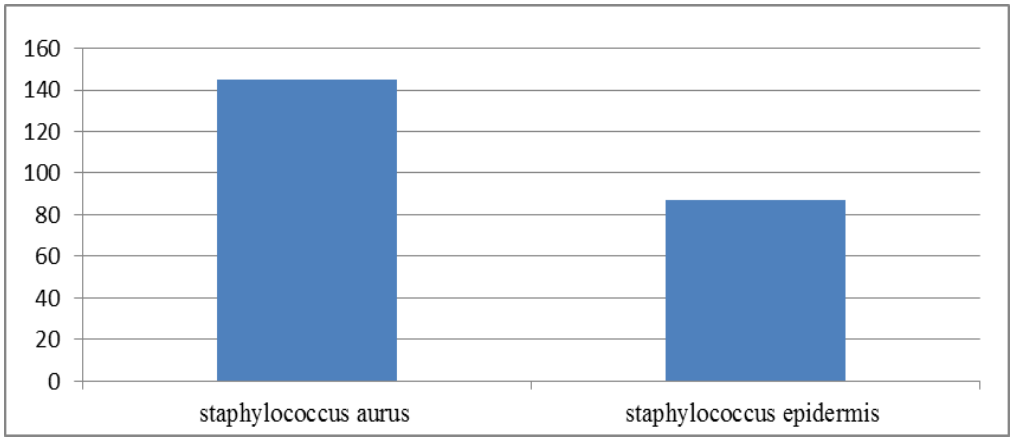

Figure (1): The frequency of *Staphylococcu.*

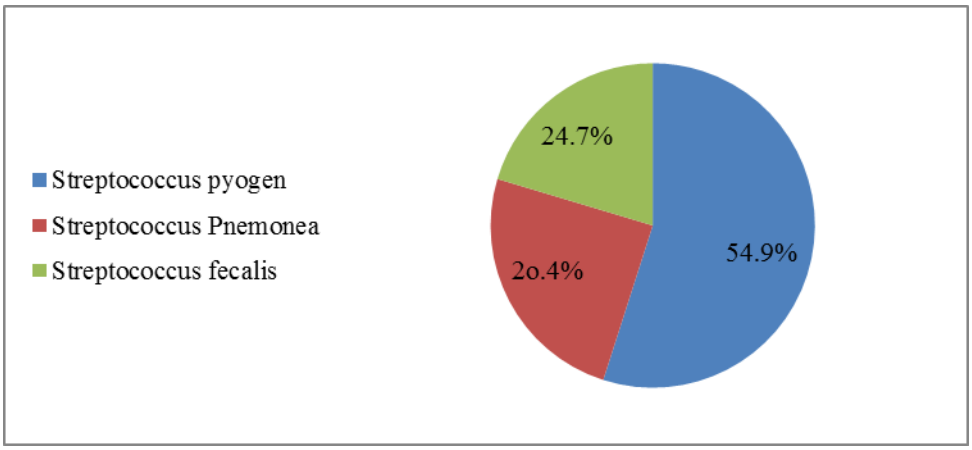

Figure (2): The frequency of *Streptococci spp*.

Table (6): The frequency of isolated bacteria from cows with reproductive problem.

Cows with reproductive problem	Isolated bacteria			
	Staphylococcus spp	***Streptococcus spp***	***E.coli***	***Pseudomonas aureginosa***
Repeat breeding	86(63.2%)	59(59%)	51(59.3%)	21(63.3%)
Abortion	8 (5.9%)	7(7%)	7 (8.1%),	2 (6.1%)
Retained placenta	30 (22.1%)	25(25%)	22 (25.6%)	9 (27.3%)
Reproductive infection	12 (8.8%)	9(9%)	6 (7%)	1 (3%)
Total	136(100%)	100(100%)	86(100%)	33(100%)

Sero-prevalence of Bovine *Brucellosis*: Using rose Bengal plate test, the overall seropositive cows for brucellosis was 66 (16.2%). In cows with infertility problems the seroprevalence was 20.8% while the seroprevalence in randomly selected cows was 11.4%, this difference was found to be statistically significant with P≤ 0.05. The probability of a cow with infertility problem to test positive for brucellosis was twice the randomly selected cows, table (7).

