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A BACTERIOLOGICAL STUDY ON BOVINE ENDOMETRITIS, WITH SPECIAL REFERENCE TO ITS TREATMENT WITH HONEY INFUSION

(With 4 Tables)

By

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دراسة بكتريولوجية عن الالتهابات الرحمية في الأبقار مع الإشارة إلي علاجها بعسل النحل

محمد محمد عبد الحفيظ ، حسين على عبد القادر ، أحمد مصطفي سيد ، شحاتة حسن شحاتة

بفحص عدد ٥٦٦ بقرة في مزرعتين للأبقار الحلابة بمحافظة أسيوط وجد عدد ٣٨ بقرة مصابة بالتهابات رحمية صديدية أو صديدي مخاطية. وقد أسفر الفحص البكتريولوجي عن عزل عدد ٤٤ عترة (٩١.٦%) للميكروب المكور العنقودى وعدد ٤ (٨.٣%) للميكروب المكور السبحي والتي تم عزلها في صورة مشتركة مع الميكروب المكور العنقودى. وبالتعرف على عترات المكور العنقودي وجد أنها كانت تمثل ٣١.٢% الميكروب العنقودي الذهبي ، ٢٩.١% الميكروب المكور العنقودى الابيدرميدس ، ١٨.٧٥% الميكروب العنقودي الانترميدكس ، ١٢.٥% الميكروب المكور العنقودي السابروفيتكس. وقد أسفرت نتائج اختبارات حساسية المضادات الحيوية عن أن السيفالوسين يليه حامض النالديكسيك كانا أعلي المضادات الحيوية حساسية ولكن أظهرت الاختبارات عدم فاعلية الديوريسيف ضد اغلب العترات المعزولة . وبالتحليل الكمي المعملي لعسل النحل أسفرت النتائج عن أقل تركيز للعسل مثبط لنمو العترات المعزولة كان ١٠% في حالات المكور العنقودي الذهبي و ٢٠% في بقية عترات المكور العنقودي. وبحقن العسل داخل أرحام مجموعتين من الحيوانات المصابة , كانت نسبة الإخصاب الكلية ٧٥% وكانت تمثل ٨٣.٨% في الحيوانات التي لم تتلق أية علاجات من قبل بينما مثلت ٧٠% في الحالات التي أظهرت مقاومة لفاعلية التتراسيكلين في حين كانت النسبة في مجموعة ضابطة لم تعالج بالعسل صفر% .

SUMMARY

Out of 566 lactating cows of two dairy farms in Assiut Governorate, 38 cows were suffering from purulent or mucopurulent endometritis. Bacteriological examination revealed the recovery of 44 (91.6%) Staphylococci strains and 4 (8.3%) streptococci strains; the latter were present in combination with *Staph.* sp. The *Staphylococcus* strains were identifed as *Staph.aureus* (31.2%), *Staph.epidemidis* (29.1%), *Staph. intermedicus* (18.75%) and *Staph. saprophyticus* (12.5%). *In vitro* antibiotic sensitivity testing to 13 antibiotics showed that cephalothin followed by naledixic acid were the most effective antibiotics against most of isolated strains, while duricef was the least effective. *In vitro* examination of the minimum inhibitory concentration (MIC) of the honey against the isolated staphylococci strains was 10% for Staph.aureus while for the other isolated strains was 20%. Three successive intra-uterine honey therapeutic infusions were given to two groups of cows with endomertritis and the conception rate (CR) of all the treated cows was 75%; CR of cows which did not receive any treatment before was 83.3%, that of resistant cows treated with tetracycline was 70% while that of control untreated cows was 0%.

Key words: *Bovine endometritis, honey infusion.*

INTRODUCTION

Endometritis is one of the major gynecological problems and among infectious causes of infertility in cattle, it ranks first in both cows and heifers (Anjaneyulu et al., 1999). Cows with a history of endometritis may show decreased productive performance (Dohmen et al., 1996). The economic viability of a dairy herd depends upon normal reproduction in these farm animals whereas the pathological changes in their reproductive tracts caused by microorganisms appear to be the main factor of infertility (Krishnan et al., 1994). When endometritis develops, conception rates will be impaired with significant correlation to bacteriological status (Takacs et al., 1990). Despite the widespread use of either local or systemic antibiotics, the rate of recovery from endometritis had not increased appreciably as none of these antibiotics is fully sensitive against the organisms causing endometritis (Hussain and

Daniel, 1991). The intra-uterine administration of a common antibiotic is used indiscriminately according to its availability with conflicting reports on its efficacy (Ambros and Pattabiraman 1993). However, when the causative organisms are resistant to these antibiotics, the presence of the drug in the uterus might suppress phagocytosis process enhancing the growth of the uterine pathogens (Mulei and Gitau, 1993). Besides most of the common used antimicrobial agents for the treatment of septic infections have adverse side effects (Hamdy et al., 1989). Such matter compelled some authors in several works to search for other natural antimicrobial agents without having side effects.

