

# Intrauterine honey infusion in Holstein Frisian cows with purulent endometritis

## Abstract

Endometritis is one of the major gynecological problems affecting reproductive performance and economy of milk production in dairy cows. Since multidrug resistant bacteria (MDR) is widely global spread, alternative therapeutic agents is urgently needed. By the gynecological investigation of 566 Holstein Frisian lactating cows, 38 suffering from repeat breeder (RB) were showing purulent exudative endometritis (PE). Different *Staphylococcus* and *Streptococcus spp.* were isolated, identified and subjected to antimicrobial assessment, where all tested strains were MDR with overall multi antimicrobial resistance index (MAR) 0.42, while when be tested against Egyptian cotton honey achieved only 10 & 20% minimum inhibitory concentration (MIC). Twenty four cows of the affected cows were included in the study were divided to three groups; (A): 6 cows did not receive any previous treatment, while (B): 10 cows did not respond to long different classic treatment and (C): 8 cows did not receive any management and left as a control group. Both groups A & B received I/U of 100 ml of full strength Egyptian cotton honey infusion day /day for three successive infusions until purulent exudate clearance or up to three successive infusions. Success results were judged by conception rate (CR), where it was 80% for group A and 70% for group B with overall conception rate for all honey treated cows of 75%. It was concluded that I/U honey is a very effective successful management for bovine PE especially those antibiotic none-responding since it would clear the pyogenic infection with high conception rate.

**Keywords:** endometritis, *Staphylococcus*, intrauterine infusion, honey

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**Abbreviations:** MDR, multidrug resistant bacteria; RB, repeat breeder; PE, purulent exudative endometritis; MAR, multi antimicrobial resistance index; MIC, minimum inhibitory concentration; CR, conception rate

## Introduction

Endometritis is highly prevalent disease where approximately 40% of dairy cows develop a uterine disease<sup>1</sup> having a substantial influence on bovine health and reproductive performance,<sup>2</sup> with significant sizable economic impacts and lowering profitability to the dairy industry.<sup>3</sup> It is frequently treated with antibiotic intrauterine infusion, but with the emergence of MDR bacterial strains the alternative antimicrobial therapy for bovine metritis and endometritis is required.<sup>3,4</sup> Several therapeutic alternative agents proved *in vitro* antimicrobial activity were recommended to be tried as intrauterine infusion such as lactic acid,<sup>4,5,6</sup> herbal essential oil extracts (garlic,<sup>7</sup> neem, ashwagandha, turmeric,<sup>8</sup> tulsi and giloy,<sup>2</sup> hyper immune serum,<sup>9-11</sup> endotoxins such as lipopolysaccharide of *E. coli*,<sup>9,10,12</sup> low dilutions of H<sub>2</sub>O<sub>2</sub>,<sup>13-15</sup> chitosan<sup>16</sup> or silver nanoparticles.<sup>3</sup> Honey with the wonderful broad spectrum antimicrobial action<sup>17-19</sup> was tried in mares.<sup>20</sup> The present work aimed to study the potency of honey against pyogenic MDR endometritis pathogens as well as its influence for reproductive re-performance and conception rate post purulent endometritis in dairy Holstein cows.

## Material and methods

Sampling, bacterial isolation and identification: Theriogenological rectal investigation of total 566 lactating Frisian cows belonging to

two automatic milking dairy farms in Assiut Governorate revealed that thirty eight cows of them were suffering from exudative purulent or mucopurulent endometritis. Uterine swabs were collected aseptically for bacteriological examination and inoculated into nutrient broth media which were incubated aerobically at 37°C for 24Hs. The incubated broth was subcultured by streaking on both 10% sheep blood and Mc Conkey's agar plates which were incubated aerobically at 37 °C for another 24 Hs. The suspected colonies were identified morphologically and biochemically.<sup>21</sup>

## *In vitro* antimicrobial resistance study

Antimicrobial sensitivity testing was adopted against 13 different antimicrobial agents.<sup>22</sup> Resistance was judged by the inhibition zone diameter  $\phi$  to calculate the MAR values.<sup>23</sup>

## *In vitro* honey minimum inhibitory concentration (MIC) assessment

Pure unprocessed Egyptian cotton honey was diluted with sterile nutrient broth to prepare four dilutions (50,20,10 and 5%) freshly prepared just before isolated strain broth inoculation to determine its honey MIC.

