



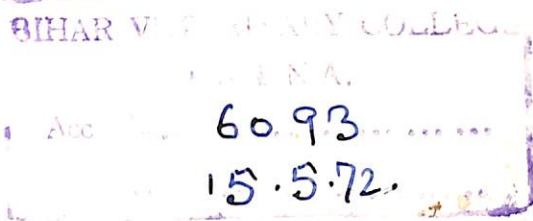


Comparative Studies On  
The Efficacy of Drugs Against Endometritis in  
Cattle with Special Reference to In Vitro and  
In Vivo Antibiotic Sensitivity Tests of  
Causative Organisms

*A Thesis*

*Submitted to the Faculty of Veterinary Science*  
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Most filially dedicated  
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*This is to Certify that ARUN KUMAR SINHA has worked under my supervision and guidance for his Thesis entitled "COMPARATIVE STUDIES ON THE EFFICACY OF DRUGS AGAINST ENDOMETRITIS IN CATTLE WITH SPECIAL REFERENCE TO 'IN VITRO' AND 'IN VIVO' ANTIBIOTIC SENSITIVITY TESTS OF CAUSATIVE ORGANISMS" for the degree of M.Sc. (Veterinary) with Gynaecology, Obstetrics and Artificial Insemination as major subject and that it incorporates the results of his independent study.*



Date : The 7th March, 1972.

( B. K. SINGH )



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## **INTRODUCTION**



## I N T R O D U C T I O N

High fertility is of paramount importance for efficient and economic livestock production. For livestock breeders there is no greater economic problem than that associated with the condition which impairs fertility of their animals. Livestock is utilised by man for converting animal feeds and fodder into milk, meat, wool and various other things. All these potentialities are very much dependent on their reproductive efficiency. Every animal that loses this efficiency is a liability to its owner. Dairy cattle which fail to conceive within three or four months following last calving cause economic loss to the owner. Delay in establishment of pregnancy consequently increases the time interval between calvings, decreases the number of offsprings during the lifetime of the animal and also causes decreased milk production. Post-partum oestrous should occur on an average three months after parturition and the animals should conceive after one or two inseminations to ensure normal calving interval.

According to Lagerlof (1948) and Gibbons (1963) causes of infertility in the cow vary from congenital defects, functional or hormonal causes, specific and non-specific infections. These infections bring about a large percentage of cases of infertility by producing inflammatory conditions of the genitalia particularly the uterus. Begg (1932) estimated that uterine affections inflict greater loss on dairy stock owners than do the affections of all other organs put together. The specific infections include diseases such as brucellosis, vibriosis, and



trichomoniasis. Such infections of the female genital organs occur without predisposing cause and are mainly enzootic in nature. However, brucellosis is now effectively controlled by calfhoo vaccination while enzootics of trichomoniasis and genital vibriosis are much less common because artificial insemination has eliminated the use of communal bull and has reduced "traffic" in bulls to a minimum (Arthur, 1964). Apart from occasional herd infection therefore, there remain the individual cases of non-specific endometritis, which require a predisposing cause. In cattle the organisms concerned are Streptococci, Staphylococci, E. coli, Corynebacterium pyogenes and others which constitute Saprophytic microbial flora of the genital tract of cows. Generally the organisms gain access at the time of parturition or breeding and if conditions are conducive for their growth, they establish themselves temporarily and cause varying degrees of endometritis. The uterus may also become infected by haematogenous dissemination of the micro-organisms. In fact it is probable that a mild degree of endometritis develop during puerperal period. But the uninjured endometrium possesses amazing powers of resolution and by the time involution is complete, such infection is overcome naturally. Any factor which delays the onset of or the course of involution will tend to favour and prolong such infection. These infections are most likely to occur in cases of dystokia, prolapse of genitalia, retained placenta or contaminations during normal calving. The involuting uterus provides very good medium for the multiplication of organisms and thus inflammatory processes are all the more accentuated. The



increase in the incidence of non-specific endometritis may be attributed to the fact that with the continuing efforts of the breeders to develop high yielding dairy cows, more trouble is occurring at parturition and that placental retention and delayed uterine involution are now more common than formerly (Arthur, 1964).

Inflammatory changes in the uterus may be localised to its different layers and for them different nomenclatures are used. According to Millar and West (1967) the following terminology have been put forward :-

- (1) Metritis or Puerperal fever - Inflammation of the uterus, may be acute or chronic.
- (2) Endometritis - Inflammation of mucous membrane only.
- (3) Mesometritis - Inflammation of mucous membrane and uterine muscular wall.
- (4) Parametritis - Inflammation localised to the peritoneum.
- (5) Polymetritis - Inflammation of all the three coats of the uterus.

Endometritis, that is inflammation of the mucous-membrane of the uterus, caused by the micro-organisms or their toxic products (Decamp, 1935), has been classified in different manners by Beaver et al. (1922), Richter (1926), Nielsen (1926), Tutt (1933), Polding and Lall (1945), Azizuddin (1948) and Dawson (1951). With some minor individual variations authors have generally retained Richter's (1926) classification which



is based on the type and extent of discharge and accordingly endometritis has been classified into three degrees. First degree is that where discharge is absent or intermittent. A continuous mucopurulent discharge is present in the second degree. A purely purulent type of discharge with some tendency to accumulate represents the third degree of endometritis. Dawson (1950) considered Richter's (1926) second degree endometritis as chronic or catarrhal endometritis and further stated that this condition merges with the less severe form, lacking a continuous discharge on one hand, and, on the other, with the more serious conditions of pyometra, when large quantities of pus and slime accumulate in the uterus.

Endometritis has been listed as one of the most important factors causing failure of conception. In 1941 the Survey Committee of the National Veterinary Medical Association (now the British Veterinary Association) concluded that 90% of the infertility affecting the dairy cows in Great Britain was due to varying degrees of endometritis (Arthur, 1964). Moore (1954), and Schutze (1957) stated that this disease involved in 85% of infertility cases and was thus one of the most important entities in the entire field of veterinary practice. Hardenbrook (1958) found that it was involved in 80% of breeding failure in Illinois and Anderholm (1958) also believed that it was the commonest cause of infertility. The above mentioned informations suggest the importance of endometritis in causing infertility and warrants detailed studies so that this problem can be tackled and economic losses occurring from this condition are kept to



the minimum.

There have been two prominent and divergent school of thoughts regarding the primary focus of abnormality causing endometritis.

Swiss workers, Zschokke (1900), Hess (1909) and others held that ovarian abnormality was the primary condition followed by uterine abnormalities, and for treatment of such cases they employed ovarian manipulations supplemented by general massaging of these organs and of the uterus.

The Danish - German school of thought led by Albrechtsen (1917) and followed by Richter (1920) and Nielsen (1924) reported the views of swiss workers. Albrechtsen (1917) believed that primary cause was infection of the tubular portion of the tract, particularly the uterus, by pyogenic organisms and that ovarian changes were secondary. He recommended disinfection of genital tract with moderate to weak solution of iodine for the purpose of treatment.

The diseases of various reproductive organs are intimately linked together, as according to Williams (1943) the reproductive system largely behaves as a unit, and serious disturbance of any one of the organs tends to cause interference with the proper functioning of all the others. If the ovaries are seriously diseased, the uterus loses its physiological function; if the uterus is extensively diseased (mummified foetus, trichomonad pyometra) there is cessation of ovarian functions. Dawson



(1950) showed explicitly that abnormalities of ovarian cycle are common in the severe forms of the disease and generally the first degree and most of the second degree cases of endometritis do not bring any change in the ovarian function and oestrous cycle. The abnormality in ovarian function is almost always observed in cases of third degree endometritis.

Since endometritis is one of the most important causes of infertility in domestic animals, attempts were made in the present study to find out suitable remedial measures against this disease. For this purpose a total number of five drugs were selected namely; Lugol's iodine solution, three antibiotics of tetracycline group and one herbal preparation to evaluate their comparative efficacy against this condition.

For chemotherapy to be a success selection of proper antibiotic is imperative, particularly these days, when frequent and irrational use of antibiotics has increased the resistance of bacteria. Therefore, a part of the present study deals with isolation of bacteria from the uterine discharge of cows suffering from endometritis and subjecting the isolates to antibiotic sensitivity test. These in-vitro studies were further extended and were tried on the particular animal to confirm their efficacy.

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## **REVIEW OF LITERATURE**



## REVIEW OF LITERATURE

### Classification of Endometritis :

An involuting uterus is highly susceptible to infection as there are large areas of raw surface containing remnants of disintegrating placental tissue and numerous blood clots. It is open to infection from both the cervix and blood circulation. Any such infection causes tissue response varying with the organisms responsible and the duration of infection. The reaction may be of any type from a simple inflammation i.e., endometritis, to a purulent necrosis i.e., advanced pyometra. From time to time many workers have classified endometritis into various sub-groups ranging from simple inflammation to purulent necrosis.

Beaver et al. (1922) classified endometritis in three groups -

- (a) Catarrhal metritis - acute and chronic.
- (b) Suppurative metritis - acute and chronic.
- (c) Pyometra and cystic degeneration of uterine glands.

The most widely accepted classification was given by Richter (1926). He classified endometritis on the nature and character of discharge as follows :

- (a) First degree endometritis - when uterine discharge was absent or intermettent.
- (b) Second degree endometritis - when there was continuous mucopurulent discharge.



- (c) Third degree endometritis - when there was purely purulent discharge with some tendency of accumulation.

Nielsen (1926) recognised five types of endometritis on the basis of nature and character of discharge;

- (a) Puerperal endometritis.
- (b) Endometritis of the first degree - a slight inflammation, at the most accompanied by only very small amounts of purulent material; there was no conspicuous flow.
- (c) Endometritis of second degree i.e., chronic catarrhal endometritis.
- (d) Endometritis of third degree i.e., pyometra.
- (e) Endometritis of fourth degree i.e., chronic endometritis with cystic degeneration of the ovaries. The latter four groups might occur any time three weeks after parturition.

Tutt (1933) reported that the chronic form of endometritis was originally divided for the purpose of description into two main classes :

- (a) Chronic corporeal (Interstitial) endometritis - when the inflammatory changes were restricted to stroma.
- (b) Chronic cervical endometritis - when the inflammatory changes were regarded to be confined to the cervix. The author finally concluded that for



all practical purposes the best classification of various types of endometritis was given by Nielsen (1926).

Polding and Lall (1945) classified endometritis on the basis of severity and duration of infection as (a) acute; (b) subacute; and (c) chronic. Azizuddin (1948) described four types of endometritis on the basis of macroscopic lesions present in the uterus :

- (a) Catarrhal endometritis - simple inflammation of uterus with or without some non-purulent discharge.
- (b) Necrotic endometritis - where the uterine walls were flabby and there was complete lack of tone, the tissue were very soft and friable and were easily crushed and torn when pressed by forceps or fingers. The exudate was curdy containing flakes of necrotic tissue.
- (c) Purulent endometritis - early stage of pyometra, almost always associated with Corynebacterium pyogenes infection. Discharge was of thick, mucopurulent, creamy nature scattered over the mucosa, occurring specially as small clumps around cotyledons. Occasionally there were small pieces of necrotic tissue mixed with pus.
- (d) Pyometra - uterine horns were symmetrically enlarged but involvement of only one horn was also observed. The tubal end of the horn was round making a pouch



like structure. The discharge was creamy with small blackish floccules.

Dawson (1951) conducted a detailed study of 88 cases of catarrhal endometritis occurring from three weeks following parturition. The conditions were classified on the basis of the nature of discharge and the absence or otherwise of oestrous, into three main groups.

Group - A : Marked pathological impairment of the oestrous cycle, copious continuous discharge mainly purulent, which tends to accumulate and produce pyometra.

Subgroup- I. Discharge odourless and apparently sterile. Disinfectant irrigations have no influence on course of disease, prognosis good.

Subgroup-II. Discharge with characteristic foul odour and containing C. pyogenes usually in large numbers. Disinfectant irrigations have marked ameliorative effect on course of disease.

Group - B : Little or no impairment of normal oestrous cycle, discharge scanty, contains pus and mucus. Bacteriological findings inconclusive.

Subgroup - I. Discharge continuous from puerperium prognosis good.

Subgroup - II. Discharge appearing transiently only after heat.

Subgroup- III. Copious discharge after trichomoniasis.



Subgroup- IV. Scanty discharge, prognosis good on "Stimulant Therapy". Evidence of mild reproductive endocrine dysfunction.

Group - C : Little or no impairment of oestrous cycle. Copious discharge consisting solely of watery mucus.

#### Diagnosis of endometritis :

Most workers have depended upon the rectal and vaginal examination of the genitalia for the diagnosis of endometritis excepting the Richter's (1926) first degree cases where these examinations are inconclusive. Devine (1920) differentiated the cases of chronic endometritis where there was sufficient thickening of uterine horns which he said to be indicative of inflammatory condition. This inflammatory condition might be manifested either by a flabby uterine horns without tone or conversely, by one that was noticeably too rigid. The absence of any exudate, with the possible exception of a slight exudate during the period of oestrous coupled usually with an enlarged and firmer cervix, served to indicate chronic endometritis. But Lothe (1921) reported that the rectal examination revealed little or nothing, the uterus, ovaries and oviducts were normal. The most authentic diagnosis could be made by inspecting the vagina and os-uteri. In normal cases these organs were quite dry and pink in colour except at the time of oestrous, when the membranes were very moist and of deeper colour. In chronic catarrhal endometritis the secretions were increased in amount and changed in appearance;



ranging from a slight cloudiness, to muco-pus. For further examination he brought the os-uteri as back as the vulva to see the changes and examine the exudate.

