

## NONCONVENTIONAL THERAPY FOR CLINICAL ENDOMETRITIS WITH GARLIC EXTRACT INFUSION IN DAIRY COWS

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### ABSTRACT

Antibacterial efficacy of garlic extract was studied in vitro and in vivo. This was judged on the basis of in vitro inhibition of microbial growth of isolated microorganisms on blood agar and the recovery of clinical cases of endometritis by intrauterine infusion of garlic extract. Bacteriological samples before and after treatment were taken from 15 cows affected with 2nd and 3rd degrees of endometritis. Initial bacterial count ranged from  $10^8$  to  $10^{12}$  Colony forming units / ml (cfu /ml) before treatment was markedly reduced after garlic treatment to be  $10^3$  to  $10^4$  cfu/ml. The isolates were cultured on blood agar media with different concentrations of garlic extract ( 2,3, 4,5,6,7,8%). Concentration of 8% inhibited all isolated strains . The in vitro study revealed a constant pH value of 5.8 by incubation at  $37^\circ\text{C}$  for one hour . The activity of garlic extract was found 18 times neomycin sulphate . The in vivo investigation disclosed that all the cows having 2nd degree endometritis responded well after single intrauterine infusion of garlic extract. All the cows showing 3rd degree endometritis required two infusions of the garlic extract for complete clinical recovery.

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### INTRODUCTION

Garlic (*Allium sativum* , family Liliaceae ) has been firstly known and used in Asia, then was transferred to other countries . It has been used as common spices, food additive and folk medicine since ancient times . At present , several investigations have been conducted to provide better understanding of its scientific value and beneficial therapeutic application . Thus, it has been found to possess antibacterial (1,2) , antifungal (3,4) , antiviral, antitumor, anthelmintic insecticidal, and anticoagulant activities<sup>(4)</sup>.

In addition , it has been found to contain prostaglandins<sup>(5,6)</sup> ( PGA , PGB and F ) , thus having oxytocic activity <sup>(7)</sup>. It has since long been recommended for cure of a number of ailments viz : wounds, foululcers, pneumonia , bronchitis , atopic dyspesia , gastrointestinal disorders and infectious genital tract disorders <sup>(8)</sup> . With voids and gabs its in vitro and in vivo antibacterial and antifungal property has also been demonstrated in animals and poultry <sup>(2,8,9)</sup> .

In the present investigation, preliminary attempts have been conducted to study and elucidate both in vitro and in vivo antibacterial property of crude extract of garlic. Also to evaluate its therapeutic efficacy against uterine infections which cause clinical endometritis in dairy cows .

## EXPERIMENTAL

### Materials and Methods :

Fifty Friesian - Holstein and crossbred cows which suffer endometritis were used in this study . The age of cows varied from 4 to 6 years old . The animals were housed in open yards under similar conditions . Natural service was used in a ratio of a bull for 20 cows . Through routine postpartum breeding soundness examination . The clinical diagnoses were categorized into 2 major groups : Group 1 ( 33 cows ) affected with 2nd degree endometritis ( separately, all the cows were infused once with 50 - 100 ml of garlic extract by using uterine catheter ) while Group 2 ( 17 Cows ) suffering from 3rd degree endometritis, ( each infused intrauterine with the same dose of garlic extract twice with one week apart ). Using each animal as its own control thirty bacteriological samples were taken from only 15 cows representing all degrees of endometritis, one before garlic infusion and the other 72 hours after garlic infusion by using biopsy knife instrument <sup>(10)</sup> .

### Preparation of Garlic Extract :

100 gm of frozen cloves were grinded thoroughly in a mixer after addition of 120 ml distielled water then left for 0.5 hour for soaking and subsequently seived through muslin cloth . The obtained garlic extract was incubated at 37 ° C for one hour ( to allow the transformation of inactive allin

to active allicin )<sup>(11)</sup> . The pH of the garlic extract was measured before and after incubation .

Determination of the Minimum Bactericidal Concentration (MBC) was carried out by mixing different concentration of garlic extract (2,3,4,5,6,7 and 8%) with nutrient agar . The solidified plates were divided, the different isolates were cultured on the plates and incubation at 37 ° C for 24 h .

After 72h streaks showing no signs of growth were aseptically transferred to nutrient broth and further incubated for 48h . MBC was considered on the lowest concentration showing no signs of growth on nutrient broth after subcultured and incubation .