## I/U honey therapeutic infusion

Twenty four lactating Frisian cows with exudative PE with different RB periods were divided to 3 groups; (A): 6 cows which did not receive any treatment, (B): 10 cows which formerly received oxytetracycline deep I/M and I/U lotagen application without any improvement and (C) 8 cows were left without interference to be considered as a control group.

Cows of both (A) and (B) groups were received I/U infusion of 100ml of pure full strength honey through rectovaginal technique using a sterile nelaton plast 40cm –CH 22 catheter which be introduced by the aid of sterile metal Lugol’s catheter. I/U infusion were carried day by day up to cessations of purulent exudate or up to three successive applications. All studied cows were followed up to five months to estimate their conception rates.

## Results

Bacterial isolation was manifested in Table 1, while their antimicrobial resistance pattern and honey MIC against them were shown in Table 2. Individual cow data (clinical exudate, duration of RB, etiological pathogens, replicate of honey intrauterine infusion and treatment success through conception rates) were illustrated in Table 3.

**Table 1** Bacterial isolates form purulent endometritis (PE) exudates

Investigated lactating cows	Cows with PE	Bacterial isolated strains					
		Total	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus intermedicus</i>	<i>Staphylococcus saprophyticus</i>	<i>Streptococcus Spp.</i>
566	38	48	15	14	9	6	4

**Table 2** Antimicrobial resistance pattern of different isolated *Staphylococcus spp.* strains and MIC of honey against them

Antimicrobial agent	Percentages of <i>Staphylococcus spp.</i> antimicrobial resistance			
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. intermedicus</i>	<i>S. saprophyticus</i>
Amekin 30µg	ND	57	ND	0
Cefobid 75µg	53	43	0	40
Cephalothin 30µg	0	71	0	ND
Chloramphenicol 20µg	80	71	14	ND
Duricef 30µg	100	100	100	0
Garamycin 30µg	53	29	14	0
Naledixic 30µg	20	71	0	60
Netlimycin 30µg	33	17	72	0
Polymyxin 30µg	80	100	0	0
Spectrama 10µg	ND	50	ND	50
Tetracycline 30µg	53	50	14	ND
Tobramycin 30µg	ND	57	ND	0
Unasyn 20µg	80	71	72	40
MAR index	0.70	0.61	0.30	0.10
MIC of Cotton honey	0.42			
	10%	20%		

ND, not done

**Table 3** Bacterial etiological agents of cow E.P. and influence of I/U Egyptian honey infusion management

Therigenological examination & vaginal discharges		RB (month)	Bacterial Isolates	I/U honey	Conception status	Conception Rate	
<b>Group A: Cows received I/U honey infusion without previous treatment</b>							
1	PE	2	<i>S. epidermidis</i>	Twice	Conceived		
2	PE	2	<i>S. saprophyticus</i> + <i>Strept sp.</i>	Single	Conceived		
3	PE	12	<i>S. epidermidis</i>	Single	Conceived		
4	Cervitis & PE	7	<i>S. aureus</i>	Twice	Conceived	83.8%	75%
5	Cervitis & PE	1	<i>S. saprophyticus</i>	Twice	Conceived		
6	Bloody PE	1	<i>S. intermedicus</i> + <i>Strept sp.</i>	Single	Failed		
<b>Group B: Cows received I/U honey infusion post long antibiotic treatment</b>							
7	PE	8	<i>S. saprophyticus</i>		Conceived		
8	PE	2	<i>S. saprophyticus</i>		Conceived		
9	Mild PE	8	<i>S. aureus</i> + <i>S. intermedicus</i>		Conceived		
10	Mild PE	1	<i>S. epidermidis</i> + <i>S. intermedicus</i>		Conceived		
11	Mild PE	2	<i>S. saprophyticus</i>		Conceived		
12	Cervitis & PE	16	<i>S. epidermidis</i>		Conceived		
13	Cervitis & PE	2	<i>S. aureus</i> + <i>S. epidermidis</i>		Conceived	70%	
14	PE	3	<i>S. epidermidis</i>	Triple	Failed		
15	Brown bloody PE	17	Negative		Failed		
16	PE	8	<i>S. intermedicus</i>		Failed		
<b>Group C : Control untreated cows</b>							
17	PE	1	<i>S. aureus</i> + <i>S. intermedicus</i> + <i>Strept sp.</i>		Failed		
18	Cervitis & PE	9	<i>S. epidermidis</i>		Failed		
19	Cervitis & PE	2	<i>S. epidermidis</i>		Failed		
20	Mild PE	1	<i>S. epidermidis</i> + <i>Strept sp.</i>		Failed		
21	Mild PE	7	Negative		Failed		
22	Mild PE	6	Negative	untreated	Failed	0 %	
23	PE	1	<i>S. aureus</i> + <i>S. epidermidis</i>		Discarded*		
24	Mild PE	3	<i>S. aureus</i> + <i>S. intermedicus</i>		Discarded*		

RB, repeat breeder.