Devine (1927) stressed the importance of training the sense of touch to aid in diagnosis of chronic endometritis so that changes in the tubes and in the uterus were readily apparent upon examination per rectum. Apart from manual vaginal and rectal examinations, Küst (1933) reported a number of special instruments of great value. He used a set of six plain nickel plated the specula of graded sizes for inspecting the vagina and cervix.

According to Tutt (1933) the uterine cornua were asymmetrical and the cervical canal was dilated except in some of the mild cases. Oestrous and ovulation were usually regular. In case of endometritis of second degree (as classified by Nielsen, 1926) manipulation per rectum revealed enlarged uterus and per vaginum the os-uteri externum was larger than normal. There was an abundant mucus discharge with small flocculi or pus alone. In some of the cases of this degree the oestrous was irregular or absent. He further stated that infertility arising from endometritis was due to two causes (a) the discharge which was inimical to the spermatozoa and (b) the unhealthy endometrium which did not offer any favourable place for embedding the fertilized ovum, if at all the fertilization had taken place.

Craig (1936) reported that the recognition of the disease is not difficult in severe cases. Per rectum the enlarged relaxed uterus, which might be uniformly involved and its



thickened wall could be easily determined for a diagnosis. A catarrhal or purulent discharge through vagina was of considerable importance in diagnosing endometritis. In the very mild catarrhal endometritis, however, the uterus did not present any marked change on rectal examination and little discharge could be pressed out through os-uteri. Regarding oestrous cycle the author stated that in a lighter cases there was no change in heat periods but in more severe forms oestrous was either absent or was only noted at irregular intervals. Laing (1955) diagnosed the case of endometritis on the occurrence of vaginal discharge and the enlargement of uterus as detected by the vaginal and rectal examination.

The accurate diagnosis of chronic metritis according to Williams (1943) demanded (a) an authentic history of the animal and (b) a detailed clinical study like vaginal examination, palpation of the reproductive organs per rectum and exploratory douching of uterus. The vagina might contain pus which usually emanated from the uterine cavity or cervical canal. For the examination of cervix he also followed the method earlier described by Lothe (1921). By rectal palpation a definite thickening of the uterine wall and sclerosis might be identified. The uterus might be of approximately the same size and similar in degree of hardness to that of oestrus; it was frequently extremely atonic and flaccid. Chambers (1952) diagnosed endometritis in older cows on the basis of a soft, flabby and atonic uterus. Regarding exploratory uterine douching Williams (1943) stated that this method might be of help in giving highly important



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evidence for diagnosing chronic metritis.

Roberts (1956) stated that frequently the clinical symptoms of an endometritis could not be demonstrated by rectal or vaginal examination. However, in the more severe cases there might be a mucopurulent discharge from the uterus, especially noted at the time of oestrus by flakes of pus in the oestral mucus. Often the mucus of oestrus might be cloudy or milky instead of clear or translucent. In some cases douching of uterus might wash out flakes of mucopurulent material and then diagnosis was more certain. On rectal examination the uterus might feel slightly large, heavy and thick walled. These changes might be limited to one horn. The oestrous cycle and periods were usually normal in length. In some cases the cycle varied due to early embryonic death and maceration of foetus as in trichomoniasis, vibriosis and possibly other infections. The cycle length may not vary in cases of endometritis where fertilization does not occur or implantation of the morula is rendered impossible due to unhealthy endometritis. Repeated services and failure of conception were common symptoms of endometritis.

Biopsy of the uterus was tried by Miller (1950) and Brus (1954) for diagnosing the cases of mild degree of endometritis where the result of rectal palpation and vaginal examinations were inconclusive. In the study by Brus (1954) on 110 sterile cows, 27 of 31 cows that showed some clinically diagnosable abnormalities had an endometritis. In 79 sterile cows without clinically detectable abnormalities 63 percent had endometritis. The most common lesions found in endometritis in



cattle, according to Miller (1950), were a periglandular fibrosis and subsequent glandular degeneration and dilation.

Bacteriological examination of the uterine discharge may also serve as an aid to the diagnosis of endometritis as Dawson (1960) compared the organisms recovered from normal and diseased cows. He found that the percentage of "organism free" cows fell steeply in the presence of disease, and that there was a distinct rise in recoveries of both types of Staphylococci and of E. coli, a considerable rise in C. pyogenes; and a slight rise in Streptococci.

#### Therapeutic methods used against endometritis :

Dawson (1960) stated that the methods applied for the treatment of endometritis have involved mainly the local application of drugs to the endometrium. The chief groups of drugs employed have been chemical disinfectants or counter irritants, reproductive hormones and antibiotics. Enucleation of corpus luteum for the treatment of endometritis became quite popular among swiss veterinarians from about 1875. By 1890 the growing acceptance of germ theory led Ehrhardt to introduce the use of disinfectants and uterine irrigations by catheter.

Zschokke (1900) suggested that this disease might be due to the failure of some principle which caused the rupture of a ripe follicle in the normal cow. He also noted a connection between endometritis and prolonged anoestrous, and that in this latter condition a persistent corpus luteum could accompany both



catarrhal endometritis and pyometra. His routine treatment based on these observations, consisted the cyst rupture and enucleation of corpus luteum, with cervical dilation in addition. He also made limited use of weak disinfectant uterine washes. Hess (1909) rejected the practice of uterine irrigation especially when it needed a brutal force in passing a catheter through a closed cervix and considered it a very bad practice. He favoured the view of corpus luteum enucleation and stated that the uterine disease was secondary to primary ovarian abnormality.

Albrechtsen (1917) thought that the endometritis was caused by a primary microbe infection in the uterus, either blood borne or picked up via vagina. This could have a secondary effect on the ovaries through infection ascending to them. Though he practiced plugging the cervix with tincture iodine wads "as an adjuvant", treatment was essentially an attempt to disinfect the tract with moderate to weak solutions of iodine through a catheter. Albrechtsen devised also the valuable technique of cervical retraction right into the vulva, and had no hesitation in subjecting this organs to drastic expansion measures with scissors and large-bore catheters. He laid more stress on his "primary uterine infection" theory, which conflicted sharply with the theory of Hess (1909). The reasons why ovarian changes were secondary to uterine infection which Albrechtsen (1917) gave were as follows : (a) ovarian cysts and retained corpus luteum were not seen uncomplicated by endometritis. (b) Endometritis existed for weeks and months without cysts, which however, had strong tendency to eventual development and (c) On curing



endometritis by the application of disinfectant, the ovarian condition resolved. Albrechtsen (1917) obtained very good clinical results and his views were almost unanimously accepted by German workers, Richter (1920) and Oppermann (1922). The latter introduced the practice of preparing vaccines from the bacteria present in the vagina to be used as prophylaxis and treatment of endometritis.

Lothe (1920) was also of the opinion that ovarian trouble was secondary to the primary uterine inflammation. He found that excepting a few, several hundred barren cows with ovarian trouble also had some form of uterine trouble, and the treatment of ovarian trouble only did not give good results. The treatment methods used by Lothe (1921) consisted irrigation of uterus with antiseptics such as 3% Lugol's solution in sterile water, 0.25% chlorazane etc., and disinfection of cervical canal by the application of full strength of Lugol's solution or other antiseptics, on a cotton swab and uterine dressing forceps. The uterus was irrigated by means of a specially designed return flow steel catheter known as "Albrechtsen's uterine catheter". The solution was run into the uterus until the return flow became clear then the catheter was taken out leaving the remainder solution in the uterus.

Although Nielsen (1926) was convinced of "primary uterine infection" theory, he admitted the significance of anoestrus in endometritis, and that the effect produced in reinitiating a cycle contributed to the value. He was the first to note that



iodine irrigation treatment appeared to have some effect in reinitiating a lapsed cycle. He believed that iodine probably acted as an irritant, on the endometrium, with a consequent hastening of uterine involution. He advocated the use of iodine irrigation of uterus as the treatment of first choice, with ovarian treatment as an important adjuvant. But De vine (1927) believed the ovarian trouble of primary nature in cases of endometritis and recommended crushing of ovarian cysts and the removal of false corpora lutea, massaging the ovaries and uterus for the treatment. He stated these treatments to be of greatest value and the treatment consisting of douching the uterus with normal saline or other non-irritating solutions of very little value. Further, he used bacterins extensively and regarded them as indispensable in treating herds at a distance where frequent calls were not possible.

Quinlan (1929) followed the idea of Abrechtsen (1917) and paid more stress on the point that retention of corpus luteum was secondary to an accompanying endometritis. With the same idea Meyer (1932) used only iodine solution varying in strength from 2 to 5% according to the degree of metritis present and got good results. Tutt (1933) was also of the idea that treatment was to be guided by the degree of endometritis present. He followed the line of treatment as advocated by Nielsen (1926) and advised to carry out a preliminary flushing of uterus with normal saline solution before irrigating it with iodine in second degree cases of endometritis. He did not find enucleation of any persistent corpus luteum of much benefit, and regarded such a



procedure in most of the cases to be unnecessary. He stated that when pyometra subsided the corpus luteum either disappeared or became so far absorbed that it had no effect on oestrous cycle. But on the other hand the author admitted that in some cases of mild degree of endometritis only ovarian massage with the rupture of any cyst present or enucleation of corpora lutea and effected recovery. He concluded that the percentage of recoveries would be doubled if careful uterine injection was carried out, even in mild degrees of endometritis.

Küst (1933) used exclusively warm normal saline solution for treatment of endometritis and assisted the outflow by uterine massage per rectum. As he considered that all the common uterine infections were amenable to chemotherapy it was not of practical importance to find out bacterial species involved. After irrigation he inserted a few enzon bars and some times, in appropriate cases, employed non-specific stimulation therapy as well. Further he advised the enucleation of any persisting corpus luteum to procure the beginning of a new ovarian cycle. Baumann (1933) used colloidal silver for disinfectant irrigations. He noted a high incidence of ovarian pathology in endometritis cases and employed manipulation freely in its treatment. Stader (1934) advocated the use of weak solution of sodium hypochlorite at hourly intervals for 24 to 48 hours. He concluded that this method was easy and needed a single professional visit. Jaeger (1934) recommended mechanical emptying of the uterus followed by disinfection with 1% chinosol as effective treatment in pyometra cases.



Erisman (1934) showed that endometritis catarrhalis was closely related with the function of ovaries. Metritis vaccination alone and also in combination with the treatment of the ovaries and vagina produced a favourable curative effect upon catarrhal endometritis. Pregnancy was induced in 77% of cases treated. Decamp (1935) favoured the use of azamine as chemical disinfectant because it dissolved in the uterine liquid and penetrated the tissue frequently in the uterus. He cited Begg (1932) estimating that uterine affection inflicted greater loss on dairy stock owners than do the affections of all other organs put together. Kuipers (1935) recognised more clearly than Nielsen (1926) the usefulness of iodine irrigations in reinitiating an oestrous cycle. He thought it helpful to give preliminary irrigation 24 hours previously with a half saturated salt solution in order to dissolve mucus, thus permitting iodine, in his own words, to "werk dieper in". Craig (1936) also tried Nielsen's (1926) method of treatment and preferred iodine to other antiseptics, but in its higher dilution. The author often combined the above treatment with enucleation of corpora lutea with the idea of causing contraction of uterus.

Riedell (1935) was the first man who sounded a challenging note when he declared, he held no theory as to the true nature of disease in the "first degree" cases of Richter's (1926) classification; he was not convinced that bacterial infection was ever necessarily present. Iodine irrigation gave good results in his hands, and he was convinced that they acted not as disinfectants, but as counter irritant as indicated by Nielsen (1926).



Hignett (1940) also believed that the curative action of iodine was counterirritant or stimulant, not disinfectant. He condemned the use of strong iodine solution in cows because it reduced her milk yield for a long period. In some cases it might cause severe damage to the uterine mucosa and certainly suppressed oestrous for some considerable time. Because he believed that the action of iodine was stimulant he stressed that fairly weak solution of iodine can perform this action as well as do strong solutions. Wille (1942) used cantharides spray on the cervix as counter-irritant in endometritis cases. 74 % of 82 cases subsequently conceived quite quickly, having previously been sterile for periods upto three months and longer.

Brownlie (1941) found good results by the intra-uterine use of 150-200 ml. of 1 : 300 Lugol's iodine solution. In severe cases he preferred to give three irrigations at intervals of seven to ten days. Williams (1943) suggested repeated douching of uterus with 2 to 3 percent of Lugol's iodine solution as recommended by Albrechtsen (1917) for the treatment of endometritis. In some cases he preferred to give a mixture of bismuth subnitrate, iodoform and mineral oil through intra-uterine route. He stated that bismuth and iodoform dissolved the mucus and remained in the uterus for some days whereas the Lugol's solution and other soluble disinfectants were promptly expelled or absorbed.

