Studies on endometeritis in cows and honey as future alternative therapy

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Abstract
This study was conducted on 29 pleuriparous Friesian cows with a history of endometritis, kept at a private dairy farm at Kaliobia Governorate. Each cow was subjected to clinical, bacteriological, virogical and biochemical examinations. For field trials of treatment, cows were treated by oxytetracycline, the traditional method in the farm (Group A); gentamycin (Group B) and honey (Group C). Cows were re-examined after 14 days, inseminated at the 2nd estrus of treatment and pregnancy was checked after two months post-insemination. All cows were returned to the normal uterine condition except two cows of group A. From the bacteriological examination, there is a highly significant decrease in mean total bacterial count between each group before and after treatment; 70 strains were isolated and the most common of which were **Trueperalla (Arcanobacterium) (Actinomyces) pyogenes** (34.3%). Gentamycin was the most effective antibiotic for treatment of endometritis. The result of in vitro studies of honey showed that, it had a highly antibacterial effect on both Gram – positive and Gram – negative bacteria isolated.
From the virological examination, 4 swabs were positive for BHV-1 isolation on MDBK and immunofuorescent antibody test .Also, 16(50.1%) serum samples were positive for BHV-1 antibodies detection by ELISA test.
From the biochemical analysis of the uterine flushing and blood samples, there was an increase in the total proteins, AST, ALP, AP , bilirubin , LDH, ESR and TLC , meanwhile there was a decrease in TEC, Hb% and PCV in cases of endometritis. As the result of treatment, there was a decline in total bacterial count, the rate of bacterial isolation and the uterine flushing; blood parameters were returned to normal rates. Also, honey was much more efficient than gentamycin and the traditional methods of treatment without any side effects as emphasized by the increase of conception rates. The present study suggested that microbiological examination of the uterine discharge and biochemical analysis of the uterine flushings and blood are good tools for diagnosis of endometritis which mainly caused by infection with **T. (A.) pyogenes** that can be exaggerated by **BHV-1 virus** and could be controlled safely by intrauterine infusion of honey instead of the use of hazardous antibiotics. Moreover, we advise to use intrauterine infusion of honey routinely postpartum for nourishment of the myometrium; enhancement of myometrial contractility leading to effective drainage of the uterus and diminishing future bacterial uterine infection.

Key words: *klebsiella, chickens, polygalacturonase (pehX) gene, Antibiotic resistance.*

Introduction

Endometritis in cattle is a common problem of infertility caused mainly by specific or non – specific potential pathogens (**Dohmen *et al*., 1995 and Gilbert *et al*., 2005**). A wide variety of bacteria has been recovered from uteri of postpartum cows , but most of aerobic pathogens have little effect on fertility and **Trueperalla (Arcanobacterium) (Actinomyces) pyogenes** was the major one isolated that mayplay an important role in the pathogenesis of endometritis **(Maarouf and El-Bealawy,**

2005; Williams *et al*., 2005 and Dolezel *et al*., 2010).Meanwhile **Ziliatis *et al*.(2oo4)** revealed that **E.coli** usually asserts itself at the beginning of inflammation and together with endotoxins (lipopolysaccharide) facilitates a subsequent infection with **T.pyogenes** . Moreover, viral infection as **Bovine herpes virus -1 (Infectious pustular vulvovaginitis**) is associated with vaginitis ,endometritis , abortion and infertility in cattle **(Hafez *et al*., 1976 ; Madbouly and Hussien ,1997; Muylkens *et al*., 2007 and Nandi *et al*., 2009).**

The blood picture and biochemical profile are a mirror of the metabolic status and fertility of dairy animals **(Rowlands *et al*., 1977 and Abd El-Aziz *et al*., 2002)**.There is less information on the biochemical changes in the uterine flushings of cows with endometritis. So, *there* has been an increasing interest in defining the biochemical nature of the intraluminal environment of the bovine female genital tract; in which spermatozoa, ova and zygotes survive; maybe a good tool for diagnosis of reproductive disorders (**Poung *et al*., 1992).**