Table (7): The association between *Brucella* infection and type of sampled cows.

Cow	*Brucella*		Total	Chi\ S	Sig	Risk value
	Positive	Negative				
With infertility problem	43(20.8%)	164(79.2%)	207(100%)	6.55	0.011	2.03
Randomly	23(11.4%)	178(88.6%)	201(100%)			
Total	66(16.2%)	342(83.8%)	408(100%)			

The seroprevalence of bovine brucellosis in the East Nile area was 24.7%, while that in Bahri north was 10.6%. There was significant association between the area and the prevalence of brucellosis, and the relative risk of a

cow to be serologically positive for brucellosis from East Nile is twice that of Bahri north area, table (8).

Table (8): The association between *Brucella* and area of sampled cows.

Location	*Brucella*		Total	Chi\ S	Sig	Risk value
	Positive	Negative				
East Nile	40(24.7%)	122(75.3%)	162(100%)	14.46	0.000	2.3
Bahri north	26(10.6%)	220(89.4%)	246(100%)			
Total	66(16.2%)	342(83.8%)	408(100%)			

The seroprevalence of brucellosis in cows with reproductive problem was 26(60.5%), 3 (7%), 10 (23.2%) and 4 (9.3%) from cows with repeat breeding (RB), retained placenta (RP), reproductive infection (RI) and abortion (Ab) respectively, figure (1).

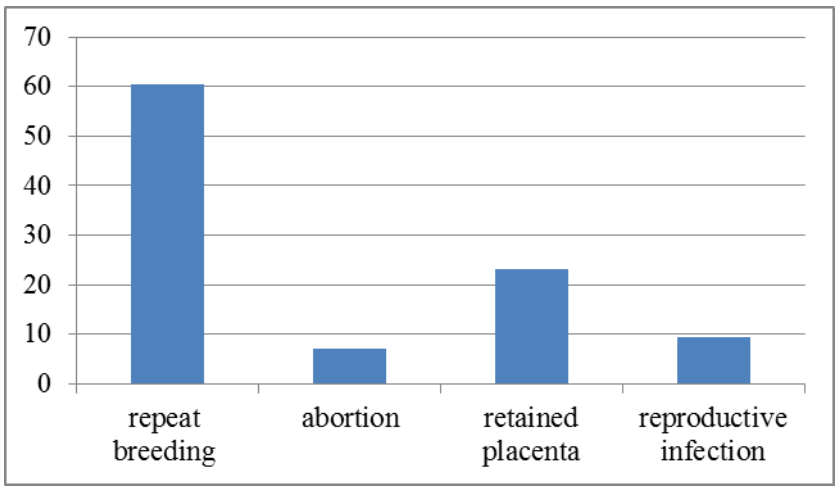

Figure (3): The Seroprevalence of brucellosis in cows with reproductive proble.

All gram +ve bacteria isolated in this study were sensitive to Vancomycine, Gentamycine and Amoxyclave. On the other hand *Staphylococcus aureus* and *Staphylococcus epidermis* were sensitive to tetracycline but the *Streptococcus spp* had lower sensitivity to tetracycline. *Streptococcus fecalis* was sensitive to Ciprofloxacin, *Streptococcus pnuemone* and *Streptococcus pyogen* sensitive to Erythromycin. All gram +ve bacteria were resistant to Fusidic Acid, Chloroamphenicole and Penicillin G except *Streptococcus pyogen* which was sensitive Penicillin G.

Gram negative bacteria isolated from dairy cows, were sensitive to Ciprofloxacine, Ampcilline, Gentamycine and Cotrimoxazole but with lower sensitivity to Chloramphincole, Tetracycline, Nitrofurantole, Streptomycine and Colictin. *Escherchichia coli* were sensitive to Nalidixic.