\*Discarded: Cows were herd discarded, be fattened and slaughtered.

## Discussion

Bovine endometritis and prolonged luteal phase leading to RB (infertility complains) is significantly increased in cows with persistent infections.<sup>11,24</sup> Well managed and profitable dairy farming requires cows with good reproductive performance,<sup>25</sup> but uterine postpartum infection with MDR pathogens which is so common<sup>24</sup> threaten fertility causing major cause of economic loss.<sup>25</sup> Increased multidrug resistance has led to the increased severity of diseases caused by bacterial pathogens.<sup>26</sup> When MAR value of *Staphylococcus spp.* just  $\geq 0.2$ , is considered high<sup>23</sup> and might be originated from

environments with misuse of antibiotics where resistance developed and spread<sup>27</sup> causing major problems in treatment of uterine infections in dairy animals.<sup>28</sup> So, it is obvious that the isolated *Staphylococcus spp.* - in the present study - were stubborn showing high MAR values (Table 2) might be managed with an alternative antimicrobial agent other than antibiotic. Against these stubborn pathogens, honey had potent antimicrobial activity since the achieved *in vitro* MIC value was (10–20% - Table 2). Otherwise, as the chronic uterine infections that are resistant to antimicrobial agents may be due to biofilm production<sup>14,29</sup> and honey is well documented that it has a tremendous antibiofilm activity,<sup>19,30,31</sup> it maximized its *in vitro* antimicrobial action

which might be added to its immunomodulation activation.<sup>32</sup> As mentioned above, intrauterine alternative proved the *in vitro* activity against bovine uterine pathogens<sup>3,8</sup> were advised without *in vivo* studies, while others failed to improve the reproductive performance when tried *in vivo*.<sup>6,28,33,34</sup> Certain studies<sup>10,11</sup> on intrauterine infusion did not differ from antibiotic application, while some of them proved to be having *in vivo* activity when be used alone.<sup>1,4,5</sup> or in combination with others.<sup>4,14,15,35</sup> Some tried intrauterine alternative agents in healthy cows to improve the reproductive performance<sup>6</sup> away from bacterial endometritis. Moreover, most of these studies recommended alternative medicinal agents basing on the *in vivo* activity were judged only by clearance of uterine infection neglected the full reproductive performance not as the present work.

As honey has wonderful antimicrobial<sup>19,36</sup> and tremendous antibiofilm activities,<sup>19,30,31</sup> intrauterine honey infusion was tried recently in 7 old age mares (16–27years) with endometritis<sup>20</sup> as 70% honey solution concluded that it was promising remarkable technique as improving endometrial cytological and ultrasonographic image, but did not improve fertility which might be due to animal senility. The study was biased against honey infusion where conducted with senile mares with not enough number statistically.

Contrarily in the present study, group (A) which did not receive any treatment showed the most positive results as purulent exudate clearance was achieved (3 cows post single infusions and 3 post twice only) with conception rate 80% (Table 3) and the only cow failed to conceive had bloody PE revealing other reproductive problem. Also, all cows of group (B) which were antibiotic none-responding received triple honey infusion where 7 cows had purulent exudate cessation post triple honey infusions with conception rate 70% (Table 3). The concluded overall conception rate of both groups (A & B) was 75% post honey intrauterine infusion regardless to RB duration since five cows got received despite long RB periods (7–16months).

Consequently, among all alternative intrauterine infusion techniques, honey application appeared to be the most convenient highly effective greatly safe and economic management. It is recommended to be used routinely 30 days post parturition (before the complete uterine involution and cervix closure) to avoid development of PE causing infectious RB.

## Conclusion

In case of bovine PE, intrauterine of 100 ml of full strength honey day/day for three successive infusions is a very effective successful management even in cow antibiotic none-responding as it would clear the pyogenic infection with high conception rate (75 %).

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## Conflicts of interest

Authors declare that there is no conflict of interest.

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