Many trials have been carried out for treatment of endometritis in cattle that aimed to reduce the load of pathogenic bacteria and enhance the process of uterine defense and repair. Some used the systemic and / or local treatment with antibiotics (**Amobros and Pattabiraman, 1993; Thurmond *et al*., 1993 and Galvão, 2009a)** and others used chemotherapeutic agents **(Koujan et al., 1996 and Dolezel *et al*., 2010)** . But the use of antibiotics and / or chemotherapeutics may retard the bovine leukocytic activity too much to have a detrimental effect on fertility (**Mulei and Gitau , 1993)** and the milk disposal after treatment make their use uneconomic . So, alternative therapies (biological sources) which stimulate the natural uterine defensive mechanisms have been suggested **(Asbury, 1992; Valdes *et al*., 1993; El-Azab and Maarouf, 2002 and Maarouf and El-Bealawy 2005).** The use of honey for controlling endometritis in cattle was selected on basis of its availability and its efficacy as antibacterial (**James *et al*., 1972; Molan, 1992; Anyanwn, 2011 and Sulaiman *et al*., 2012**), antifungal **(Crane, 1980 and Zighloul, 1990)** and antiviral **(Crane, 1980; Al-Wailli, 2004 and Ghapanchi *et al*., 2011)** as well as its previously use for controlling reproductive disorders in cattle **(Maarouf, 1998).**

Therefore, the present study was designed to determine the microbiological causes of endometritis in cows; the in vitro antibacterial activity of honey and also to evaluate its therapeutic efficacy in controlling endometritis in cattle, side by side to investigate some biochemical alterations in the uterine flushings and blood picture of cows for screening the endometritis cases.

Material and Methods

A total of 29 pleuriparous Friesian cows with a history of endometritis kept at a private dairy farm at Kaliobia Governorate were used in this study. Each cow was subjected to clinical gynaecological examination rectally and vaginally.

Two uterine swabs from each cow were collected for bacteriological examination following the technique adopted by **Noakes *et al*., (1989).** The first swab was immediately inoculated in 10 ml. sterile peptone water while the second into nutrient broth. Also, 29 vaginal swabs were taken with vigorously rubbed against vaginal mucosal surfaces suspended in phosphate buffer saline (PBS) for virological investigation. The swabs were transported to the laboratory in ice bag within 2-4 hr.

One ml from the tube containing swab with peptone water was taken into tube containing 9 ml peptone water, then serial dilutions were applied for total bacterial count flowing **Cruickshank *et al*., (1975).**

The broth tubes were incubated at 37^{o} C for 24 hr., then subcultured by streaking on the following media: nutrient agar; brain heart infusion agar with 10% sheep blood; McConkey's agar and blood agar . The plates were incubated aerobically at 37^{o}C for 24-72 hr., and then subcultured for colonies purifications. The purified colonies were identified morphologically