DISCUSSION

The main objective of this study was to investigate the risk factors and bacterial infections associated with reproductive failure in dairy cows, The organisms isolated in this study from bovine reproductive tract were 39.1% *Staphylococcus spp*, (62.5% *Staphylococcus aureus* and 37.5% *Staphylococcus epidermis*), 27.2% *Streptococcus spp*, (54.7% *Streptococcus pyogen*, 24.7% *Streptococcus Pnemonea* and 20.6% *Streptococcus fecalis.*), (25.2%) *E.coli spp* and (8.5%) *psuedomonas spp*. This result was matching with the result obtained by (Sadig, 2010), who stated that the most common bacterium isolated from postpartum cow's vaginal swabs were *Staphylococcus spp*, *Streptococcus spp* and *E. coli*, he also reported that *Staphylococcus aureus* was the most common bacteria isolated in Khartoum University Farm in Sudan, that may be due to improper hygienic practices during milking or improper personal hygiene during parturition or placenta removal therefore *Staphylococcus aurous* play important role in reproductive failure. This finding was almost in line with other finding obtained by (Casarin *et al*., 2018), who evaluated the reproductive tract of slaughtered cows with vaginitis, cervicitis, and endometritis, the bacteria isolated in their study were, *Trueperella pyogenes*, *Escherichia coli*, *Enterococcus faecalis and Staphylococcus epidermidis*.

This study revealed that the major isolated bacteria from repeat breeder cows were *Staphylococcus spp*, *Streptococcus spp*, *E.coli spp* and *Psuedomonas spp*. This was partially in agreement with result reported by (Gani, *et al*., 2008), who stated that the bacteria isolated from uterine samples collected from cows suffering from repeat breeding were; *Staphylococcus* (predominant) followed by *Bacillus*, *E. coli* and *Pseudomonas.*

Sharma *et al.*, (2009), collected samples from the cervical mucus of repeat breeder cows in India; the dominant bacterial isolates were, *Escherichia coli, Staphylococcus aurous and Pseudomonas aeruginosa,* but they isolated other bacterial species such as Bacillus *spp., Proteus spp., Enterobacter spp.,* and *Corynebacterium spp.*

In this study the main isolated bacteria from reproductive infection cows were *Staphylococcus spp, Streptococcus spp, E. coli* and *Psuedomonas spp*, was similar to other results reported by (Konigsson, 2001). Also the current result was in line to other findings reported by (Williams, 2005, Seals, *et al.,* 2002, Knudsen, *et al.,* 2014 and Azawi 2008); they identified *Bacteroides spp., Fusobacterium necrophorum, Escherichia coli, Streptococcus spp., Clostridia spp., Pseudomonas aeruginosa,* and *Staphylococcus spp* as the bacteria most likely to be associated with bovine uterine infection.

In this study the seroprevalence of brucellosis using the Rose Bengal plate test (RBPT) was found to be 16.2%, This finding was lower than other result reported by (Salman and Nasri 2012) and (Angara, *et al.,* 2016) they showed that the prevalence rate of bovine brucellosis in Khartoum State was found to be 27.5% and 27.2% respectively, the lower prevalence rate in this study may be due to culling of cows with positive brucellosis and the improvement in the hygienic practices in dairy farms. In this study cows with reproductive problems got higher prevalence rate of brucellosis (20.8%) when compared to that randomly selected (11.4%), this difference was found to be statistically significant with ($p \leq 0.05$), and the relative risk of problematic cow to get brucellosis is twice the randomly selected cows, that means reproductive problems may be due to brucellosis, or infected cows were more sensitive to brucellosis than other non-infected cows. The seroprevalence of bovine brucellosis in the East Nile locality was higher 24.7%, than that in Bahri north locality 10.6%. This difference was of highly statistical significance; the risk of a cow to be serologically positive for brucellosis from East Nile is twice that of northern Bahri, This finding was disagreeing with (Angara *et al.,* 2016); they recorded the prevalence of bovine brucellosis in the Nile East locality was 23.4%, while that in Bahri North locality was 30.5%. Also the present result was not in line with the result obtained by (Elfadil, 2012), who reported the prevalence rate of bovine brucellosis in the East Nile locality 10.85%. the higher Sero-prevalence of bovine brucellosis in the East Nile locality compared to Bahri north locality, may be due to the fact that farmer in Bahri North were more educated and aware of the consequences of brucellosis than farmer in the East Nile locality, in addition most farms in East Nile complexes were crowded and more adjacent to each other and sometime use the same bull for insemination.