Kust and Schaetz (1943) used iodine irrigations specifically for their "tissue stimulant" effect, and not as a disinfectant. Furthermore they were the first to combine "stimulation" with "disinfectant" therapy, for they combined Surfen, a



complex organic bacteriocide, with iodine in solution. They used a mixture of 8 ml. of 2% surfen and 4 ml. of 5% tincture of iodine to 1 litre of water and got all the cases of endometritis cured by two application. Holl (1944) also tried the same mixture and got good results by curing 77.5% of catarrhal cases in a single treatment.

Kudryashov (1945) reported that daily subcutaneous injection from 1-5 in number, of 15-30 mg. stilboesterol (0.5% solution) or 20-30 mg. synoestrol (1% solution) were equally effective in curing endometritis in cows. Of 11 cows sterile as a result of chronic catarrhal endometritis 9 were cured. In a group of 14 cows which had catarrhal endometritis together with persistent corpora lutea, 9 were cured. Hornaday (1947) stated that since there was a cleansing process of mucus-membrane of the uterus during each heat period, endometritis was seldom found in cows that had normal heat cycles. Thus he believed that the condition of endometritis could be controlled by inducing regular heat periods.

Dawson (1951) carried out 87 treatment trials on cases of catarrhal endometritis in bovine with Lugol's solution, oestrogens or corpus luteum enucleation, local sulphanilamide, propamidine and combination of later three. Results indicated that sulphanilamide, propamidine and endocrine treatment all had a good effect on the condition. Even in cases long standing the combination of endocrine methods with propamidine appeared to be the treatment of choice.



Roberts (1956) and Hardenbrook (1958) reported that most of the treatments were based on either stimulating the uterus or overcoming the suspected or known intra-uterine infection. They supported the views of Riedell (1935), Hignett (1940) and others that the Lugol's solution and other solutions used as antiseptics were actually acting more as uterine stimulants rather than disinfectants. They stated that intra-uterine or intra-muscular injection of oestradiol used for the treatment of endometritis also produced stimulant action on uterus.

Pietzsch (1958) treated 50 cows suffering from endometritis by infusing 200 ml. of Lugol's solution and 50 cows were treated with a similar volume of saturated Sodium Chloride solution. From the Lugol's iodine group 30 and from Sodium Chloride group 40 became pregnant after subsequent service.

Easley et al. (1951) for the first time published their reports on the use of antibiotics against bovine endometritis. They used 200,000 units of penicillin with or without 1 gram of streptomycin and one or two sulfonamide compounds. Each of these combinations seemed to give some good effects. Hoppe (1955) found 400,000 units penicillin with 1 gram streptomycin useless given at insemination time, though Hamnell (1954), using 300,000 units of penicillin with or without half gram of streptomycin obtained encouraging results both at and between oestrous periods. These experiments were entirely on first degree cases, whereas Merkt (1953), Evertz (1955), Genegal (1955) and Stach (1957) also treating 2nd and 3rd degree cases, employed larger doses. Merkt (1953)



used a minimum dose of half a million units, some times repeated after 24 hours, some times together with a similar dose of streptomycin. His most satisfactory preparation was one supplying 400 mg. penicillin in ointment form. Evertz (1955) and Genegal (1955) both found one million units of procaine penicillin giving better results than Lugol's iodine solution, while Stach (1957) combined 250,000 units of penicillin with 4-amino-benzol sulpho-thiocarbamide salt of 4-amino-methylbenzol sulphonamide (Marbadal) and usually 0.25 gm. streptomycin and used through intra-uterine route.

Roberts (1956) reported that the antibiotics which were used in cases of endometritis were penicillin, streptomycin, aureomycin, terramycin, chloromycetin and others, alone or in combination. Usually 500,000 to 1 million units of penicillin and 0.5 to 1 gram of streptomycin or 0.5 to 1 gram of the broad range antibiotics were infused in 10 to 50 ml. of distilled water. The author treated 599 cases of "repeat breeding" cows with different treatment methods, which has been given in Table No. 1.

Sacchi et al. (1958) found oxytetracycline to be promising in the first degree cases; they used it at oestrous or 24-72 hours later in a dose equivalent to 100 mg. of biological activity. Link (1956) claimed that 543 of 548 cows he treated with terramycin bred again but a series of untreated control was not maintained. Ohm (1955) and Genegal (1955) employed on cases of all degrees of endometritis 426 mg. aureomycin in 7.1 gm. of



TABLE NO. 1

Results of douching or infusing uteri of "Repeat Breeding" cows.

Products used.	Year	No. of cases	Av. no. of services before treatment	Cows conceiving first service		Cows conceiving first & second services	
				No.	%	No.	%
1	2	3	4	5	6	7	8
"Preservisal" (isotonic Ringers sol.+ dextrose) 500 ml. as douche of uterus, several hours before service.	1944-49	101	3.74	39	38.6	56	55.4
Lugol's solution 250 to 500 ml. of 1 to 3 percent Lugol's in distilled water as douche of uterus at time of oestrous.	1945-50	133	3.84	68	51.1	85	63.9
Tyrothricin solution, .05% in distilled water, 40 ml. injected into uterus at the time of oestrous.	1950-51	45	3.6	17	37.8	23	51.1
Penicillin and Streptomycin solutions.	1950-53	160	3.65	74	46.3	96	60.0
Total		439					
Penicillin 500,000 units Streptomycin 0.5 to 1 gm. in water and oil emulsion at the time of oestrous or during dioestrous		44	4.1	19	43.2	23	52.3
Penicillin 500,000 units Streptomycin 0.5 to 1 gm. in 20 to 40 ml. distilled water during the early part of oestrous.		86	3.2	40	46.5	53	61.6



Cont'd. Table No. 1.

1	2	3	4	5	6	7	8
Penicillin 500,000 units Streptomycin 0.5 to 1 gm. in 20 to 40 ml. of 5% so- dium sulfonamide (Sulfamerazine + sulfamethazine) from 24 to 48 hours after service.							
		30	4.2	15	50.0	20	66.6
Total		160					

ointment base; it seemed superior to Iugol's solution giving results about equivalent to those from a million units of penicillin. Bierschwal and Uren (1956) showed that aureomycin was rapidly absorbed into the blood stream after infusion into the lumen of the uterus. Greatest aureomycin activity in the blood serum occurred approximately 2 hours after infusion.

Gibbons et al. (1959) treated cows harbouring infection in the uterus with a uterine infusion of 400 mg. of tetracycline in solution. Treatment during the luteal phase of oestrous cycle failed to improve the conception rate (c.r.); indeed the 23 controls harbouring pathogens had a better c.r. (1.78) than the 32 treated cattle (1.96). Brown et al. (1959) treated post-parturient cows which had experienced difficulty in parturition, with aureomycin and sulfamezathine. It was found that breeding efficiency of aureomycin treated cows approached the level of normal cows. Naumov and Atanasov (1961) studied the comparative efficacy of different drugs in cases of endometritis. They used



through intra-uterine route a combination of penicillin, streptomycin and stilboestrol in one group; Lugol's solution and stilboestrol in second group; and chlorhexidine alone in third group and got the conception rate respectively as, 60 out of 147, 15 out of 23 and 15 out of 51. Van Waveren (1962) found slightly better results with iodine or chlorhexidine treatment as compared to antibiotic (a mixture of penicillin and streptomycin) therapy. Bahrs (1962) treated 221 cows that had failed to conceive, some of them were having uterine catarrh. A dose of 20 ml. of one of two preparations was infused into the cervix one day after second insemination during the same oestrous. Preparation E 860 contained 500,000 i.u. benzathine penicillin, 0.5 gm. sulphotolamide, 0.5 gm. chloramphenicol and 60,000 i.u. Vitamin A in 20 ml. vegetable oil. Preparation E 831 was the same without Vitamin A. Among 99 untreated control 22% became pregnant after the first insemination compared with 54 % of the 221 treated cows. Results for each of the two preparations were similar.

Wohanka and Hubrig (1962) concluded that pathogenic bacteria, introduced into the genital system during or after parturition, constituted a danger only when involution process was already impaired through metabolic or other disorders resulting from deficiency and stress during pregnancy. Rommel (1963) reported that bacterial infection of the genital tract in cows depends not only on amount, species and virulence of micro-organisms involved and the defence mechanism of the genital system, but also on the phase of oestrous cycle. Central nervous reaction to uterine inflammation caused by bacteria is similar to



that produced by pregnancy and may result in prolongation of corpus luteum phase. Inflammatory processes in turn create an environment favourable for most pathogenic bacteria.

Boiter et al. (1968) cured 208 cases out of 230 cows suffering from metritis by repeated intra-uterine injections of oxytetracycline in citrated blood, after intramuscular injection of 1 mg. per kg. of Romtiazin (Promazine maleate). They concluded that the tranquillizer produced elimination of mucus and inflammatory exudate and had no effect on the cervix of pregnant cow. Sane and Deshpande (1971) tried Mastalone-U in the transient form of infertility of cows. They tried this drug in 41 cases of infertility in cows due to uterine infections and found encouraging results.

Sabir and Bhide (1971) categorized the pharmacological action of Berberine under two broad headings (1) the stimulant actions, some of which are due to its anticholinesterase mechanism and (2) the inhibitory actions, many of which could be due to inhibition of depolarization and repolarization of the excitable tissues. They further stated that on some individual organs the net effect would then depend upon which of the two actions dominates at a given dose of berberine. The dose suggested by Bhide (1971) was 0.5 gm. twice daily through intra-uterine route for cattle.

#### Bacterial agents associated with cases of endometritis:

Until the latter half of the 19th century the workers



were too preoccupied with disinfectant treatment to show much interest in the nature of the organisms found associated with uterine pus. Albrechtsen (1917) briefly noted the recovery of Streptococci twice from pus. Quinlan (1929) made cultures from endometritis cases, and found E. coli, various types of cocci and C. pyogenes, and Bauman (1933) also got the same organisms.

In a comparative study on bacterial flora isolated from genital organs of healthy and diseased cows Sykora (1932) observed that there was no difference as regards the number of species obtained but there was preponderance of growth during cultivation from diseased cow in comparison to the slow growth from the content of healthy cows. The mucus from the cervix uteri of cows with healthy genital organs was sterile in 25% of cases; in 75% of cases cultures of Staph. aureus were isolated, but in very low numbers. From the exudate of cervix uteri of animals with diseased genital organs, the following bacteria were isolated : S. aureus (83% of cases), S. albus (33%), S. citreus (16%), Streptococcus (33%), Coliform bacillus (50%) and Gram negative bacilli (33%).

Out of 51 cases of pyometra studied by Jaeger (1934) 18% were found sterile and 25% were containing C. pyogenes. The remainder percentage comprised of trichomonads and cocci of various types. The works of Runge (1942) reported the finding of four types of microbes from pyometra cases such as B. abortus, E. coli, Cocci and C. pyogenes but did not incriminate any of the four types to be the causative agent in particular. Whereas,



Holl (1944) tagged C. pyogenes with pyometra cases in particular and stressed its association with such cases as a distinct clinical entity. His findings were also in consonance with that of Kust (1933), Jaeger (1934) and Hignett (1940). On the contrary to the above findings Kostner (1939) while analysing the isolates from 18 pyometra cases found only E. coli and Cocci and he could not isolate C. pyogenes from any one of them. His 9 cases were negative for any microbes.

Azizuddin (1950) injected bacterial suspensions of C. pyogenes, C. equi and Staph. albus into ligated segments of guinea pigs. C. pyogenes and Staph. albus gave rise to lesions comparable to those met with in natural cases of endometritis in cows. Plazas - Morales (1955) isolated the following organisms in order of frequency from the normal cervix of 20 virgin heifers, 20 pregnant cows and 60 cows in oestrous, E. coli (62), Non-haemolytic Staphylococci (55), Bacillus subtilis (43), Bact. aerogenes (13), Haemolytic Staphylococcus (11), Haemolytic Steptococcus (9), Small rod shaped Gram positive organisms (8), Paracolon (6), Proteus (6), Mycetes (3), Streptococcus faecalis (1) and Salmonella (1).

A total of 100 cows were examined by Pietzsch (1958) and bacteria in pure cultures were obtained from 18 cases of endometritis. He isolated E. coli, and Streptococci - but not S. pyogenes, Pseudomonas, Bacillus mesentericus, Proteus and Sarcina. Seven samples were bacteriologically sterile and the rest were positive for organisms of ubiquitous faecal nature. Kiesel and Dacres (1959) took 1,359 cervical swabs from cows in



40 herds which had failed to conceive to 3 services and incubated on blood agar, sabouraud's and thiol ( $\text{CO}_2$  tension) media. 383 proved positive; the most common organisms being non-haemolytic Staphylococci, E. coli, haemolytic Streptococci, especially group E and vibrio species both positive and negative to catalase.

Gavrilets (1959) isolated 28 species of bacteria from uterus and vagina of 36 clinically healthy cows; Staph. aureus was isolated from 16 cows, Staph. citreus from 15 and haemolytic Streptococci from 5. Gibbons et al. (1959) made 207 cultures from cervical mucus of 125-130 adult cattle and from 20 classified as infertile. The presence of bacteria in the genital organs had no effect on subsequent breeding.