by Gram – stain and biochemically following **Holt *et al*., (1994) and Quinn *et al*., (1994).**
A mixture of 16 hours broth culture of strains isolated from each animal case was used to carry out the antibiotic sensitivity test following **Koneman *et al*., (1997).**The in-vitro antibacterial activity and the minimum inhibitory concentration of honey against isolated strains was applied **(Zighloul,1990** and **Maarouf, 1998**).
The vaginal swabs were inoculated into Madin -Darby Bovine Kidney cell line (MDBK) for virus isolation according to **Edwards's *et al*., (1983)** and **Brunner *et al*., (1988)** and the viral isolates were identified by direct immunofluorescence test following **Terpstrac (1979)** and **Moore *et al*., (2000).** Also, 29 serum samples from studied cows were used for antibody detection against Bovine herpes virus-1 by indirect ELISA following the **Institute Pourquier ELISA** kit**.**
A total of 29 uterine flushings from studied cows beside 10 normal ones were collected for biochemical analysis. The flushing was collected from each animal by infusing 40 ml of sterile normal saline into the uterus through Foley's balloon catheter, then collected in a sterile container by gently massaging the uterus .The flushings were centrifuged at 3000 rpm for 15 minutes to remove the cellular debris and the supernatant fluid was transferred for storage at -20 C until analysis.
The biochemical analysis included: The total proteins and bilirubin following **Doumas (1971) and Harrison and Barlow (1989)** as well as the enzymatic activities of a separate amino transferase (AST); alanine amino transferase (ALT); lactate dehydrogenase and alkaline phosphatase (AP) according to **Reitman and Frankel (1957); McQueen (1972) and Szasz (1976)** respectively. Blood samples were obtained from all cows (29 diseased and 10 healthy) for haematological examination: Total erythrocyte count (TEC); erythrocyte sedimentation rate (ESR); haemoglobin percentage (Hb %); packed cell volume (PCV) and total leukocyte count (TLC) were carried out as described by **Coffin (1953).**
For field trails of treatment, animals were assigned randomly into three groups viz : Group A, consisted of 9 cows treated by the traditional method in the farm by intrauterine infusion (I/U) of 0.5 gm oxytetracycline in 20 ml sterile water for successive 4 days. Group B, consisted of 11 cows treated by the I/U infusion of 40 ml gentamycin 10% in 3 doses with 3 days interval. Group C, consisted of 9 cows treated by the I/U infusion of 100 ml pure honey in 3 doses with 3 days interval. Beside topical application of pure honey on vaginal erosions and ulcers.
After 14 days of treatment, clinical, bacteriological; virological and biochemical examinations were reapplied as described above. Animals were inseminated artificially in the second estrus after treatment and the pregnancy was estimated rectally after two months of the last insemination.
The obtained data was statistically analyzed according to **Sendecor and Cochran (1980)** using the computer software program **(SPSS, 2001).**

Results

The clinical examination showed that all studied cows with endometritis of 2nd and 3rd degrees were characterized by an enlarged cervix, thick, firm uterine wall (rectally) and congested cervix, profuse, turbid mucopurulent to purulent sometimes with foul smelling uterine discharge, beside 4 cases showed erosions and ulcers on vagina (vaginally). After treatment, all cows returned to the normal uterine condition as presented by the soft consistency and slight turgidity of uterine wall, beside the disappearance of abnormal discharges except two cows of group A.
As shown in Table (1), there is a highly significant decrease in mean total bacterial count between each group before and after treatment. Table (2) revealed that, a total of 70 strains were isolated from 29 studied cows and the common strains were **Trueperalla** (**Arcanobacterium) (Actinomyces) pyogenes**

(34.3%) meanwhile, **E.coli; Staphylococcus aurus; Streptococcus pyogenes; Proteus vulgaris.; Pseudomoneus aeruginosa** and **Klebsiella pneumonae** were isolated in lower incidences (15.7%;12.9%;12.9%;10.0%;7.1% and 7.1%) respectively. So , **T**.(**A). pyogenes** was considered as the most important pathogenic bacteria in cases of endometritis.

From Table (3), it appears that gentamycin was the most effective antibiotic (82.8%) followed by enrofloxacin (75.9%) and lincomycin (72.4%) whereas erythromycin was the lowest effective one (34.5%). Gentamycin was the antibiotic of choice used for treatment of the 2nd group (B).

The results of in vitro studies of honey showed that pH values of honey was 3.9 and proved to have highly antibacterial effect as it gave (++++) zone of inhibition . Moreover, Table (4), showed that honey had an antibacterial effects against the isolated bacteria and the least concentration 1% was ineffective, MBC of honey for **E. coli, Strept. pyogenes, Staph. aureus, pr. vulgaris and Kl. pneumonae** was 5% while for **T**. (**A.) pyogenes and Ps. aeruginosa** was 10% after which further dilution resulted in no growth.