The use of honey as a therapeutic substance has been rediscovered by the medical proffessions and is widely documented as an antimicrobial agent which rapidly clears up the infection and promotes the healing process (Molan 1992). Honey provides an excellent treatment for local suppuration (Rahal et al., 1984 and Hamdy et al., 1989).

The present work aimed to recognize the bacterial pathogens causing endometritis in Frisian cows as well as to determine whether the antimicrobial activity of honey would overcome them and whether the intra-uterine (I/U) honey therapeutic infusion would increase the cow's conception rate.

MATERIAL and METHODS

1- Sampling, bacterial isolation and identification:

Rectal examination of 566 lactating Frisian cows belonging to two dairy farms in Assiut Governorate with natural serving management revealed that thirty eight cows were suffering from purulent or mucopurulent endometritis. According to Zemjanis (1970) and Mulei & Gitau (1993), the uterine infection was detected by observing the foul smelling vaginal discharges (purulent or mucopurulent) as well as, noticing the hard enlarged cervix and uterus was much thicker and firmer on the rectal examination.

Uterine swabs through rectovaginal technique were collected aseptically for bacteriological examinations. The swabs were inoculated into nutrient broth media which were acrobically incubated at 37°C for 24 h., then subcultures were made by streaking on both 10% sheep blood and Mac Conkey's agar media which were also aerobically incubated at 37°C for 24 h. The suspected colonies were identified morphologically by Gram's stain and biochemically according to Quinn

et al. (1994); catalase, coagulase and polymyxin resistance tests were applied for complete identification.

2- *In vitro* antibiotic sensitivity test:

It was carried out by the standard diffusion technique for the isolated strains against 13 different antibiotics [amikin (30 µg), cefobid (75µg), cephalothin (30µg), chloramphenicol (20 µg), duricef (30 µg), garamycin (30 µg), naledixic acid (30 µg), netlimycin (30 µg), polymyxin (30 µg), spectrama (10 µg), tetracycline (30 µg), tobramycin (30 µg), unasyn (20 µg)]

3- *In vitro* honey assessment:

50%, 20%, 10% and 5% concentrations of pure marketed cotton honey were prepared to determine the minimum inhibitory concentration (MIC) of honey adequate for complete growth inhibition of each isolate. The technique was applied according to White (1975).

4- I/U honey therapeutic infusion:

To evaluate the honey intra-uterine therapeutic infusion, twenty four cows with endometritis were devided to 3 groups.

A- Six cows which did not receive any treatment before

B- Ten cows which were formerly recieved available treatment (deep I/M oxytetracycline and I/U lotagen application) for long time without any improvment.

C- Eight untreated cows were considered as a control group.

I/U infusion of 100 ml pure cotton honey was applied for members of both A & B groups through the rectovaginal technique using a nelaton plast 40 cm-CH 22 catheter and its corresponding syringe. I/U catheter introducing was done by the aid of the metal lugol's catheter which was introduced into the whole length of plast catheter to be rigid and fit for I/U infusion easily applicable. I/U infusion was carried out day by day if needed and the uterus was still exuding purulent uterine discharges. All groups were followed up- five months- to estimate their conception rates.

RESULTS

The obtained results are illustrated in Tables 1 to 4.

DISCUSSION

Uterine bacterial infection in cows is the most important cause of infertility (Dholakia et al., 1987) which constitutes a great hazard after calving as 30% of cows still have moderate or serious infections at the end of involution (Takacs et al., 1990). Postpartum uterine infection in cows, as well as the abnormal parturition mostly can be followed by persistent endometritis (Hussain and Daniel 1991), but the role of different bactetria in pathogenesis of endometritis and their effect on reproduction are not fully understood (Dohmen et al., 1996). It is well established that great losses were associated with bovine endometritis. In Egypt, the economic losses resulting from cow and buffaloe infertility was found to be 100 million Egyptian pounds annually (Osman and Attalla, 1984).