Dawson (1960) summarised the reports of several workers and mentioned that in cases of endometritis Staphylococci (non-haemolytic more frequently than haemolytic) and E. coli were frequently found. Further he concluded that Streptococci (usually alpha and beta haemolytic) were not much less common and a large number of anthracoid isolations were reported. Bacillus proteus, Pseudomonas pyocyanea and Neisseria species were put in the group of minority organisms. On the basis of comparative data of isolation from normal cows it was revealed that there was a steep fall in percentage of "organism free" cows, a distinct rise in recoveries of both types of Staphylococci and E. coli, a considerable rise in C. pyogenes and a slight rise in Streptococci. Isolation of micro-organisms in mixed cultures were of considerable importance.

Trotter (1961) examined uteri from 74 cows which had



been culled for failure to breed and found bacteria including *Pseudomonas* and haemolytic *Staphylococci* from 45 uteri. Van - Waveren (1962) made 884 swabs from 426 cows having a mucopurulent discharge, 446 were bacteriologically sterile. Of the remainder *C. pyogenes* was present alone in 228 and with various other bacteria in 27, other *Corynebacteria* in 4; *E. coli* alone in 18 and with other bacteria in 20; non-haemolytic *Streptococci* in 26, haemolytic in 9; *B. abortus* in 33, *Staphylococci* in 32, fungi (unspecified) in 5; *Proteus* in 4; *Salmonella dublin* in 1, *Pseudomonas* in 1 and *Shigella* in 1.

Conto (1962) recovered *E. coli* at oestrous from the uterus of 3 of 20 normal cows, 3 of 4 cows with vaginal discharge that had been served 3 times or more without conceiving, and from the uterus of 5 of 6 cows that had aborted 24 hours previously. *C. pyogenes* was isolated from one of the remaining cows with vaginal discharge and one from cows that had aborted. *Proteus* species and *Staph. albus* were isolated from 3 normal cows.

Winkenwerder (1966) reported isolation of non-haemolytic *Streptococci* in 23.2% of 2962 samples from genital organs of cows. *Streptococci* apparently enter the genital organs of cattle as saprophytes and exert a pathogenic effect only during the weakened resistance of the host animal.

#### Sensitivity to antibiotics of bacteria associated with cases of endometritis :

The use of antibiotics in the treatment of genital affections was scarce upto the first half of the 20th century.



Disinfectants were the treatment of choice. But now a days antibiotics are very commonly used in the treatment of endometritis. It would always be of great advantage if proper examinations are made for isolating the organisms from the uterine discharge of endometritis suffering cows and antibiotic sensitivity is done. The result will be definitely better, when specific antibiotic is applied for specific type of organisms in vivo also. Unfortunately very little informations are available regarding antibiotic sensitivity test in connection with treatment of endometritis.

#### E. coli :

Escherichia coli was isolated from faeces of a breast-fed infant by T. Escherich in the year 1885. He considered it to be a saprophyte. Jensen (1896) considered it to be responsible for endometritis in dogs.

Pulvertaft (1952) made tests with penicillin, streptomycin, aureomycin, chloramphenicol and terramycin on growing cultures of E. coli. With penicillin enlargement of the organism occurred at all concentrations followed by lysis, with the other four antibiotics death occurred at high concentrations and at low concentrations enlargement of the organism and monster formation without division occurred. Smith and Crabb (1956) found all the strains of E. coli obtained from the cases of white scour to be sensitive to chloramphenicol.

Glantz (1962) performed the in vitro sensitivity test



of 287 strains of E. coli isolated from animals and poultry to antibiotics and nitrofurans. The most effective compounds were colistin, chloramphenicol, furazolidone, N- (5-nitro-2 furfurylidene)-1-amino-2-pyrrolidone, thiofuradene and polymyxin. The activity of dihydrostreptomycin, chlortetracycline, tetracyclin and oxytetracycline were intermediate. Penicillin, oleandomycin, furaltadone and nidroxyzone were least effective.

Malik (1963) isolated about 140 strains from sheep, cattle, horses and dogs and found that most effective antibiotics against their isolates were neomycin, tetracycline, oxytetracycline and chloramphenicol; both streptomycin and chlortetracycline were only moderately effective and penicillin and erythromycin had no action.

Glantz (1965) tested 530 strains of E. coli for their in-vitro sensitivity to 13 sulfonamides by broth tube and disc method. The majority of strains were sensitive to 0.25 to 1 mg./ml. levels of all compounds tested. McKay et al. (1965) listed the results of sensitivity tests on animal pathogens conducted during the period of 1956-63. The strains of E. coli and salmonella species showed evidence of marked increase in resistance. They tested a total of 3,898 E. coli strains. The results disclosed that while in 1957 the percentage of resistant strains of E. coli isolated from clinical pathological specimens was 60%, it rose to 90% by 1963. The tetracycline resistant strains increased from 0% to 29% during these years. The corresponding increase in percentage of neomycin and chloramphenicol resistant strains during these years were 10% to 50% and 8% to 22%



respectively.

Truszczyński et al. (1966) conducted in-vitro sensitivity tests of 200 E. coli strains isolated from oedema disease or enterotoxaemia in pigs. Among them all were resistant to penicillin and erythromycin, moderate resistance was observed to chlortetracycline and tetracycline while least number of strains were resistant to streptomycin and chloramphenicol.

Kondracki (1967) performed sensitivity of 192 E. coli isolates with neomycin, streptomycin, chloramphenicol, oxytetracycline, chlortetracycline and penicillin. It was found that 6.8, 31.3 and 52.3% of the isolates were highly sensitive to neomycin, streptomycin and chloramphenicol; 55.2, 28.6, 15.1, 2.1 and 0.5% were moderately sensitive to each of the first five antibiotics mentioned and 34.9, 25.5, 0.5, 7.9, 5.8 and 3.6% were weakly sensitive to each of first six antibiotics.

✓Bugeac (1969) tested 158 strains of E. coli isolated from unweaned piglets or sows with metritis against seven chemotherapeutic agents. The tests indicated that polymyxin, neomycin, furazolidone, chloramphenicol and kenamycin were most active against the bacteria. About 70% of the strains were resistant to the tetracyclines.

✓Andreani et al. (1969) found the most effective drug to be nitrofurazone in a sensitivity test carried out on 215 strains of E. coli isolated from various species of animals. Many of the strains were resistant to streptomycin, chlortetracycline and chloramphenicol.



Tiwary (1969) carried out sensitivity test of 61 calf strains of E. coli to seven antimicrobial drugs and found that the most effective drugs were furadentine (100%), chloromycetin (98.3%) and ampicillin (95%). Erythromycin and streptomycin were moderately effective and steclin and ledermycin were less effective.

### Salmonella :

Several workers have reported a significant increase in the proportion of salmonella that exhibit resistance to tetracyclines and to chloramphenicol. Rahman (1957) tested various bacteria to penicillin, oxytetracycline, chloramphenicol and other chemotherapeutic agents and suggested chloramphenicol to be the best drug for salmonella infection.

Joachin et al. (1959) studied the action of chloramphenicol, tetracycline, oxytetracycline and chlortetracycline against 110 strains of salmonella and found only 8 strains resistant to tetracycline and 1 to chloramphenicol. He concluded that the organism was highly sensitive to above mentioned antibiotics. Nystrom (1960) compared the action of 12 antibiotics, sulfadiazine and nitrofurantoin on 322 salmonella strains and reported chloramphenicol to be most effective. Smith (1960) found all except one of 106 strains of salmonella from pigs to be sensitive to oxytetracycline, streptomycin, chloramphenicol and furazolidone.

Ramsey and Edwards (1961) reported that whereas only 5% of 100 strains of S. typhimurium isolated from man in 1956-57



were resistant to tetracyclines, 14% of 158 strains isolated in 1959-60 were resistant. Manten et al. (1961) reported a slight decrease between 1958-60, but a significant increase between then and 1961. Mc Whorter et al. (1963) found that S. typhimurium showed a higher incidence of resistance to chlor-tetracycline than did other serotypes. Of 48 strains from cattle, 45 (4%) were resistant. Mc Kay et al. (1965) reported the results of sensitivity tests on animal pathogens conducted over period of 1956-1963. They reported that in 1957, strains of Salmonella typhimurium infection in the bovine was quite sensitive to streptomycin. Since then a high incidence (90% in 1963) to this drug was observed. In most cases a good response to treatment was obtained by the use of neomycin, chloramphenicol, nitrofurazone, chlorhexidine or a combination of these drugs in salmonellosis in all animal species.

Settens (1968) found that none of the 1024 salmonella strains was resistant to chloramphenicol or to nitrofurazolidone, whereas 4 were resistant to sulfamethizole, 8 to streptomycin and 6 to tetracycline. Popa et al. (1968) reported all the strains to be relatively or totally resistant to streptomycin and penicillin, high or moderate sensitivity was shown by 48% of the strains to chloramphenicol and by 30% to tetracycline.

Chung (1969) reported no strain of salmonella to be resistant to either tetracycline or chloramphenicol. Tiwary (1969) found salmonella to be resistant to ledermycin, streptomycin and steclin but with ampicillin, erythromycin, chloromycetin and furadentine they were sensitive.



Staphylococci :

Abraham et al. (1941) reported majority of Staphylococci to be sensitive to penicillin. But with the passage of time and increased use of antibiotics, it was brought to notice that the acclaimed penicillin was found ineffective in some of the staphylococci infections. Fleming (1942) occasionally encountered insensitive strains of Staphylococci. Similar observation was also made by Hobby et al. (1942).

Aynsley (1953) examined 500 cultures from cases of bovine mastitis using ditch plate technique, the ditch containing 2 units/ml. of penicillin and reported that 14 cultures of Staphylococci were resistant to penicillin. The inhibiting concentration of penicillin fell between 0.02 and 5.0 units/ml. when these 14 strains were tested by serial dilution method.

Calaprice (1959) isolated pathogenic Staphylococci from the infected genital tracts of cows and tested for their antibiotic sensitivity. The majority were markedly resistant or only slightly sensitive to penicillin, streptomycin and sulphathiazole. Some of these were also resistant to erythromycin and chloramphenicol. Rehder (1960) tested 671 isolates of Staphylococci from cases of mastitis against penicillin, streptomycin, chlortetracycline and oxytetracycline. Only ten showed resistance to chlortetracycline and all were sensitive to sulphonamides.

Mondini and Gasparini (1960) found that out of 51 Staphylococci strains, isolated from cow's milk and considered



pathogenic, 43 were sensitive to sodium penicillin, 46 to penicillin V and tetracycline, 45 to streptomycin and spiramycin, 48 to chloramphenicol and 14 to sulphonamides.

Overjero et al. (1960) stated tetracycline erythromycin and chloramphenicol to be highly active against strains of staphylococci isolated from gangrenous mastitis. Penicillin showed little activity. St. George et al. (1962) isolated 746 coagulase positive Staphylococci from 4,834 quarter samples out of which 39 were resistant to penicillin, 110 to streptomycin, 29 to chlortetracycline and 25 to oxytetracycline.

Panduranga Rao et al. (1966) tested 137 strains of Staphylococcus aureus of bovine mammary origin for their sensitivity to penicillin, streptomycin, aureomycin, chloromycetin and terramycin. Of these 120 (87.59%) were sensitive to penicillin, 119 (88.86%) to streptomycin and all to aureomycin, chloromycetin and terramycin. Of the strains resistant to either penicillin or streptomycin, 14 (10.22%) were resistant to both. Ostashevskii and Obrastsov (1968) tested 74 strains of Staphylococci for their sensitivity; 49 were sensitive and 18 were resistant to penicillin, 60 sensitive and 11 resistant to chlortetracycline, 52 sensitive and 7 resistant to oxytetracycline.

Dittus (1969) carried out sensitivity tests on 1,000 strains of Staph. aureus from bovine mammary origin against penicillin, streptomycin, tetracycline, chloramphenicol, erythromycin, kenamycin and cloxacillin. 100% were sensitive to one or more antibiotics. Out of 1,208 strains of Staph. aureus from



bovine udder Ziv (1969) found 30% to be sensitive to penicillin G, 60% to dihydrostreptomycin, 80-85% to chloramphenicol and tetracycline.

### Pseudomonas :

The micro-organism, Pseudomonas pyocyanea was first isolated from "blue pus" by Gessard in 1882 and from time to time it has been recorded to be associated with numerous affections in man and animals. It has also been known to be a causative agent of infertility in bulls (Narsing Rai and Natrajan, 1958). This organism was also isolated from the uterus of cows suffering from endometritis (Pietzsch, 1958) while Getty and Ellis (1967) produced endometritis, cervicitis and vaginitis by inseminating virgin heifers with the semen contaminated with Pseudomonas.