The results of virological examination Table(5) showed that out of 29 vaginal swabs 4(1.16%) were positive for virus isolation on MDBK,where the CPE characterized by grape like clusters of rounded cells gathered around a hole in the monolayer and sometimes giant cells with several nuclei maybe observed. These four isolates were positive by direct immunofuorescent antibody test.Also,16(50.1%) serum samples out of 29 ones were positive for BHV-1 antibodies detection by ELISA test (Table,6).After treatment, no CPE were detected on MDBK cell layer.

The results of biochemical examination (Table, 7) showed a significant increase in the total proteins, AST, ALT, AP, bilirubin and LDH activity in uterine flushings of cows affected with endometritis when compared to those of healthy controls. Moreover, Table (8) appeared a high significant increase in ESR and TLC but there is a significant decrease in TEC, Hb% and PCV in diseased cows before treatment when compared with healthy control ones. After treatment , the parameters were returned to normal levels and honey was more efficient than oxytetracycline and gentamycine .

Table (9), showed that the rate of bacterial isolation significantly declined from a total of 70 strains before to 17 strains after treatment.

The efficacy of the three ways of treatment were evaluated depending upon: The decrease in mean total bacterial count after treatment, where there is a significant decrease of mean TBC between group A($40.18 \pm 9.36 \times 10^3$) and group C ($13.56 \pm 5.05 \times 10^3$) meanwhile, there is no significance difference between group B ($22.59 \pm 8.56(\times 10^3)$ group C(Table,1); the decrease in number of isolates in each group after treatment, such efficacy was 45.8% for group (A), 90.9% for group (B) and 91.7% for group (C) as shown in Table (10)and also, the results of biochemical analysis after treatment, as the parameters were returned to nearly normal levels and honey was efficient than gentamycin and oxytetracycline. Moreover, Table (11) appeared that the conception rate was higher in group C (77.8%) than that in B (72.7%) and significantly higher than group A (44.4%).

Table (1). Mean total bacterial count (TBC) in 29 studied cows (before and after treatment) and efficacy of different treatment.

Animal groups	No.of cows	Mean TBC before treatment ($\times 10^7$)	Mean TBC after treatment ($\times 10^3$)
Oxytetracycline group(A)	9	52.67 ± 4.53	40.18 ± 9.36***a
Gentamycin group	11	49.91 ± 3.19	22.59 ± 8.56***ab
Honey group(C)	9	46.67 ± 4.59	13.56 ± 5.05***b

***high significance decrease between before and after treatment in each group at $p < 0.05$.
Different litters means significance between different groups

Table (2). Total number and percentage of bacterial species isolated from 29 cows with endometritis.

Bacterial species	Number	Percentage
Trueperalla pyogenes	24	34.3
E. coli	11	15.7
Staphylococcus aureus	9	12.9
Streptococcus poygenes	9	12.9
Proteus vulgaris	7	10.0
Pseudomoneus aero-	5	7.1
Klebsilla pneumonae	5	7.1
Total	70	100.0

* Percentage in relation to total number of isolated bacteria.

Table (3). In vitro efficiency of the different antibiotics in treatment of endometritis**.**

Antibiotics	Sensitivity		Resistivity	
	No.	%*	No.	%*
Gentamycin	24	82.8	5	17.2
Enrofloxacin	22	75.9	7	24.1
Lincomycine	21	72.4	8	27.6
Amoxicillin	17	58.6	12	41.4
Ampicillin	17	58.6	12	41.4
Doxycycline	16	55.2	13	44.8
Colistine	14	48.3	15	51.7
Oxytetracy-cline	14	48.3	15	51.7
Streptomy-	13	44.8	16	55.2
Erythromy-	10	34.5	19	65.5

* Percentage in relation to total number of bacterial mixture isolated from 29 cows

Table (4). Minimum bactericidal concentration (MBC) of honey on different isolates.