#### Activity of Garlic Suspension :

This was assessed by mixing the nutrient agar medium with a suspension of 1 % ( v/v ) of *Staphylococcus aureus* ATCC ( 6538 p ) . The molten agar medium was poured in sterile petri dishes to a depth of 3-4 mm . The inoculated plates were dried for 30 min at room temperature before use . Holes (cups) were done in the seeded agar with sterile cork - borer 8 mm diameter . Cups were filled with the dose (14 µ g/ml) of standard preparation of neomycin sulphate (CFR) which produces the same degree of inhibition (British Pharmacopoeia 1980, XIV , PA - 121)

#### Culture of Collected Specimens :

The specimens were collected in sterile test tubes containing one quarter Ringer's solution . The collected specimens were then serially diluted and appropriate dilutions were plated on blood agar and MacConkey's agar with and without garlic extract ( 5% ) . The inoculated media were incubated at 37 ° C for 24 hs . The initial bacterial count was determined before and after treatment and the obtained colonies of different species of microorganisms were further purified and identified .

## RESULTS

In vitro investigations revealed that pH value of the garlic extract was

5. 8 before and after incubation for one hour at 37 ° C . The initial bacterial count of the different collected samples from all cases of endometritis was found ranging from  $10^8$  to  $10^{12}$  cfu / ml before treatment , while after treatment it was  $10^3$  to  $10^4$  cfu / ml . The activity of the garlic extract was determined as compared to the standard neomycin sulphate and it was found to be 18 times of the neomycin sulphate. The Minimum Bactericidal Concentration ( MBC ) was determined. and the results for different isolates were depicted in table 2. The in vitro study showed evidently that 8% of garlic extract inhibited most the isolates obtained. Interestingly, the media contained garlic extract ( 5% ) showed no of bacterial growth except 4 resistant strains ( *Actinomyces pyogenes*, *Achromobacter* spp, *Pseudomonas multiphilia* and *Citrobacter diversus* ). The results of aerobic bacteria isolated and identified are summarized in table 1. It is worth mentioning that, bacterial isolation occurred less frequently in cows after garlic infusion . Thus, in all 15 samples collected after treatment were sterile except three samples from cows that had 3rd degree of endometritis which yielded some bacterial growth after the first dose of the garlic extract.

Seemingly , after the garlic treatment , all the cows showing 2nd degree endometritis responded well following one infusion , while all the cows having 3rd degree endometritis required two infusions with one week apart. In the meantime the clinical response was evidenced by the disappearance of detectable clinical lesions , mucopurulent and the purulent discharges also disappeared completely and almost absence of bacteria in the specimens collected after garlic therapy and finally the animals were clinically and apparently recovered.

## DISCUSSION

Along cherished dream of bovine practitioners has been to find out and use of nonantibiotic alternatives for manipulation of the uterine infections , since antibiotics have prevailed for many years as the treatment of choice for genital infections. Increasing awareness of treatment failures, the risk for bacterial resistance , the risk for residues in tissue and milk and the possibility of undesirable effects on the natural defence mechanisms have increased the interest in alternative (non - antibiotic) methods (12) .

Moreover, local use of antiseptics, which is another possible nonantibiotic approach (e.g., iodine chlorhexidine and other similar antiseptics) may adversely influence the local defense mechanisms<sup>(13)</sup> and their restricted use is therefore has been recommended. Therefore, our motivation for this new non - conventional garlic therapy is to manipulate uterine infections by intrauterine infusion of such considerable safe medicament with no apparent side effects and lower price as well as its availability are the advantages of this kind of antimicrobial agent.

The most common microorganisms isolated and incriminated in bovine endometritis are summarized and tabulated in Table 1. These findings are somewhat consistent with many authors<sup>(14 -16)</sup>. With an exception, it appears to be no available circumstantial evidence that garlic therapy can be curative for uterine infections in bovines. The efficacy of garlic therapy against infections of genital tract was studied in buffaloes and cows suspected of vaginitis, endometritis and postpartum metritis,<sup>(8)</sup>. It was reported that this treatment did cause much improvement with high clinical response and cure for such genital disorders. Our clinical findings are somewhat similar to the latter work<sup>(8)</sup>. Therefore, it could be suggested that the marked antibacterial activity of the garlic extract may, in part be related either to its bactericidal and bacteriostatic property<sup>(1,2)</sup> or to its acidic pH (5.8) which is unfavourable for bacterial growth and multiplication as well as to its oxytocic activity<sup>(6)</sup>. Finally its prostaglandin activity<sup>(5)</sup> may play a part in the process. All these factors might act synergistically together to form suppressive, unsuitable uterine ecology and microenvironment that favours the effectiveness of uterine immune response and inhibition of growth that strongly enhanced clinical responses and recovery of the cases.