Tests for antimicrobial sensitivity in this study revealed that Gram-positive and Gram-negative isolates were sensitive to Gentamycine and Ciprofloxacin, this finding agrees with other result reported by (Fisher *et al*., 2005), they found that the Gentamycine appeared to be a rational antibiotic choice for intra uterine infusion and Enrofloxacin, one of the fluoroquinolones, is active against Gram-positive and Gram-negative organisms. Also agrees with results recorded by (Singh *et al.,* 2014 and Resum and Singh 2016), they reported that the Gentamycine, Enrofloxacin and Cephalexin antibiotics remain a drug of choice on the reproductive performance of repeat breeder cows.

In this study tetracycline was found to be of lower sensitivity in both Gram-positive and Gram-negative bacteria except for *Staphylococci*. This may be due to the excessive use of Tetracycline in dairy farms. Generally most farms that visited in this study uses Oxytetracycline to treat uterine infection via intrauterine infusion or intramuscular administration with lower doses for less than 3 days that emphasized lower sensitivity to Tetracycline in this study. This result was in agreement with the result detected by (Okker *et al*., 2002 and Smith and Risco, 2002), they suggests that parenteral Oxytetracycline is a poor treatment for postpartum metritis. In conclusion *Staphylococcus spp, Streptococcus spp* and *E. coli* were the most common bacterium isolated from cows with reproductive problem and brucellosis was higher in cows with reproductive problems.

REFERENCES

1. Alton GG, Jones LM, Angus RD, Verger JM (1988). Techniques for the brucellosis laboratory. Institut National de la Recherche.
2. Angara, T.E., Ismail, A.A, Ibrahim, A. M., Osman, S. Z. (2016). Assessment of the economic losses due to Bovine brucellosis losis in Khartoum State, Sudan, International Journal of Technical Research and Applications e-ISSN: 2320-8163, www.ijtra.com 4(2): 85-90.
3. Ariza, J., Cascio, A., Colmenero, J.D., Corbel, M. and Falagas, M.E., (2007). American Journal of Tropical Medicine and Hygiene, 15: 523-530.
4. Azawi, O. I., (2008). Postpartum uterine infection in cattle. Anim Reprod Sci, 105: 187–208. [PubMed].
5. Casarin J.B.S., Martini A.P., Trentin J.M., Fiorenza M.F., Pessoa G.A., Barros S.S. & Rubin M.I.B. (2018). Bacteriological, cytological and histopathological evaluation of the reproductive tract of slaughtered cows. *Pesquisa Veterinária Brasileira, 38(1): 53-58.* Departamento de Medicina de Grande Animais, Universidade Federal de Santa Maria, Avenida Roraima 1000, Santa Maria, RS 97105-900, Brazil. E-mail: juliaspreckelsen@gmail.com.
6. David, G. W., Richard, C.B., Slack, J, and Peuthere, F., (2002). A Medical Microbiology, 10th edition, 262-275.
7. Elfadil, M.H.M., (2012), Some infertility problems and their economical impacts in dairy farm in