Isolated strains	Concentration of honey				
	1%	5%	10%	15%	50%
Trueperalla pyogenes	+	+	+	_	_
E. coli	+	+	_	_	_
Staphylococcus aureus	+	+	_	_	_
Streptococcus poygenes	+	+	_	_	_
Proteus vulgaris	+	+	_	_	_
Pseudomoneus aero-	+	+	+	_	_
Klebsilla pneumonae	+	+	_	_	_

Table (5). Viral isolation on MDBK cell culture and identification of the viral isolate by direct fluorescent antibody technique.

No. of samples	Virus isola-tion	%	Direct fluores-cent	%
29	4	1.16	4	1.16

Table(6). Distribution of antibodies to BHV-1 by using Institute Pourquier ELISA kit:

No. of samples	No. of +ve samples	% of +ve samples
29	16	55.1

S/P = 100 (OD sample-OD negative/OD positive-OD negative)

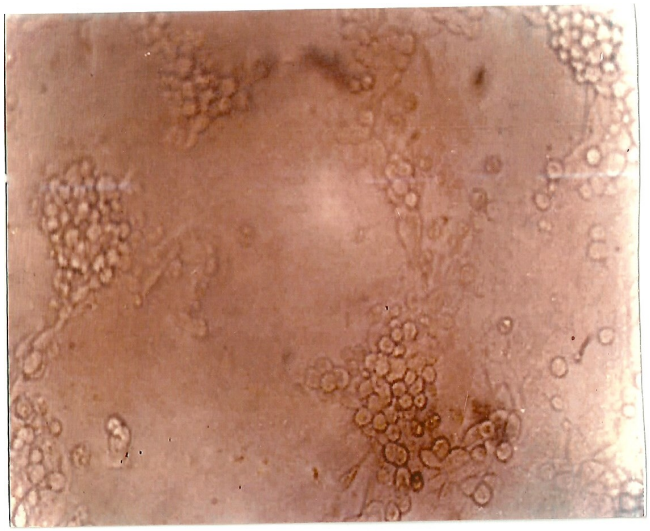

Figure (1). MDBK cell culture showing CPE of BHV-1 (grape like appearance)

Figure (2). Identification of BHV-1 by fluorescent antibody test.

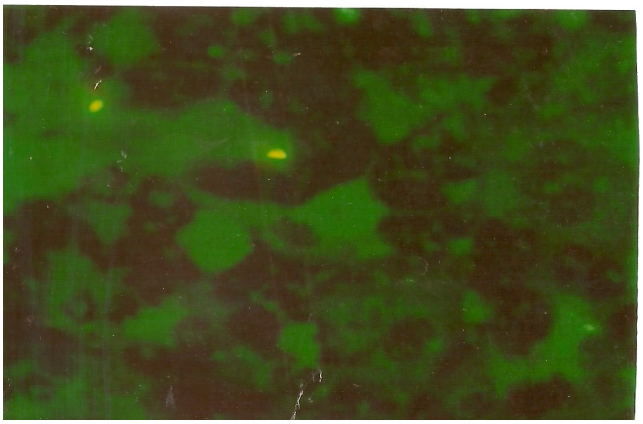

Table (7). Biochemical analysis of uterine flushings before and after treatment of 29 cows with endometritis.