Through the present study, cow purulent endometritis revealed isolation of 44 (91.6) *Staph.* sp. strains and 4 (8.3%) *Strept.* sp. strains which were found to be with *Staph.* sp. (Table 1). *Staphylococcal* endometritis was common in cattle (Luginbull, et al., 1981; Dholakia et al., 1987; Kudryavtsev et al., 1991; Krishnan et al., 1994 and Dohmen et al., 1996) while streptococcal cow endometritis was met with a good number of workers (Luginbull et al., 1981; Diker et al., 1989; Dholakia et al., 1987; Takacs et al., 1990; Bonnete et al., 1991; Mulei & Gitau 1993 and Dohmen et al., 1996). Osman et al. (1991) recorded that *Staph.aureus* together with *Strep.sp.* were the more predominant pathogens in buffaloe cows of infected preperium. They added that there was no differences between bacterial types isolated from genital tract of cows and that of buffaloes. In the present study three cows revealed negative bacterial isolation inspite of persistance of purulent vaginal or uterine discharges. The condition would be attributed to the fact that isolation of bacterial organisms from the inflammed cow uterus depends upon the stage of healing process during which micro-organisms would disappear first and later of the inflammatory signs resulting in sterile discharge (Bois, 1986).

Staphylococcal strains identification was achieved as 24 strains reacted as coagulase positive while the rest 20 strains were coagulase-negative. By polymyxin resistance test, coagulase-positive strains were identified as 15 (31.2%) *Staph.aureus* which were the most prevalent strains and 9 (18.75%) *Staph. entermedicus*, while those 20 coagulase-negative strains were identified as14 (29.1%) *Staph.epidermidis* and 6

(12.5%) *staph.saprophyticus* (Table 1). The presence of staphylococci themselves does not inevitably cause infection as the staphylococcal disease arises when the defensive mechanism of the cow is breached by hormonal inbalance-estrogen with bacteriostatic action giving rise to staphylococcal endometritis (Lamming et al., 1981). It is well established that Staph-coagulase positive strains are more virulent than others. Staphylococcal virulence cannot be explained in terms of a single factor; enzymatic or membrane damaging exotoxin amounts, the high dose of invading organisms and the low deffensive mechanism of the host to face this invasion which magnify the organism virulence (Anderson, 1986). So, the staphylococci coagulase negative would not be neglected in the current study. The role of coagulase-negative staph. would not be neglected in the current study. The present *in vitro* antibiotic sensitivity test revealed that *Staph.aureus* strains were sensitive to cephalothin followed by nalidixic acid, while *Staph.epidermidis* strains were sensitive to netlimycin followed by garamycin (Table 2). As regards *Staph.intermedicus* strains were highly sensitive to Cephalothin, naledixic acid, cefobid and polymyxin while *Staph.saprophyticus* stains showed high sensitivity to tobramycin, garamycin, polymyxin, netlimycin and amikin (Table 2). It is obvious from these findings that cephalothin, netlimycin and nalidixic acid were the most effective antibiotics. The use of cephalthin in the treatment of cow's endometritis was recommended by Sharma et al. (1993) and Abd-Elgwad et al. (2000), but its use in the veterinary field is unadvisable due to the high cost of treatment, as well as the frequency of its administration and milk disposal after treatment- until blood complete antibiotic withdrawal is achieved- which make its use uneconomic (Hussian and Daniel 1991). Tetracycline showed- in the present investigation- a moderate effect on most of the isolated strains contradicting with the findings obtained by Osman et al. (1991); Goswami et al. (1992) and Mulei and Gitau (1993). The authors concluded also that chloramphenicol showed a high sensitive reaction similar to that of tetracycline, while in the present study, chloramphenicol was less effective than tetracycline and the most resistant antibiotic was duricef. Penicilinase production-which is the most common form of staphylococcal antibiotic resistance-can interfere with the action of the semisynthetic penicillin or cephalosporins such as duricef (Anderson 1986). Therefore, staphylococcal antibiotic resistance against wide spectrum antibiotics is considered to be the major problem of staphylococcal endometritis; the condition which was faced through