Eastern Nile Locality, A Thesis for Master degree, Sudan University of Science and Technology.
8. Fisher, J.S., (2005). The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis.m Theriogenology, 63: 818–30.
9. Gani, M.O.; Amin, M.G.; Alam, M.G.; Kayesh, M.E.; Karim, M.R.; Samad, M.A. and Islam, M.R. (2008). Bacterial flora associated with repeat breeding and uterine infections in dairy cows. Bangl. J.Vet. Med., 6(1): 79-86.
10. Kather, N.Y., Hasan. A., SH., Dawood, W. S., and Mohammed, S., N., (2012). Bacterial flora isolated from genital tract of cows submitted for artificial insemination in Balad district, Kufa Journal for Veterinary Medical Sciences, (3): (1).
11. Kim, I.H., Kang, H.G., (2003). Risk factors for postpartum endometritis and the effect of endometritis on reproductive performance in dairy cows in Korea. J. Reprod Develop, 49: 485-491.
12. Knudsen, L. R. V., Schou, K. K., Jensen, T. K., & Angen, Ø. (2014). Molecular characterisation of the uterine microbiome of dairy cows suffering from endometritis, metritis, and pyometra. Kgs. Lyngby: Technical University of Denmark (DTU).
13. Konigsson, K. R., Gustafsson, H. and Kindhal, H. (2001). Clinical and bacteriological aspects on the use of oxytetracycline and flunixin in primiparous cows with induced retained placenta and post partum endometirity. *Reprod. Domest. Anim*, 36(5): 247- 256.
14. Monica, C., (2006). District Laboratory Practice in Tropical Countries, 2nd edition, 136-138.
15. Nasir, A.A., Perveen, Z., and Ikram-ul-Haq, M., (2005). Comparative study of standard and modified serum agglutination tests for diagnosis of Brucellosis in animals. Pakistan Vet. J., 25(1): 33-34.
16. Okker, H.; Schmitt, E.J. P.and Vos, L.A.M., Scherpenisse, P., Bergwerff, A.A, Jonker, F.H., (2002). Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous pos, tpartum administration in lactating dairy cows. J. Vet. Pharmacol Therap, 25: 33-38.
17. Resum E.C., and Singh J. (2016). Low prevalence of *Salmonella* in Swedish dairy herds highlights differences between serotypes. Prev Vet Med, 125: 38–45.
18. Ross, J.D.C., (2002). An update on pelvic inflammatory disease. Sex Transm Infect, 78: 18–19.
19. Sadig, N.B.M., (2010). Identification of Aerobic bacteria isolated from vagina of cross-bred dairy cows during early postpartum, A thesis of the Master degree department of microbiology faculty of veterinary Medicine, U of K.
20. Salman A. M and Nasri A. E., (2012). Evaluation of four serological tests to detect prevalence of bovine brucellosis in Khartoum State. Journal of Cell and Animal Biology, 15 May, 2012; 6(9): 140-143.
21. Salman, M. A., Elniema, A. M., Amona, M. H. and Lmyaa M. H., (2014). Application of Different Serological Tests for the Detection of the Prevalence of Bovine Brucellosis in Lactating Cows in Khartoum State, Sudan *Journal of Applied and Industrial Sciences, 2014; 2(5): 213-218, ISSN: 2328-4595 (PRINT), ISSN: 2328-4609 (ONLINE)*ssan4.
22. Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M. and Zinsstag, J., (2003). Brucellosis and Q – fever seroprevalances of nomadic pastoralists and their livestock in Chad. Prev. Vet. Med., 61: 279-293.
23. Seals, R.C., Matamoros, I., Lewis, G.S., (2002). Relationship between postpartum changes in 13, 14-dihydro-15-keto-PGF2alpha concentrations in Holstein cows and their susceptibility to endometritis. J Anim Sci., 80: 1068–1073. [PubMed]
24. Sharma, S., Singh, M. and Vasishta, N.K. (2009). Isolation and antimicrobial susceptibility of aerobic bacteria recovered from the uteri of dairy cows suffering from endometritis. *Indian J. Anim. Sci*., 79: 278-282.
25. Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., Schuberth, H.J., (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle, Biol Reprod, 8: 1025-1032.
26. Singh, C.S.P.; Singh, S.K. and Singh, B. (2014). Studies on incidence of infertility in cows. Indian Veterinary Journal., 58: 909-912 (veterinary Bulletin 51: 7266).
27. Smith, B.I. and Risco, C.A., (2002). Clinical manifestation of postpartum metritis in dairy cattle. Comp Contin Educ Pract Vet, 24: S56-S63.
28. Zinsstag, J., Schelling, E., Roth, F., Bonfoh, B., Savigny, D. and Tanner, M., (2007). 'Human benefits of animal interventions for zoonosis control', *Emerging Infectious Disease*, 13: 527–532. http://dx.doi.org/10.3201/eid1304.060381, PMid: 17553265.