parameters	Before treatment		After treatment		
	Healthy Control	endometritis	oxytetracycline	gentamycine	honey
Total protein gm/dl	4.58 ± 0.09 [b]	5.66 ± 0.05 [c]	5.86 ± 0.05 [c]	5.16 ± 0.09 [b]	4.54 ± 0.10 [a]
AST u/L	28.96 ± 0.35[a]	47.84 ± 0.89[b]	27.52 ± 0.73[a]	27.00 ± 0.91[a]	26.48 ± 0.95[a]
ALT u/L	14.46 ± 0.23[a]	31.36 ± 0.22[b]	15.05 ± 0.12[a]	14.90 ±0.48[a]	14.62 ± 0.33[a]
ALP u/L	77.18 ± 0.45[a]	94.26 ± 0.93[c]	79.28 ± 0.55[b]	76.9 ± 0.6 [a]	76.9 ± 0.68 [a]
Blirubin mg/dl	3.2 ± 0.08 [a]	4.3 ± 0.09 [b]	3.6 ± 0.1 a	3.3 ±0 .09[a]	3.1 ± 0.08[a]
LDH u/L	277.2 ± 1.50[a]	332.2 ± 1.95[b]	270.4 ± 2.04[a]	273.4 ± 2.77[a]	280.6 ± 1.81[a]

abc Values within the same raw followed by different superscript letters were significantly different ($P \leq 0.05$) , while values within the same raw followed by the same superscript letter were not significantly different ($P \leq 0.05$) .

Table (8). Haematological analysis of 29 cows with endometritis before and after treatment.

parameters	Before treatment		After treatment		
	Healthy control	endometritis	oxytetracycline	gentamycine	honey
ESR mm/24hr	7.74 ±0.20 [a]	14.88 ± 0.27 [b]	8.02 ± 0.19 [a]	8.24 ± 0.25 [a]	7.56 ± 0.20 [a]
Hb gm%	13.7 ± 0.18 [c]	9.7 ± 0.10 [a]	11.56 ± 0.22 [b]	11.64 ± 0.17 [b]	12.5 ± 0.16 [c]
PCV %	42.24 ± 0.53 [c]	37.86 ± 0.47 [a]	39.12 ± 0.49 [b]	41.38 ± 0.58 [c]	42.8 ± 0.86 [c]
TEC million/ul	5.72 ± 0.12 [a]	4.78 ± 0.09 [b]	5.65 ± 0.09 [a]	5.90 ± 0.08 [a]	5.78 ± 0.09[a]
TLC thousand /ul	5.65 ± 0.07 [a]	6.99 ± 0.09 [b]	5.82 ± 0.18 [a]	5.55 ± 0.07 [a]	5.76 ± 0.06 [a]

abc Values within the same raw column followed by different superscript letters were significantly different ($P \leq 0.05$), while values within the same raw followed by the same superscript letter were not significantly different ($P \leq 0.05$).

Table (9). Efficacy of treatment of 29 cows, in relation to total number of isolates.

Bacterial isolates	Before treatment			After treatment		
	No.	%*	%**	No.	%*	%**
Trueperalla pyogenes	24	82.8	34.3	9	31.0	12.9
E. coli	11	37.9	15.7	2	6.9	2.9
Staphylococcus aureus	9	31.0	12.9	2	6.9	2.9
Streptococcus poygenes	9	31.0	12.9	3	10.3	4.3
Proteus vulgaris	7	24.1	10.0	1	3.0	1.4
Pseudomoneus aerogenosa	5	17.2	7.1	0	0.0	0.0
Klebsilla pneumonae	5	17.2	7.1	0	0.0	0.0
Total	70		100.0	17		24.3

* Percentage in relation to total number (29) of studied cows .
** Percentage in relation to total number (70) of isolated strains

Table (10). Efficacy of different treatments on the number of isolates.

Animal groups	Number of isolates		Efficacy		
	Before treatment	After treatment	No	%*	%**
Oxytetracycline group(A)	24	13	11	45.8	15.7
Gentamycin group(B)	22	2	20	90.9	28.6
Honey group(C)	24	2	22	91.7	31.4
Total	70	17	53	75.7	75.7

* Percentage in relation to total number of isolates in each group.
** Percentage in relation to total number (70) of isolated strains before treatment

Table (11). Effect of different treatment of endometeritis on the conception rate .