Schalm (1957) studied the sensitivity of 27 strains of Pseudomonas aeruginosa to tetracycline, aureomycin, terramycin, penicillin, erythromycin, chloramphenicol and dihydrostreptomycin. He found all the strains resistant to these antibiotics with the exception of chloramphenicol, dihydrostreptomycin and neomycin which inhibited 26.1%, 38.5% and 17.4% respectively. Farrag (1959) carried out sensitivity of 10 strains of P. aeruginosa from various affections of animals against six various antibiotics and found that all the strains were resistant to penicillin but sensitive to dihydrostreptomycin, one strain was sensitive to tetracycline while 2 strains were sensitive to chloramphenicol.



Gancik (1959) found that with the addition of a mixture of streptomycin, penicillin and polymyxin the bull semen infected *Pseudomonas* became free from this organism. Thus it suggested the synergistic effect of the three antibiotics. Farrag and Oof (1967) studied the sensitivity of 26 strains of *C. pyogenes*, 5 *C. ovis*, 15 *Staphylococcus aureus*, 14 *Streptococcus pyogenes*, 12 *E. coli* and 9 *Pseudomonas aeruginosa* isolated from cases of goat and bovine mastitis against eight different antibiotics. With the exception of *P. aeruginosa*, tetracycline and chloramphenicol gave the best results in inhibiting the majority of the organisms tested. Penicillin, erythromycin and novobiocin had no inhibitory effect on *E. coli* and *P. aeruginosa*, whereas streptomycin inhibited the majority of strains of these two organisms. *P. aeruginosa* was more or less resistant also to kanamycin and neomycin.

Cristea *et al.* (1969) tested 155 strains of *Pseudomonas* of animal origin for their sensitivity against various antibiotics. They found all the strains resistant to benzylpenicillin but 26% sensitive to streptomycin. 88% were resistant to chlor-tetracycline. Hogle (1970) reported this organism to be the most resistant to antibiotics and nitrofurazone in comparison to *E. coli* and *Staphylococcus aureus*.

### Streptococcus :

Relatively little work has been reported regarding the sensitivity of this organism to different antibiotics.



Farrag (1948) carried out studies on the action of penicillin in-vitro against 50 strains of Streptococcus agalactiae and found that they differed widely in sensitivity to penicillin and this variation may offer an explanation for the frequent failure in treatment of Streptococcal mastitis. But Ford and Wilson (1953) studied in-vitro action of penicillin on 281 strains of Strept. agalactiae and observed that none was resistant. Edwards (1952) studied the in-vitro and in-vivo activity of penicillin and found that it was most effective against Gram positive organisms especially Streptococcus agalactiae and other mastitic Streptococci as compared to Gram negative organisms.

Whitby and Hynes (1956) reported Strept. pyogenes to be very susceptible both to sulphonamides and many antibiotics like penicillin, streptomycin, tetracycline, etc. Strept. faecalis was resistant to sulphonamides but moderately susceptible to antibiotics other than penicillin. Minimum inhibitory concentration for penicillin, streptomycin, erythromycin, chloramphenicol and tetracycline were 3, 50, 2, 10 and 0.5 microgramme per ml. respectively. Rahman (1957) found that Strept. pyogenes, Strept. equi, and Strept. zooepidemicus all the three were sensitive to penicillin, and to chlortetracycline, oxytetracycline and chloramphenicol in increasing order.

Burrows (1959) reported that chemotherapeutic activity of sulphonamides was overshadowed by antibiotics, especially penicillin. The haemolytic streptococci were more susceptible to these agents in-vitro than the Staphylococci. Berger (1959) tested 103 strains of beta haemolytic Streptococci in-vitro for



their nitrofurazone sensitivity and found strains of serological group B and D to be resistant. Kuntev (1962) investigated sensitivity of strains of Streptococci, E. coli, C. pyogenes and Staphylococci to antibiotics and sulphonamides. All organisms were sensitive to a high or moderate degree to penicillin, streptomycin, chloramphenicol, oxytetracycline and sulphonamides.

Prasad and Prasad (1963) reported that out of 45 strains of Streptococci isolated from milk sample of cows approximately 2.2% were found to be resistant to penicillin concentration of 0.5 unit per ml. While Kortum (1963) found 0.22% penicillin resistant strains out of 452 strains of Streptococci isolated from bovine mastitis. The author also found 0.9% resistant to chloramphenicol, 5% tetracycline, 33% streptomycin and 53% to neomycin. Overgoor (1966) reported that most of the 104 streptococcal strains from pneumonia cases were sensitive to penicillin, streptomycin, chloramphenicol and tetracycline. Jacobs and Hanselaar (1967) concluded that all the strains of Streptococci of bovine mastitis origin were more or less sensitive to penicillin, ampicillin and cephaloridine and resistant to streptomycin, neomycin, tetracycline and novobiocin.

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## **MATERIALS AND METHODS**



## MATERIALS AND METHODS

Experiments were carried out on non-descript cows which came for gynaecological check-up at the Department of Gynaecology, Bihar Veterinary College, Patna. In each case history of the animal was recorded in relation to number of calvings, number of artificial inseminations or natural services done, oestrous cycle, whether regular or irregular. After obtaining the history the animals were subjected to the detailed gynaecological examinations to diagnose endometritis. Only those cases were taken up for the purpose of study which were having a continuous mucopurulent uterine discharge resembling second degree endometritis (Renter, 1926). These cases were also in close approximation to Dwason's (1951) group B having mucopurulent uterine discharge with little or no evidence of pathological cyclic impairment. The precedence followed by Nielsen (1926) was adopted in considering all cases in existence beyond 3 weeks post-partum, as "chronic" rather than "puerperal" endometritis. Most of these cases had attracted the attention of animals' owners, but some were only detected during the course of routine clinical examination.

Endometritis was confirmed on the basis of recto-vaginal examinations and relevant history pertaining to the disease.

History of the case : - Owners were inquired regarding the last calving, whether it was a normal or assisted delivery;



regarding regularity of oestrous cycle; regarding number of inseminations or natural services tried without leading to conception. If the abnormal uterine discharge was apparent and had attracted the attention of owners, they were asked regarding the time when they first noticed it.

Speculum examination : - The vulva and perinium of the animal was washed and dried. The vaginal examination was carried out with the help of a well sterilized speculum. In almost all the cases when the speculum was applied a mucopurulent discharge was evoked. The side wall of the vagina was examined for the presence of granules, inflammation or any other abnormality. The os-uteri was examined and found to be partially opened, enlarged and inflamed in most of the cases.

Rectal examination : - It was carried out to manipulate the genitalia. Inflammation of os-uteri was confirmed by detectable enlargement in the size and induration of the organ. The horns of the uterus were examined after feeling the body. The horns were manipulated for any enlargement in their size either unilateral or bilateral, tone and thickness of the wall. The fallopian tubes were then examined for any enlargement in their size. Manipulation of the ovaries was done for any persistent corpus luteum or cysts, their size and shape, and adhesions if any.

Cervicitis either in moderate or severe form was found invariably present along with endometritis. Both the cornua in cases of endometritis were generally found to be enlarged,



atonic, doughy and thick-walled (Devine, 1920; Nielsen, 1926; Tutt, 1933; Craig, 1936; Williams, 1943; Roberts, 1956 and others). In some cases a tone in the uterus similar to the condition of normal heat was also detectable. The ovaries were palpated to be normal and functioning, the size and shape were not identical because of individual variations.

Cases of chronic cervicitis with marked induration, salpingitis and adhesions of ovaries were not taken-up for the purpose of treatment to maintain uniformity.

A total number of five drugs were tried in the present study to obtain their relative efficacies against second degree chronic endometritis. Five groups were made and each group comprised of 21 animals. Thus a total number of 105 cases were undertaken in the present study.

Group - A : - In this group 20 ml. of Lugol's iodine solution was used. Its concentration was kept to 0.5% as suggested by Dawson (1960). It was prepared as follows :-

Iodine.	2.5 gm.
Potassium iodide.	6 gm.
Sterile distilled water.	500 ml.

The iodine and potassium iodide were mixed together in a mortar with the help of a pestal. 500 ml. of sterile distilled water was added to it and kept in a amber coloured bottle.



Group - B : Uni-Berbyl Tablets (Unichem).

Each tablet contained 200 mg. of berberine hydrochloride and 800 mg. of glucose. 5 such tablets were dissolved in 20 ml. of sterile distilled water and was given intra-uterine.

Group - C : Aureomycin oblets (Cynamid).

2 oblets each containing 500 mg. of chlortetracycline hydrochloride were dissolved in 20 ml. of distilled water (sterile) and was administered through uterine route.

Group - D : Steclin bolus (Squibb).

2 bolus each containing 500 mg. of tetracycline hydrochloride were dissolved in 20 ml. of sterile distilled water and administered through uterine route.

Group - E : Mastalone - U (Pfizer).

Composition - Each vial contained :

Oxytetracycline hydrochloride I.P.	200 mg.
Oleandomycin Phosphate N.F. equivalent to of oleandomycin base.	100 mg.
Neomycin sulphate I.P. equivalent to of Neomycin base.	100 mg.
Prednisolone I.P.	5 mg.
Chlorpheniramine melete.	100 mg.

To each vial 10 ml. of sterile distilled water was added. After reconstitution the entire contents were drawn



into the syringe and introduced into the uterus.

Distilled water was sterilized in autoclave at 120°C and 15 lb. pressure for 20 minutes. To maintain uniformity in treatment each drug was administered intra-uterine every alternate day and five intra-uterine medications were done with each drug. Thus comparative efficacy of drug was studied within the stipulated period of 9 days.

The cows were examined every alternate day during the course of treatment and if the animal was found to be cured, no further treatment was applied even though it had not completed its course of five treatments. After completion of five treatments schedule the owners of the animals were requested to bring them when they were observed to be in heat subsequently. In oestrous the animals were again examined recto-vaginally and if the discharge was found obviously normal and the genitalia revealed all the features of follicular dominance the animals were used to be sent for insemination. Owners of the animals were requested to bring the animals again after 1½ to 2 months for pregnancy diagnosis.

The cases were taken to be cured when the abnormal uterine discharge disappeared during the course of treatment and the oestral flow subsequent to treatment became obviously normal. The cases were categorised as incurable or refractory to treatment when the abnormal uterine discharge persisted even after 5 medications or when the first oestral flow on completion of treatment schedule still contained pus flakes or was



obviously pathological.

Statistical analysis : - After the completion of trials with the drugs, observations were subjected to statistical analysis by Chi Square test according to the formula given by Panse and Sukhatme (1961).

$$\text{Chi Square} = \frac{1}{n_1 n_2} \frac{(an_2 - bn_1)^2}{a + b}$$

where a and b stand for the frequencies in the two classes and  $n_1$  and  $n_2$  for corresponding totals in the respective classes.

Bacterial agents associated with cases of endometritis :

From among the cases of endometritis in cows reporting to Department of Gynaecology, Bihar Veterinary College, Patna for treatment, 19 cases were sorted out.

Uterine discharge appearing at the cervix was collected for bacteriological examination and antibiotic sensitivity test in-vitro. The particular organism sensitive to a particular antibiotic in-vitro was tried in-vivo also.

Method of collection of material : - Cotton swabs were prepared on a straight copper wire of 14"-18" in length. It was placed in a test tube (6"x3/4") and plugged with cotton. All the swabs thus prepared were sterilized at 180°C for one hour.

At the time of collection all possible precautions were taken regarding sterilization of hands, protective clothings and





Photograph showing the swab used  
for collection of uterine  
discharge



globes. The vulva and perinium of the animal was washed with soap and water and dried with a piece of sterile gauze. Rectified spirit was then applied on the vulva with the help of a cotton swab and sufficient time was allowed for spirit to dry.

In order to ensure that the discharge is collected from the cervix and the vaginal secretions are eliminated as far as possible the following procedure was followed.

Left hand was introduced into the rectum and the cervix was secured. A trained attendant was asked to insert the speculum in the vagina so as to expose the cervix. The area around the cervix was washed with cotton soaked in 70% alcohol, with the help of a sterilized uterine forcep. 2 to 3 minutes time was allowed for the alcohol to dry. To hasten the process of drying the area around the cervix was mopped with the sterilized cotton. Now the plug of the test tube containing the swab was opened over the flame of spirit lamp held by another attendant as near the vulva of the animal as possible. The swab was taken out with the right hand and inserted into the vagina upto the cervix through the speculum already fixed. With the help of left hand already introduced into the rectum, the uterine horns were squeezed and the fresh discharge just coming out of cervix was collected by giving a rotatory movement to the swab. The swab was then taken out and put back into the test tube over the flame of spirit lamp and was replugged with cotton. The material thus collected was brought to the laboratory and processed for further examination.



Media used and their preparations : - The following media were used for the isolation of organisms :

(a) Nutrient Broth tube :

Composition :	Peptone -	10 gm.
	Meat extract (Lab. Lamco)	10 gm.
	Sodium Chloride.	5 gm.
	Water.	1 litre.

The ingredients were mixed and dissolved by heating briefly in the steamer. The pH was adjusted to 8.0, heated at 100°C for 30 minutes, cooled filtered and finally adjusted to pH 7.5. Poured in test tubes and sterilized by autoclaving at 120°C for 15 minutes. The test tubes broth were incubated overnight at 37°C to test for sterility.