the present work as the owner's complain was that many cows with endometritis were managed with available treatment (I/M tetracycline administration and I/U lutagen for long period), but they did not respond and the infection persisted.These chronic cases which resisted tetracycline antibiotic and lutagen antiseptic were infused I/U with honey. Honey prevented bacterial growth even where heavy infection was present and it was effective against antibiotic resistant bacterial strains (Molan, 1992). Its antimicrobial properties were shown to be more strong against cocci more than bacilli (Sedova and Usmanov, 1973) particularly against *Staph.aureus* which were widely documented (Bogdanov, 1984; Christov & Maldinov 1961; Mishref et al., 1989; Molan 1992 and Cooper et al., 1999). *Staph.aureus* is one of the most species sensitive to the antimicrobial activity of honey (Molan, 1992 and Cooper et al., 1999). Complete bactericidal action against *Staph.aureus* would be achieved by the use of 1.3% to 50% honey concentration (Mishref et al., 1989), while complete growth inhibition could be obtained by the use of 0.3% (Dustmann, 1979), 0.6% (Christov & Maldinov 1961), 10% (James et al., 1972), 50% (Jeddar et al., 1985) or 100% honey concentration. These differences may depend on the type of honey used as not all honey samples have the same degree of antimicrobial activity (Molan, 1992). Mountain honey has the strongest antimicrobial properties followed by honey from cotton (Sedova and Usmanov, 1973). Cotton honey was recommended also by Seleim et al. (1988). Honey's osmotic pressure is one of its antimicrobial activity by water withdrwal against bacteria. Also, its pH is low enough to slow down or prevent the growth of many species of bacteria (White, 1975), but hydrogen peroxide can be considered as the main substance responsible for the antimicrobial activity of honey. Its production is achieved when the honey is diluted by body fluids and occurs by the action of hydrogen peroxidase enzyme contained in honey (Bogdanov, 1984). The enzyme is inactive in undiluted honey, unchanged and suitable for storage for long periods if it is kept away from heat and light (Jeddar et al., 1985). Due to the previously mentioned marked variations, honey prepared for therapeutic use should be assayed for its antimicrobial activity before use (Molan, 1992). Honey used in the present study showed MIC as 10% against *Staph.aureus* and 20% against the other isolated *staph*.sp. strains (Table 3). These findings were greatly encouragable to I/U administration of honey especially it is more effective *in vivo* than *in vitro* as the dead leucocytes would release

hydrogen peroxide which would maximize the action of the catalase present in the honey (Effem, 1988). Also its I/U use has valuable advantage as it permits undisposal of milk yielded by the treated cows. Its success in therapy was judged not only by clearing up the infection but also referring to conception rate which showed 83.8%, 70% and 0% for groups A, B & C respectively (Table 4). It is clear that honey treated groups (A&B) showed satisfied conception rates especially group B which is the tetracycline resistant group, and the overall conception rate for both treated groups was 75% in contrast to the 0% of the untreated control group. Honey antimicrobial action appeared to be effective against antibiotic resistant strains of bacteria (Molan, 1998). Within group A, only one cow failed to get concieved although a single I/U honey therapeutic infusion was enough to stop the uterine discharge completely; it may had infertility problems other than uterine infection. In group B, three cows failed to get concieved, fattened and slaughtered. Three I/U honey infusions were not enough to clear up their uterine infections even those with negative bacterial isolation. As regards the 8 cows of the control group, one of them died three weeks after investigation, it was non pregnant. Two weeks later, another cow was accidentally harmed (metacarpal fractured) and emergency slaughtered which was also non pregnant and they were excluded as they were not followed up. The other 6 cows were not pregnant concluding conception rate of 0% five months after the investigation. Sharma et al. (1993) and Abd-Elgwad et al. (2000) concluded that conception rate after using cephalothin was 72%, while gentamycin showed 75% but reached 84% if it was administrated together with prostaglandin and livamisol. By the use of betadine, conception rate was about 59% (Mujuin et al., 1994 and Abd-Elgwad et al., 2000); the authors stated that it was the cheapest drug used with somewhat satisfactory results. However honey therapeutic use is more cheeper and more effective concerning to conception rates besides its excellent effect on tissue healing process. It also gives a fast rate of tissue regeneration and suppression of the inflammation, oedema and exudation. It has a direct nutrient effect on regenerating tissues as it contains a wide range of amino acids, vitamins and trace elements in addition to large quantities of readily assimilable sugars (Molan, 1998). Also it increases the glutathione content of tissue exudates which stimulates cellular growth and devision, and in this way it promotes the healing process (Hamdy et al., 1988). Suguna et al. (1993) concluded

that the rate of tissue contractions and the rate of epithelisation were increased significantly as a result of treatment with honey.