Animal groups	Total cases	Pregnant cases	Conception rate
Oxytetracycline group(A)	9	4	44.4
Gentamycin group (B)	11	8	72.7
Honey group(C)	9	7	77.8
Total	29	19	65.5

Discussion

Endometritis constitute a major problem hindering occurrence of pregnancy in bovine. The present study revealed that microbial infection in cows was the most important cause of infertility resulted in persistent endometritis; clinically diagnosed by the presence of erosions and ulcers on the vulva, pathological discharge from the cervical orifice and biochemically by alterations of blood constituents and uterine flushings. Through the present study, cows with endometritis revealed a high significant decrease in mean total bacterial count before and after treatment. A total of 70 strains were isolated from 29 studied cows and the common strain was **Trueperalla (Arcanobacterium) (Actinomyces) pyogenes** (34.3%) meanwhile, **E.coli; Staphylococcus aureus; Streptococcus pyogenes; Proteus vulgaris; Pseudomoneus aeruginosa** and **Klebsiella pneumonae** were isolated in lower incidences. Similar results were previously reported by **Dohmen *et al*., (1995); Maarouf and El-Bealawy (2005); Williams *et al*.,(2005);Foldi *et al*., (2006); Yavari *et al*., (2007); Petit *et al*., (2009) and Dolezel *et al*., (2010).** Although **E.coli, Staphylococci and Streptococci** have been reported to be the most important pathogens in cases of endometritis (**Noakes *et al*., 1991; Anjaneyulu *et al*., 1999; Abd El-Haffez *et al*., 2001 and El-Azab and Maarouf, 2002**). The present study suggested that **T.** (**A.**) **pyogenes** is the primary pathogen for endometritis, while others maybe due to unhygienic conditions of cows. This finding came in harmony with **Farin *et al*., (1989); Dohmen *et al*., (1995); Maarouf and El-Bealawy, (2005) and Dolezel *et al*., ((2010)**.Thus; the presence of this bacterium can be considered as an indicator of pathological condition in the bovine uterus.

Gentamycin appeared in the present study as the most effective antibiotic in cases of endometritis (Table, 3). This finding came in consistence with some previous studies **(Sharma *et al*., 1993 and Maarouf and El-Bealawy 2005).**

The in vitro study of honey proved that it had highly antibacterial effect on bacterial mixture of both Gram – positive and Gram – negative bacteria causing endometritis. It had antibacterial activity against **E. coli**, **Strept. pyogenes**, **Staph. aureus**, **Pr. vulgaris** and **Kl. pneumonae** till 5% and 10% concentration for **T**. (**A.) pyogenes** and **Ps. aerogenosa**, where further dilution results in no growth inhibition. These results came in harmony with previous studies **(James *et al*., 1972; Jeddar *et al*., 1985; Zighloul, 1990; Molan, 1992a; Maarouf, 1998; Abd El-Hafeez *et al*., 2001; Anyanwn, 2011 and Sulaiman *et al*., 2012).** However, the antibacterial activity of honey maybe attributed to the hydrogen peroxide produced and accumulated in diluted honey either with water or tissue fluids by honey enzyme glucose oxidase during its action on honey glucose to form gluconolacton **(Jedder *et al*., 1985; Molan, 1992a; Kačániová *et al*., 2011);** the high osmotic pressure and potassium content of honey that leads to withdrawal of water and plasmolysis of the organisms (**James *et al*., 1972 and Sulaiman *et al*., 2012**) and its phytochemical nature, which include its content of peroxides, amylose, fatty acids, phenols, ascorbic acid, decanoic acid terpenes, benzyl alcohol and benzoic acid which exhibit strong antibiotic activity against bacterial and fungal infections (**Crane, 1980; Bogdanov, 1989 and Molan, 1992a).**