(b) Nutrient agar plates : - Nutrient agar was prepared by adding 2.2% of "agar-agar shreds" in the nutrient broth. After autoclaving it was poured in petri dishes and cooled to solidify. The petri dishes were kept overnight in the incubator at 37°C to test for sterility.

(c) Blood agar plates : - It was prepared by adding sterile blood to sterile nutrient agar that had been melted and cooled to 55°C. 10% of ox blood was added to it. The medium was poured in petri dishes and incubated overnight at 37°C to test its sterility.

(d) Mac-Conkey's Agar plates : -

Composition :	Peptone -	20 gm.
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Sodium taurocholate.	5 gm.
Water.	1 litre
Agar.	20 gm.
Neutral red solution, 2% in 50% ethanol.	About 3.5 ml.
Lactose 10% aqueous solution.	100 ml.

The peptone and taurocholate (bile salt) was dissolved in water by heating. The agar was added and dissolved in autoclave. Filtered and adjusted the pH to 7.5. The lactose and neutral red was added and mixed. Heated in autoclave with "free steam" (C.100°C) for 1 hour, then at 115°C for 15 minutes. Before using, the plates were incubated overnight at 37°C to test for sterility.

(ed) Brilliant green agar plates : - The medium was prepared as Mac-Conkey's agar but with the addition of 0.04 gm. of brilliant green per litre.

Stains used : - Gram's stain.

Composition : (a) Carbol Gentian violet :

Saturated alcoholic solution of gentian violet.	1 Part
5% solution of phenol in distilled water.	10 parts.

(b) Gram's iodine :

Iodine -	1 gm.
Potassium iodide -	2 gm.
Distilled water -	300 ml.



(c) Dilute carbol fuchsin :

Ziehl Neelsen's carbol fuchsin.	1 part.
Distilled water.	9 parts.

(d) 70% alcohol :

Absolute alcohol.	70 ml.
Distilled water.	30 ml.

Preparation of inoculum and inoculation of culture media : - The swab containing the material from the genital tract was placed in a test tube containing 3 ml. of nutrient broth (pH 7.0) and shaken thoroughly. The broth tubes containing the swab were kept overnight in the incubator at 37°C. A loopful of broth culture was taken and streaked on blood agar plates and incubated at 37°C for 24 hours. From blood agar plates different types of colonies were picked up and streaked on separate nutrient agar plates and incubated overnight at 37°C. Different types of organisms growing in various colonial morphology present on these nutrient agar plates were examined for their staining and morphological characters. The colonies giving single type of organisms were transferred to nutrient agar slants and incubated overnight at 37°C and stored in refrigerator. Colonies having mixed organisms were again purified in the same way on nutrient agar plates and stocked. The detailed staining and morphological characteristics of the organisms were studied. For this purpose smears were made on clean grease free slides and stained with Gram's stain.

Staining procedure : -

1. Stained in carbol gentian violet for 2 to 3 minutes.



2. Poured off the stain, replaced with Gram's iodine solution and allowed to act for 1 minute.

3. Dried thoroughly by blotting.

4. Decolourised with 70% alcohol, until the stain ceased to be removed.

5. Counter stained with dilute carbol fuchsin for 10 to 25 seconds. Washed with water and dried.

Gram positive - Dark violet.

Gram negative - Pink.

Cellular morphology of the organisms was observed after staining them with Gram's stains. The organisms were found to belong to a particular group of bacteria such as Gram positive cocci and Gram negative rods.

Gram positive cocci were further examined for their colonial morphology on the different media such as nutrient broth, nutrient agar plate and blood agar plates. Whereas Gram negative rods were cultured on different media such as nutrient broth tube, and nutrient agar, blood agar, Mac-Conkey agar and brilliant green agar plates.

Cultures giving colonial morphology as detailed below were attributed to the different group of organisms. Cultures giving uniform turbidity in nutrient broth and small, smooth, glistening, finely granular colonies, which on staining with Gram's stain formed a chain of Gram positive cocci were taken to be streptococci. They were further put on blood agar plates



for haemolysis test. Cultures giving uniform turbidity with a powdery sediment in nutrient broth and round, smooth, glistening, opaque, convex and of golden yellow or white colour on nutrient agar plates which gave on Gram's staining a Gram positive cocci arranged in bunches were attributed to Staphylococci. Further they were put to coagulase test for differentiating pathogenic cocci from that of non-pathogenic.

**Coagulase test** : - All the Staphylococci strains were examined for coagulase production. For this purpose, citrate phosphate buffer was prepared as mentioned below :

**Solution - I.**

Disodium hydrogen phosphate	-	71.6 gm.
Distilled water	-	1000 ml.

**Solution - II.**

Citric acid.	-	21 gm.
Distilled water.	-	1000 ml.

82.35 ml. of solution I was added to 17.65 ml. of solution II. The pH was adjusted to 7.0.

**Procedure** : - 5 drops of an overnight culture in nutrient broth were added to 0.5 ml. of rabbit plasma diluted to 1 : 10 with a mixture of one part of citrate phosphate buffer and two parts of physiological saline. The reading was taken after two hours and 4 hours incubation at 37°C and after keeping overnight at room temperature. It was observed that none of the strains



showed positive reaction on coagulase test at two hours. Maximum number of strains showed reaction on 4 hours incubation while a few remaining were positive for coagulase after keeping overnight at room temperature. All the strains yielding positive result either at 4 hours or after standing overnight were taken as coagulase positive

Cultures producing greenish blue colouration in nutrient broth and large, low convex, with an irregular spreading translucent edge with dark and greyish centre on nutrient agar and on staining with Gram's stain appeared to be Gram negative rods, were considered to be of *Pseudomonas* group.

Cultures giving pink (lactose fermenting) colonies over Mac Conkey's agar plate were transferred to nutrient broth for testing motility and morphology of the organisms. Smears from nutrient broth were stained with Gram's stain and the organisms showing Gram negative short rods were put to biochemical and sugar fermentation tests. Organisms giving Indole positive, M.R. positive, Citrate negative, Nitrate positive, V.P. negative were taken to be E. coli. Sugar fermentation tests with glucose, sucrose, mannitol, dulcitol, arabinose and salicin were carried out.

Cultures giving non-lactose fermenting white colonies over McConkey's agar plate were again streaked over brilliant green agar plate and incubated at 37°C for 24 hours. White colonies with marked discolouration of media around them were suspected to be *Salmonella* and were put to biochemical and



sugar fermentation tests. The culture which showed Indole negative, M.R. positive, Nitrate positive, V.P. negative and Citrate positive and which fermented glucose with production of acid and gas, mannitol, dulcitol and sorbitol and failed to ferment lactose and sucrose was assigned to genus *Salmonella*.

#### Fermentation of Carbohydrates : -

The fermentative activity of the isolates of coli group and *Salmonella* were tested by employing different sugars.

Peptone water medium was used for different sugar media. The percentage of sugar in peptone water medium was 1%. Andred's indicator was used for the detection of acid production. Sugar solution was first prepared as stock solution of 10% strength. It was sterilized in Koch's fractional sterilizer. The peptone water medium was sterilized in autoclave at 15 lb. pressure and  $121^{\circ}\text{C}$  for half an hour. Sugar solution was incorporated in peptone water medium so as to get a final concentration of 1% sugar in peptone water sugar medium. The medium was poured in Durham's fermentation tube and steamed for 30 minutes. The organisms were inoculated in each of the sugar medium and incubated at  $37^{\circ}\text{C}$  aerobically for 72 hours. Appearance of pink colour in the inoculated tube was taken as indicator of fermentation.

#### Methyl-red and Voges-Proskauer test :

The test strains were grown in 5 ml. of glucose-phosphate peptone water medium for 72 hours. Five drops of methyl



red indicator (0.04%) was added to culture. Appearance of red colour was indicative of positive result whereas yellow colour indicated negative result.

The Voges-Proskauer test was performed by adding about 1 ml. of 40% caustic potash solution to 72 hours grown culture of the test strain in glucose phosphate peptone water medium and was then left at room temperature overnight. Development of brick red or pink colour was taken as positive and no colouration or slightly yellow colouration was considered as negative.

#### Indole production test :

The test strains were grown in peptone water tubes for 48 hours at 37°C. One ml. of ether was added to each tube containing 5 ml. of the inoculated medium and shaken well. One ml. of Ehrlich's reagent was run down by the side of the tube. Development of deep red colour denoted the presence of indole.

#### Nitrate reduction test :

The test strain was grown in plain broth containing 2% potassium nitrate for 48 hours at 37°C. The presence of nitrite was tested by adding a few drops of 'Test solution-A' (containing 8 gms. of 0.5 percent sulphanilic acid in dilute sulphuric acid) and 'Test solution-B' (containing 6 ml. of dimethyl alphanaphthylamine in 1 litre of acetic acid) successively to about 2 ml. of the culture. Presence of red colour denoted the presence of nitrite.



### Citrate utilisation test :

The medium recommended by Koser (1923) was used for this test. It consists of -

Sodium ammonium hydrogen phosphate	-	1.5 gm.
Potassium dihydrogen phosphate.	-	1 gm.
Magnesium sulphate.	-	0.2 gm.
Sodium citrate.	-	2 gms.
Distilled water.	-	1 litre.

The test strains were grown in the above mentioned media in 5 ml. test tubes for 48 hours at 37°C. The production of a visible turbidity indicated a positive reaction.

### Sensitivity to antibiotics of bacteria associated with cases of endometritis :

The bacteria isolated from the uterine discharge were put to sensitivity test with five types of antibiotics, viz., Penicillin, Ambistryn, Chloromycetin, Ledermycin and Terramycin. Pour plate technique was followed on the methods described by Panduranga Rao et al. (1966).

Nutrient agar plates containing critical concentration of five different antibiotics were used for determining the sensitivity of the organisms isolated. In each case stock solution of the antibiotic was added to the molten agar cooled at 50°C. After mixing the plates were prepared by pouring 15 ml. of antibiotic containing agar in each plate of about 10 cms. in diameter. The plates were allowed to set and then dried in the incubator



before use.

The different antibiotics which were employed in this study were as follows :-

TABLE NO. 2.

Sl. No.	Trade Name	Compound	Form of supply.	Contents
1.	Penicillin G Sodium I.P. (Squibb).	Penicillin G Sodium I.P. Buffered crystalline.	Vials.	200,000 units
2.	Ambystin (Squibb).	Streptomycin Sulphate.	Vials.	1 gm.
3.	Ledermycin (Cynamid)	Demethylchlor-tetracycline hydrochloride.	Capsule.	250 mg.
4.	Terramycin (Pfizer).	Oxytetracycline hydrochloride.	Capsule.	250 mg.
5.	Chloromycetin (Parke-Davis).	Chloramphenicol.	Capsule.	250 mg.

Preparation of Penicillin-agar plates :

A stock solution of penicillin containing 100 i.u./ml. of the antibiotic was prepared by adding 5 ml. of normal sterile saline (N.S.S.) to a vial containing 200,000 i.u. of crystalline penicillin G. sodium. After mixing, 0.1 ml. of this was transferred to a sterile bottle containing 39.9 ml. of N.S.S. This



was stored in the refrigerator for one week and discarded thereafter. For preparation of plates 0.6 ml. of stock solution was added to 99.4 ml. of molten agar cooled to about 50°C. The concentration of penicillin in the culture medium was 0.6 i.u./ml.

#### Preparation of Streptomycin agar plates:

A stock solution containing 1000 microgram/ml. of streptomycin was prepared as follows : Five ml. of sterilised normal saline was added to a vial containing 1 gm. of streptomycin sulphate. After mixing the contents 0.2 ml. of this solution was transferred to a sterile bottle containing 39.8 ml. of N.S.S. This solution was stored in the refrigerator for two weeks, and during this period one ml. of the above said stock solution was added to 99 ml. of molten agar to get a concentration of 10 microgramme streptomycin per ml. of culture medium.

#### Preparation of Ledermycin agar plates :

A stock solution containing 1000 microgramme per ml. chlortetracycline hydrochloride was prepared by dissolving 250 mg. chlortetracycline in 250 ml. of N. S.S. The stock solution was stored in refrigerator upto 48 hours, and after that fresh solution was prepared. 0.6 ml. of the stock solution was added to 99.4 ml. of molten agar to obtain a final concentration of 6 microgramme of antibiotic per ml. of culture medium.

#### Preparation of Terramycin agar plates :

Stock solution containing 1000 microgramme per ml. of



oxytetracycline was prepared by dissolving 250 mg. of oxytetracycline hydrochloride in 250 ml. of N.S.S. This solution was then stored in a refrigerator upto 5 days and thereafter fresh solution was prepared. A final concentration of 6 microgramme oxytetracycline per ml. of the medium was obtained by adding 0.6 ml. of the stock solution to 99.4 ml. of molten agar.

#### Preparation of Chloromycetin agar plates :

Stock solution containing 1000 microgramme per ml. of chloromycetin was prepared as follows : The content of one capsule containing 250 mg. of chloromycetin was emptied into a sterile flask containing 250 ml. of N.S.S. and mixed well. The solution was sterilised by heating to boil and then cooled and stores in the refrigerator. To get a concentration of 6 microgramme of antibiotic per ml. of the medium, 0.6 ml. of the stock solution was added to 99.4 ml. of molten agar cooled to about 50°C.