From the present results it is recommended to use I/U honey infusion routinely about a month post partum to diminish future bacterial uterine infection and avoid endometritis instead of the use of hazardous antibiotics especially these developing resistance.

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Table (1): Bacterial isolates from cases of cow's endometritis samples.

Lacatating cows	Cow with endomet-ritis		Bacterial isolates										
			Staph. sp.								Strept.sp		Total
			aureus		*epidermidis*		*intermedicus*		*saprophyticus*				
566	N	%	N	%	N	%	N	%	N	%	N	%	48
	38	6.7	15	31.2	14	29.1	9	18.7	6	12.5	4	8.3	

Table (2): Antibiogram of *Staphylococcus* species isolated from cow's endometritis

Antibiotic	*Staph. aureus* $n = 15$	*Staph. epidermidis* $n = 14$	*Staph. intermedicus* $n = 9$	*Staph. saprophyticus* $n = 6$
Amikin	ND	43%	ND	100%
Cefobid	47%	57%	100%	60%
Cephalothin	100%	29%	100%	ND
Chloramphenicol	20%	29%	86%	ND
Duricef	0%	0%	0%	100%
Garamycin	47%	71%	86%	100%
Naledixic acid	80%	29%	100%	40%
Netlimycin	67%	83%	28%	100%
Polymyxin	20%	0%	100%	100%
Spectrama	ND	50%	ND	50%
Tetracycline	47%	50%	86%	ND
Tobramycin	ND	43%	ND	100%
Unasyn	20%	29%	28%	60%

The inhibition zone more than 2 mm was considered as sensitive

ND = not done

Table (3): MIC of honey against Staphylococci isolated from cow's endometritis

Honey conc.	*Staph. aureus*	*Staph. epidermidis*	*Staph. intermedicus*	*Staph. saprophyticus*
50%	S	S	S	S
20%	S	S*	S*	S*
10%	S*	R	R	R
5%	R	R	R	R

MIC (the minimal inhibitory concentration) = S*

S = Sensitive and no bacterial growth

R = Resistant and bacterial growth developed

Table (4): Bacterial isolates and conception rates of Frisian cows with endometritis

S	Vaginal exudate & rectal examination	R. B	Bacterial isolates	H.R	Conception status	CR %	
A- Cows treated with honey without previous treatment n = 6							
1	P.E	2	*S.epidermidis*	twice	concieved		
2	P.E	2	*S. saprophyticus + St. sp.*	single	concieved		
3	P.E	12	*S.epidermidis*	single	concieved	83.8	
4	Cervicitis & P.E	7	*S.aureus*	twice	concieved		
5	Cervicitis & P.E	1	*S.saprophyticus*	twice	concieved		
6	Bloody P.E	1	*S.intermedicus + St. sp.*	single	failed		
B- Cows with antibiotic resistane (Tetracycline) endometritis n = 10							75
1	P.E	8	*S.saprophyticus*	triple	concieved		
2	P.E	2	*S.saprophyticus*	triple	concieved		
3	Mild P.E	8	*S.aureus + S.intermedicus*	triple	concieved		
4	Mild P.E	1	*S.epidermidis+S.intermedicus*	triple	concieved		
5	Mild P.E	2	*S.saprophyticus*	triple	concieved		
6	Cervicitis & P.E.	16	*S.epidermidis*	triple	concieved	70	
7	Cervicitis & P.E	2	*S.aureus + S.epidermidis*	triple	concieved		
8	P.E	3	*S.epidermidis*	triple	failed		
9	Brown bloody P.E	17	negative	triple	failed		
10	P.E	8	*S.intermedicus*	triple	failed		
C- Control untreated cows n = 8							
1	P.E	1	*S. aureus + S. intermedicus + st.sp*	-	failed		
2	Cervicitis & P.E	9	*S. epidermidis*	-	failed		
3	Cervicitis & P.E	2	*S. epidermidis*	-	failed		
4	Mild P.E	1	*S.epidermidis _ Strept. sp.*	-	failed		
5	Mild P.E	7	-ve	-	failed	0%	
6	Mild P.E	6	-ve	-	failed		
7	P.E	1	*S. aureus + S. epidermidis*	-	discarded		
8	Mild P.E	3	*S.aureus + S.intermedicus*	-	discarded		

R .B = duration of repeat breeder (before investigation) in months
H.R = repetition of intra uterine honey infusion
P.E = Exudative purulent endometritis
C.R = Conception rate