Out of 29 vaginal swabs, 4 swabs showed the characteristic CPE by propagation on monolayer of MDBK cells and CPE became more pronounced after short time by excessive propagation on the cells and per nuclear yellowish green fluorescent granules was detected by FAT (Table, 5**).**Similar results were reported by **Hafez *et al*., (1976) and Madbouly and Hussien (1997**). **BHV-1** antibodies were detected in 16(55.1%) of serum samples by indirect ELISA (Table, 6). These results came in harmony with **Islas (2003) and Boelaert (2005).** The results maybe attributed to viral exposure, recent recovery or latent infection without clinical signs "normal criteria that

mayrecur under stress conditions of transportation; bad hygiene or bacterial infections **(Smits *et al*., 2000; Koeijerder *et al*., 2008 and OIE, 2010).**

The total protein concentration in uterine flushings of cows with endometritis was significantly higher than that of healthy ones (Table, 7). This might be due to an increase in the level of secretory proteins and tissue damage during the course of endometritis. The same results were previously reported **(Rao and Seshagiri, 1998 and Maarouf and El-Bealawy, 2005).** Also, levels of , AST, ALT, AP , bilirubin and LDH were higher in the uterine flushings of cows with endometritis compared to those in the normal cases. Nearly similar results were recorded by Boos et al.(1988); **Zain El-Din *et al*., (1996); Abd El-Aziz *et al*., (2002)** ; **Maarouf and El-Bealawy (2005) and Pariza *et al*., (2009).** The increase in enzymatic activities might be due to leakage of more amount of enzymes from inflamed and necrotic tissues into circulation and uterine lumen (**Coles, 1985**; **Boos *et al*., 1988 and Zain El-Din *et al*., 1996)**. Moreover, the results of haematological examination, Table(8) showed a high significant increase in ESR and TLC meanwhile ,there was a significant decrease in haemoglobin % and PCV in diseased cows before treatment compared to those of healthy control ones. These results agreeded with that reported by **Islam *et al*., (1999) and Pariza *et al*., (2009).**

Regarding the effect of treatment on mean total bacterial count and bacterial isolates, there was a highly significant decrease in mean total bacterial count and decline in the rates of isolation of different bacterial strains following treatment than before (Table, 1and 9). Moreover, our results (Tables, 7, 8 and 10), illustrated the efficacy of gentamycin over the tradional method of treatment in the farm. Also, it was obvious that honey was much more efficient than gentamycin without any side effects. This suggestion was emphasized by the results obtained with conception rate (Table, 11). In addition, the pronounced improvement in the conception rate after intrauterine infusion of honey may be due to its antibacterial and antiviral activities **(Crane, 1980; Al-Wailli, 2004 and Ghapanchi *et al*., 2011);** its acidic pH (3.9) may correct pH of healthy bovine uterus 5.5-7.0 and in case of endometritis became 7.5-8.5" and kill the bacteria **(El-Doker,1984)** ;it may be stimulate the granulation tissue formation and regeneration for healing the microabrasions that may be present in uterus and its content of sex hormones as estrogen, oxytocin and prostaglandin that exerting a pronounced influence on uterine resistance against infection by increasing blood flow , enhancement of myometrial contractility leading to effective drainage of the uterus**(El-Doker, 1984; Zighloul, 1990; Badaway, 1994 and Maarouf, 1998).** All these factors might act synergistically together to form micro environment that favours the effectiveness of uterine immune response and inhibition of microbial growth that strongly enhanced clinical response and recovery of the cases.

It is obvious from this investigation that microbiological examination of the uterine discharge and biochemical analysis of blood and uterine flushings are good tools for diagnosis cases of the endometritis which mainly caused by infection with **T**. (A**.) pyogenes** that can be exaggerated by **BHV-1** virus and could be controlled safely by intrauterine infusion of honey instead of the use of hazardous antibiotics. Moreover, we advise to use intrauterine infusion of honey routinely postpartum for nourishment of the myometrium; enhancement of myometrial contractility leading to effective drainage of the uterus and diminishing future bacterial uterine infection.

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