#### Inoculation and incubation of plates :

Five-millimeter loopfuls of 18-24 hours broth cultures were used for inoculating the antibiotic agar plates. Each plate was divided into 4 equal sectors and one sector was used for each strain. Nutrient agar plates without any antibiotic were also similarly inoculated simultaneously.

#### Reading of results :

Complete inhibition of growth was taken as indication



of sensitivity whereas any amount of growth was interpreted as an indication of resistance. The critical concentrations of different antibiotics employed in this study was taken according to Schaub and Foley (1952) which has been tabulated below:-

TABLE NO. 3

Sl. No.	Antibiotics used	Critical concentration.
1.	Penicillin.	0.6 i.u./ml. of agar.
2.	Streptomycin.	10 microgramme/ml. of agar.
3.	Ledermycin.	6 microgramme/ml. of agar.
4.	Terramycin.	6 microgramme/ ml. of agar.
5.	Chloromycetin.	6 microgramme/ ml. of agar.

#### In-vivo test of antibiotics :

Based on the results of in-vitro sensitivity tests suitable antibiotic was selected and treatment was given to the particular cow. The doses of different antibiotics used in in-vitro tests were calculated for in-vivo trial. Cows that recovered from the disease after antibiotic therapy were taken to be an indication of resemblance of sensitivity of the organisms to that particular antibiotic in-vitro and as well as in-vivo.



Those antibiotics which failed in killing the organisms in-vivo i.e., failed in curing the disease were noted to be effective only in-vitro sensitivity test.

For in-vivo trials the cows were called on the 5th day from the date the swab was taken. On the day of swabbing the posterior portion of vagina of the animals were painted with glycerine as placebo after taking all the aseptic precautions.

Out of 19 cases taken for in-vitro test only 16 cases could be tested in in-vivo sensitivity test.

The five days gap was sufficient for achieving at a conclusion after in-vitro sensitivity test. On the 5th day the particular antibiotic found effective was injected through intra-uterine route in that particular cow from where the material was taken. The owners of the animals were instructed to bring their cows on alternate days, for re-examination and further continuation of treatment, if required. To maintain uniformity in treatment each drug was administered through intra-uterine route every alternate day and five such medications were done with each drug. During the course of treatment the medication was stopped when the animal was found to be cured even though it had not completed its course of five treatments. The owners of the animals were told to report when the cows came in heat subsequently after completion of treatment. On the day of heat the uterine discharge was examined and if it was found to be obviously normal, the cows were sent for



insemination. The owners were requested to bring their animals after 1½ - 2 months of insemination for the detection of pregnancy.

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## **RESULTS**



## RESULTS

A total number of five drugs were tried in the present study to find out their relative efficacies against chronic endometritis on 105 cows which had come for treatment at the out-door clinics of Gynaecology Department of Bihar Veterinary College, Patna. The animals were divided into five groups, and each group was treated with a different drug.

20 ml. of 0.5% Lugol's iodine solution was used in Group "A". This drug was effective on 12 animals (57.1%) out of 21 animals. Lugol's solution increased the quantity of uterine discharge after its first and second application and afterwards the discharge decreased in quantity with the 3rd treatment. After 4th and 5th treatment the uterine discharge disappeared altogether. In one of the 12 cases the discharge disappeared after 4th treatment only, therefore 5th treatment was not considered necessary. The uterine discharge was found to be normal in 12 cases which came in oestrous after completion of treatment, and these animals were sent for insemination. Only 4 of them which were brought for checkup found to be pregnant.

Under Group "B" Uni-Berbyl tablets could cure 11 cases (52.4%). It was observed that treatment with this drug had stimulated uterine discharge significantly after 1st and 2nd application. Subsequently the uterine discharge diminished in quantity and disappeared altogether after 5th medication. The cured cases were advised for insemination out of which only 2 could come for



further checkup and were found to have conceived.

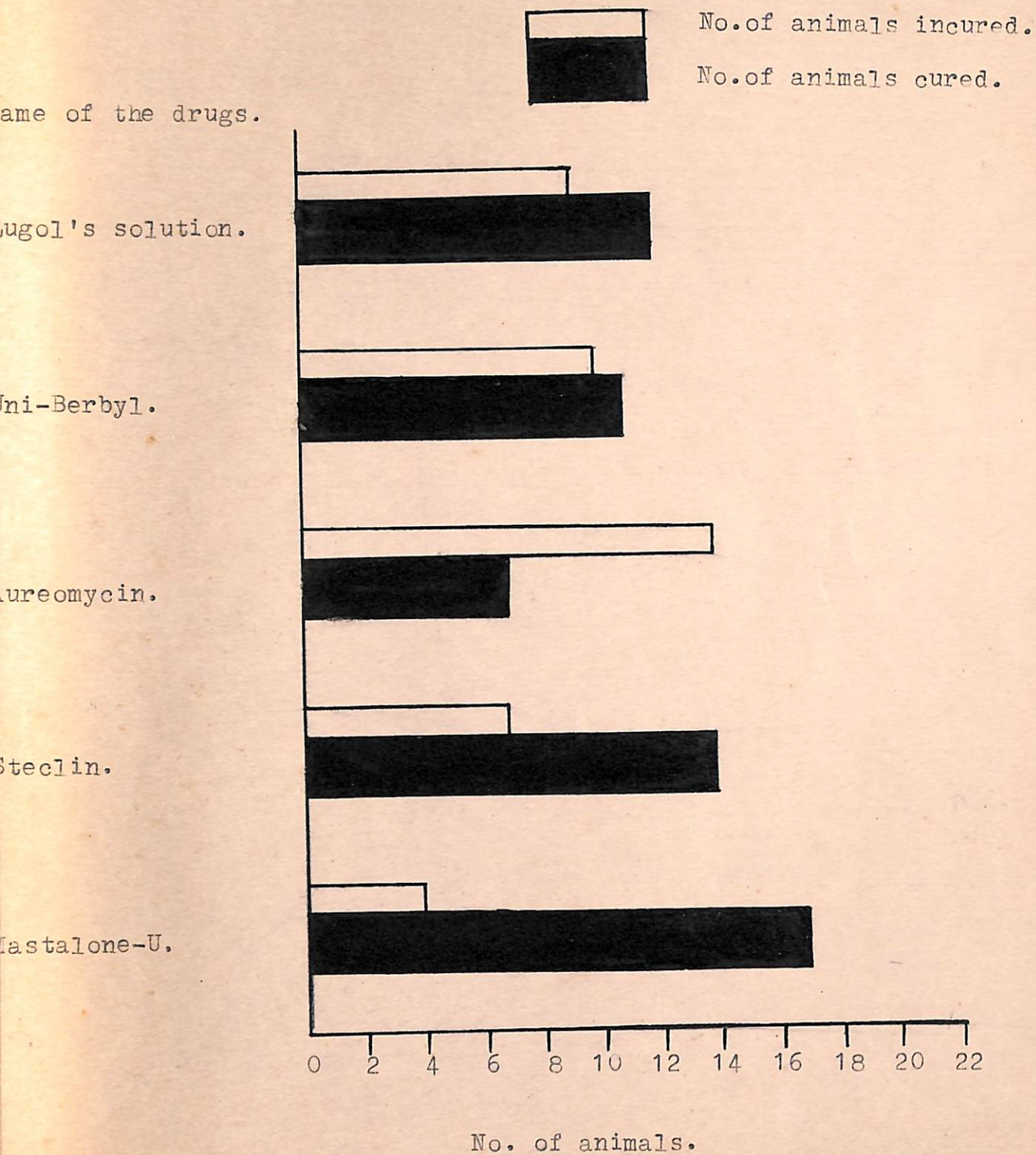
Aureomycin used under Group "C" could cure only 7 cows (33.3%). In most of the incured cases the abnormal uterine discharge persisted despite completion of the treatment schedule. On a examination of the uterine discharge it was noticed that the treatment had no effect on the course of the disease. Rest of the incured cases where the uterine discharge was not evident on the completion of treatment the oestral flow was abnormal during the subsequent oestrous. Out of 7 cured cows which were sent for insemination only 2 turned up for checkup and were found to be pregnant.

Under Group "D" steclin could be efficacious in 14 cases (66.7%). Under this treatment also the uterine discharge increased a bit after 1st and 2nd application and after that it became scanty. With this treatment 3 animals were cured only after 4 applications therefore the 5th treatment was not needed. All the cured cases were sent for insemination when they evinced signs of oestrous. Only 6 of these cases were brought for pregnancy diagnosis and were found to be pregnant.

In Group "E" Mastalone-U was observed to be effective in largest number of cases taken for the study. 17 animals effectively responded with the treatment with overall percentage of 80.90. With this treatment 6 of the 17 cured cases were cured after 4 applications of the drug. In the incured cases also the abnormal uterine discharge became significantly scanty during the course of medication but the oestral flow was not normal



# HISTOGRAM SHOWING THE EFFICACY OF DRUGS.





and for this purpose a 2x5 contingency table was drawn.

TABLE NO. 5

2x5 Contingency table.

Name of the drugs.	Impro-ved.	Not impro-ved.	Total	Propor-tion impro-ved.	Chi squ-are value.	d.f.
0.5% Lugol's solution.	12	9	21	0.571	10.719*	4
Uni-Berbyl Tablets.	11	10	21	0.524		
Aureomycin oblets.	7	14	21	0.333		
Steclin bolus.	14	7	21	0.667		
Mastalone-U	17	4	21	0.809		

\* = Significant at 5% level.

From the above 2x5 Contingency table Chi Square test was carried out. The value of Chi Square was found to be 10.719 with 4 degree of freedom and was significant (at 5% level) which indicated that the various drugs tried were found to differ significantly as regards their response in cases suffering from chronic endometritis. Mastalone-U was found to be highly effective as compared to other drugs.

To compare the response given by individual drug the



2x5 Contingency table was broken up into smaller tables. It was observed that some of the drug differed insignificantly in their therapeutic action. However, some of the drugs differed quite significantly which has been shown in the tables below :-

TABLE NO. 6

Name of the drugs.	Animals cured.	Total no. of animals	Value of Chi Square	d.f.
Aureomycin	7	21	9.72 **	1
Mastalone-U.	17	21		

\*\* = Significant at 1% level.

TABLE NO. 7

Name of the drugs.	Animals cured.	Total no. of animals	Value of Chi Square	d.f.
Uni-Berbyl.	11	21	3.85 *	1
Mastalone-U.	17	21		

\* = Significant at 5% level.

TABLE NO. 8

Name of the drugs.	Animals cured.	Total no. of animals	Value of Chi Square	d.f.
Aureomycin.	7	21	4.67 *	1
Steclin.	14	21		

\* = Significant at 5% level.



TABLE NO. 9

Name of the drugs.	Animals cured.	Total no. of animals	Value of Chi Square	d.f.
Lugol's solution.	12	21	2.79 N.S.	1
Mastalone-U.	17	21		

N.S. = Non-significant.

TABLE NO.19

Name of the drugs.	Animals cured.	Total no. of animals	Value of Chi Square	d.f.
Steclin.	14	21	2.07 N.S.	1
Mastalone-U.	17	21		

N.S. = Non-significant.

It is evident from the above tables and statistical analysis that Mastalone-U gave the best response among all the drugs tried. Although statistically its action is not significantly superior to that of Lugol's solution and steclin bolus but on an average it cured 80.9% of cases whereas the other two drugs cured 57.1% and 66.7% respectively. The other drugs in order to their efficacy were steclin bolus, Lugol's solution, uni-berbyl tablets and aureomycin oblets.





Photograph showing the normal  
uterine discharge after  
completion of pregnancy



It was observed during the course of treatment that when the condition tended to improve two phenomena concurrently occurred. Initially the quantity of uterine discharge increased and on follow-up it decreased in quantity and ultimately it did not become evident. These phenomena were conspicuously marked during medication with Uni-Berbyl. On the other hand rectal examination revealed the size and thickness of uterus returning to normal and assuming its physiological contour.

Bacterial agents associated with cases of endometritis:

In the present study, a total of 19 clinical cases of chronic endometritis in cows were examined for the presence of pathogenic organisms and in-vitro antibiotic sensitivity test was done on the isolates. The antibiotic showing antimicrobial activity for a particular organism in-vitro was also tried therapeutically in-vivo.

A total of 28 isolates belonging to five different genera of organisms such as, streptococci, staphylococci, Pseudomonas, E. coli and Salmonella were recovered. Out of 5 strains of Streptococci, 4 were beta haemolytic and 1 was non-haemolytic. Nine of 12 strains of Staphylococci were non-haemolytic and the remaining 3 were haemolytic. The incidence of different isolates were as follows : Streptococci 26.32%, Staphylococci 63.16%, Pseudomonas 15.79%, E. coli 36.84% and Salmonella 5.26%. Out of 19 cases, 9 cases (47.4%) showed mixed infection. The details of isolation of different pathogens is tabulated in Table no.11.



TABLE NO. 11

Table showing details of isolation of different organisms from cases of endometritis.