It is obvious from this investigation that intrauterine garlic extract infusion for manipulation of uterine bacterial infections in cows suffering mucopurulent and purulent endometritis is promising method and prospective as additional therapeutic nonantibiotic alternative without adverse side effects. In conclusion, the introduction of the garlic therapy as antimicrobial agent opens new horizons for treatment of genital tract infections in domestic animals.

**Table 1** : Incidence and Distribution of Microorganisms in Case of Endometritis

Microorganism	Incidence
1- E. coli	15 samples of endometritis
2- Actinomyces pyogens	11 samples
3- Citrobacter diversus	6 samples
4- Enterobacter agglomerans	5 samples
5- Staphylococcus aureus	4 samples
6- Achromobacter Spp.	3 samples
7- Streptococcus faecalis	3 samples
8- Ps . multiphilia	1 samples

**Table 2** : Minimum Bactericidal Concentration of the Garlic Extract on Different Isolates of Microorganisms .

Name of Isolated Strains	Concentration of the garlic extract (%)						
	2%	3%	4%	5%	6%	7%	8%
1- Actinomyces pyogens	+	+	+	+	+	+	-
2- Achromobacter Spp.	+	+	+	+	-	-	-
3- Streptococcus Spp.	+	+	+	+	-	-	-
4- Citrobacter diversus	+	+	+	+	-	-	-
5- pseudomonas multiphilia	+	+	+	+	-	-	-
6- Enterobacter agglomerans	+	+	+	-	-	-	-
7- E. coli	+	+	+	-	-	-	-
8- Staphylococcus aureus	+	+	+	-	-	-	-
9- Bacillus Spp.	+	+	+	-	-	-	-

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## إستخدام طريقة غير تقليدية لعلاج التهاب الغشاء المبطن للرحم في الأبقار الحلابه بواسطة مستخلص نبات الثوم .

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إستهدفت هذه الدراسة معرفة مدى تأثير وكفاءة مستخلص نبات الثوم كمضاد للبكتيريا المسببة لإلتهابات غشاء الرحم الداخلي في الأبقار سواء في المعمل أو في الحيوانات المصابة بالتهاب الغشاء المبطن للرحم وتم تقدير قوة وكفاءة مستخلص نبات الثوم في المعمل بتقدير مدى مساحة عدم نمو البكتيريا المعزولة من حالات التهاب الرحم علي منابتها بعد إضافة مستخلص الثوم اليها . وبالنسبة لمدي تأثير وكفاءة مستخلص نبات الثوم كمضاد للبكتيريا في حالات الإلتهاب الرحمي في الحيوان الحي قدرت بمدي شفاء الحيوان من التهاب غشاء الرحم بعد حقن مستخلص الثوم في الرحم . وتم ايضاً اخذ عينات للفحص البكتريولوجي قبل وبعد حقن مستخلص الثوم في الرحم لمعرفة مدى تأثير المستخلص علي البكتيريا الموجودة بالرحم في حالات التهاب الرحم من الدرجة الثانية والثالثة . وقد لوحظ تناقص كبير في عدد البكتيريا بعد العلاج بمستخلص الثوم . والميكروبات التي تم عزلها من حالة التهاب الغشاء المخاطي للرحم من الدرجة الثانية أو الثالثة تم تنميتها علي منابت الميكروبات ووضع المستخلص بتركيزات مختلفة ( ٢.٣.٤.٥.٦.٧.٨٪ ) عليها لمعرفة مدى تأثير المستخلص علي البكتيريا في المعمل ووجد أن تركيز ٨٪ قد أوقف نمو جميع أنواع الميكروبات التي تم عزلها ووجد أن تأثير المستخلص علي الميكروبات يتفوق علي تأثير سلفات النيرمايسين ثمانية عشر مره وبالنسبة للتأثير علي البكتيريا في الحيوان البع ووجد أن جميع حالات التهاب بطانة الرحم من الدرجة الثانية قد استجابت للعلاج بعد مره واحده من حقن ٥٠ - ١٠٠ مل من المستخلص في الرحم بينما جميع الأبقار التي كانت تعاني من التهابات بطانة الرحم من الدرجة الثالثة إحتاجت الي تكرار العلاج مره ثانية بعد اسبوع بنفس الجرعة السابقة ( ٥٠-١٠٠ مل ) وكانت الإستجابة الإكلينيكية للعلاج ايجابية في جميع الحالات المصابه بعد الجرعه الثانية .

ومن هذا يتضح أن مستخلص الثوم ذو فائدة في علاج التهابات بطانة الرحم لإحتوائه علي مواد فعالة ومؤثره علي الميكروبات التي تسبب عدوي والتهاب لبطانة الرحم الداخلية في الأبقار.