Animal No.	Strep-tococci	Staphylo-cocci.	Pseudomonas	<u>E. coli.</u>	Salmonella
1	-	-	-	-	-
2	+	+	-	-	-
3	-	+	-	-	-
4	-	-	-	+	-
5	-	-	+	-	-
6	-	-	-	+	-
7	-	+	-	+	-
8	-	-	+	-	-
9	+	+	-	-	-
10	-	+	-	+	-
11	-	-	-	-	+
12	-	-	-	+	-
13	+	+	-	-	-
14	-	+	-	-	-
15	+	+	-	-	-
16	-	+	-	+	-
17	-	+	-	-	-
18	+	+	-	-	-
19	-	+	-	+	-

The results of biochemical and sugar fermentation



behaviours of Salmonella and E. coli isolated have been summarised in Table Nos. 12, 13 and 14.

TABLE NO. 12

Biochemical reactions and sugar fermentation behaviours of Salmonella.

Indole production.	-	Negative.
M.R. reaction.	-	Positive.
V.P. reaction.	-	Negative.
Citrate utilisation.	-	Positive.
Nitrate reduction.	-	Positive.

Fermentation Reactions :

Glucose	-	Positive.
Sucrose.	-	Negative.
Lactose.	-	Negative.
Mannitol.	-	Positive.
Dulcitol.	-	Positive.
Sorbitol.	-	Positive.

TABLE NO. 13

Biochemical reactions of E. coli.

Indole production	-	Positive.
M.R. reaction.	-	Positive.
V.P. reaction.	-	Negative.
Citrate utilization-		Negative
Nitrate reduction -		Positive.



TABLE NO. 14

Sugar fermentation behaviours of strains  
of E. coli.

Culture No.	Glucose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin
5	+	+	+	-	+	+
7	+	+	+	-	+	-
9	+	-	+	-	+	-
14	+	-	+	-	+	-
16	+	+	+	+	+	+
23	+	-	+	+	+	-
28	+	-	+	-	+	-

#### Antibiotic sensitivity tests :

In-vitro antibiotic sensitivity test was performed using 5 antibiotics, such as, Penicillin, streptomycin, chlor-tetracycline, oxytetracycline and chloramphenicol. Most of the organisms were found to be sensitive to more than one antibiotics. In that case any of the effective antibiotic was chosen and used for in-vivo trial. Each antibiotic was dissolved in 20 ml. of sterile distilled water and introduced through intra-uterine route. Details of the in-vitro antibiotic sensitivity test has been summarised in Table No. 15.



TABLE NO. 15

Table showing antibiotic sensitivity of uterine pathogens from clinical cases of endometritis.

Cultu- re No.	Name of organisms	Strain No.	Antibiotics				
			Peni- cillin	Strep- tomy- cin.	Leder- mycin	Terra- mycin	Chloro- mycetine.
1	2	3	4	5	6	7	8
1	Pseudomonas.	1 P.	R	S	R	R	R
6	Pseudomonas.	2 P.	R	S	R	R	S
10	Pseudomonas.	3 P.	R	S	R	S	R
2	Streptococcus.	1 St.	S	S	R	S	S
11	Streptococcus.	2 St.	R	S	S	S	S
17	Streptococcus.	3 St.	S	S	R	R	S
20	Streptococcus.	4 St.	R	S	S	S	S
24	Streptococcus.	5 St.	S	S	R	S	S
3	Staphylococcus.	1 S.	R	R	S	S	S
4	Staphylococcus.	2 S.	R	S	S	S	S
8	Staphylococcus.	3 S.	S	S	R	S	S
12	Staphylococcus.	4 S.	R	R	S	S	S
13	Staphylococcus.	5 S.	R	R	S	S	S
18	Staphylococcus.	6 S.	R	S	R	S	S
19	Staphylococcus.	7 S.	S	S	R	S	S
21	Staphylococcus.	8 S.	R	S	S	R	R
22	Staphylococcus.	9 S.	S	S	R	S	S
25	Staphylococcus.	10 S.	S	S	R	S	S
26	Staphylococcus.	11 S.	S	S	R	S	S
27	Staphylococcus.	12 S.	R	R	R	S	S
15	Salmonella.	1 Sl.	R	R	S	S	S



Cont'd Table No. 15

1	2	3	4	5	6	7	8
5	<u>E. coli.</u>	1 E.	R	S	S	R	S
7	<u>E. coli.</u>	2 E.	R	S	S	S	S
9	<u>E. coli.</u>	3 E.	R	S	S	R	S
14	<u>E. coli.</u>	4 E.	R	R	S	S	S
16	<u>E. coli.</u>	5 E.	R	R	S	S	S
23	<u>E. coli.</u>	6 E.	R	R	S	S	S
28	<u>E. coli.</u>	7 E.	R	S	R	S	S

R = Resistant.

S = Sensitive.

Based on the in-vitro sensitivity, in-vivo antibiotic therapy was conducted. Out of 19 cases only 16 cases were available for this purpose. The antibiotics were administered 5 times through intra-uterine route on alternate days. But most of the cases required less than 5 treatments and many of them were cured on 3 treatments. One case was cured after two medications only, two cases were not cured even after the application of 5 treatments. The cases were categorised as cured when the abnormal uterine discharge disappeared or the oestral flow subsequent to treatment was found to be normal. The cured cases showing these symptoms were sent for insemination and the owners were requested to bring their animals after 1½-2 months for pregnancy diagnosis. The persistent cases did not show these characteristics and uterine discharge persisted in them even after completion of 5 treatment schedule and the oestral flow subsequent to



treatment was obviously not normal. The results of therapeutic trials with antibiotics are shown and summarised in Table No.16.

TABLE NO. 16

Table showing results of antibiotic therapy in clinical cases of endometritis.

Case No.	Antibiotic used	Dose	No. of administrations required.	Cured/Incured.
1	Streptomycin.	1 gm.	4	Cured.
2	Terramycin.	1 gm.	2	Cured.
3	Terramycin.	1 gm.	3	Cured.
4	Streptomycin.	1 gm.	5	Incured.
5	Chloromycetin.	1 gm.	Not reported.	
6	Terramycin.	1 gm.	4	Cured.
7	Streptomycin.	1 gm.	3	Cured.
8	Terramycin.	1 gm.	Not reported.	
9	Terramycin.	1 gm.	5	Incured.
10	Terramycin.	1 gm.	5	Cured.
11	Aureomycin oblets.	1 gm.	3	Cured.
12	Terramycin.	1 gm.	5	Cured.
13	Streptomycin.	1 gm.	3	Cured.
14	Penicillin.	20 lac. i.u.	3	Cured.
15	Aureomycin oblets.	1 gm.	5	Cured.
16	Terramycin.	1 gm.	3	Cured.
17	Penicillin.	20 lac. i.u.	Not reported.	
18	Penicillin.	20 lac. i.u.	4	Cured.
19	Terramycin.	1 gm.	3	Cured.



It is evident from the table that out of 16 cases available for in-vivo trial, 14 were cured (87.8%). Only 4 of the 16 treated animals were presented after 1½-2 months for subsequent check up and they were found to be pregnant.

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DISCUSSION



## **DISCUSSION**



## DISCUSSION

### Comparative efficacy of drugs against endometritis :

The cases of second degree chronic endometritis were chosen to study the comparative efficacy of drugs. According to owners' history almost all the cases had one or other form of trouble during last parturition like dystokia, retention of placenta, premature birth and parturition trauma. From that time abnormal uterine discharge appeared and the animal failed to "clean up" normally.

In the present study Lugol's solution could cure 12 cases (57.1%) which is superior to Aureomy<sup>c</sup>in (33.3%) and Uni-Berbyl (52.4%). Although Dawson (1951) cured all the eight cases of chronic endometritis with 6 ounces of 1:500 Lugol's solution. No report regarding treatment of 2nd degree endometritis with 0.5% Lugol's solution is available. Lugol's iodine solution has a mild irritating action on the endometrium, stimulates uterine motility, and has an antiseptic and flushing action on the mucopurulent material (Roberts, 1956). In the present study this solution was used for the same purpose. The concentration of Lugol's solution used was 0.5% which was advocated by Dawson (1960). Some workers like Meyer (1932), Williams (1943) and others have pleaded for the use of 1% and even upto 5% strength of Lugol's solution depending upon the severity of the disease but Hignett (1940) stated that the main action of iodine is tissue stimulant and fairly weak solutions serve this purpose as well as



do the stronger solutions. Further, he reported that stronger solutions of iodine may upset the endocrine balance of the animal and reduce the milk yield. The volume of Lugol's solution taken as one dose was 20 ml. according to Tutt (1933) who recommended 20 - 25 ml. of Lugol's solution in second degree of endometritis. The cases which could not be cured with this treatment may be due to endometritis caused by some obstinate type of organisms.

Uni-Berbyl tablets were used in this study for the first time through intra-uterine route in cattle. Each tablet contained 200 mg. of Berberine which is an alkaloid occurring in *Berberis aristata* (Nadkarni, 1954). The hydrochloride of Berberine was used in this study. This drug could cure 11 cases (52.4%) which is superior to Aureomycin (33.3%). It was observed during the course of study that Uni-Berbyl increased the uterine discharge 2 - 3 times after 1st and 2nd application and afterwards the discharge decreased. It cleared off the uterus from mucopurulent material during the course of 5 treatments schedule. It is thus evident that the increase in the uterine discharge played a significant role in control of disease in 11 animals. This increase in the output may have been due to the cholinomimetic action of Berberine on uterine musculature. This observation is corroborated further from the reports of Sabir and Bhide (1971) who observed that Berberine inhibits cholinesterase and pseudo-cholinesterase, thus potentiating the action of acetylcholine on the smooth muscle of gastro-intestinal tract of dogs. Nadkarni (1954) reported the astringent properties of Berberine which may have decreased the secretions from the injured surfaces of endometrium.



From the results obtained in this study, the cholinomimetic action of Berberine on the uterine musculature due to the blockade of cholinesterase enzyme and its astringent action on the endometrium may have played the decisive role in combating the condition.

The results obtained indicated that 33.3% and 66.7% of the animals were cured, post uterine administration of chlortetracycline and tetracycline respectively. These drugs were used as it is and no other drug combinations were adopted. While with oxytetracycline neomycin, oleandomycin, prednisolone and chlorpheniramine maleate combinations (Mastalone-U) were used and in this group 80.9% cases showed recovery.

The comparatively poor recovery rates obtained with chlortetracycline and tetracycline may have been due to the diffusion of these drugs to the plasma across the uterine membrane. We know that the tetracyclines have three pka values 3.3, 7.7 and 9.7 ( Sissodia and Stowe, 1964 ). At an altered uterine pH range between 7.13 - 8.18 (Azizuddin, 1953), due to inflammation, the 9.7 pka group would be extensively unionised thus facilitating diffusion across the uterine membrane. This would thus decrease the concentration at the site of action, uterus in this case. Though the pka group at 3.3 would be more ionized, Sissodia and Stowe (1964) reported that tetracyclines with pka value 3.3 and 7.7 acting jointly should show a milk to plasma theoretical ratio of 0.71 at a milk pH of 6.8. These workers however, found that the experimental milk to plasma ratio was 1.60. Thus it would be possible that the pka groups at 3.3 and 7.7 also showed significant



unionizing characteristics which would facilitate diffusion across a body membrane. Very recently Miller et al. (1972) and Banerjee (1972) has also observed that several compounds of sulphonamide group diffuse across uterine membrane to the plasma and milk in a significantly higher concentration, which may decrease the drug concentration at uterine site of action. Thus the result in this study may be expected in view of the uterine pH values and the three pka values of the tetracyclines.

The poor rate of recovery as observed with aureomycin in comparison to stecline may be attributed to the following facts:

- (a) Irritating and corrosive characteristics inherent with the chemical structure of chlortetracycline which contains chlorine. The irritant action of the chlortetracycline at the local membrane may have convincingly hindered the process of recovery as compared to other tetracyclines.
- (b) The uterine pH shifts towards alkalinity between 7.13 to 8.18 in chronic endometritis according to Azizuddin (1953). At a neutral and alkaline pH, aureomycin undergoes rapid deactivation as reported by Kanegis et al. (1950). In view of the above, it may be possible that in our study as well, the poor therapeutic response of Aureomycin may have been due to its deactivation in an alkaline pH which is inherent in the inflammatory conditions.
- (c) The other factors which may impede the action of Aureo-



-mycin may depend upon severity of the disease, resistant bacteria and age of the particular animal.

The highest percentage of recovery (80.9%) observed with Mastalone-U was quite expected owing to its broad spectrum combination. ✓ Oxytetracycline hydrochloride has got a wide range of activity against various Gram positive and Gram negative organisms. Oleandomycin is considered to be more effective against Gram positive organisms including Staphylococci which are resistant to other antibiotics. Neomycin is comparatively more efficacious in topical therapy directed against infections caused by Gram positive organisms especially members of Proteus and Pseudomonas group. Further, neomycin when topically applied over a mucous membrane is poorly absorbed (Heuber, 1969). Pseudomonas has been found in 3 cases of endometritis out of 19 cases during this study. Thus the three antibiotics covered almost all bacterial organisms commonly encountered in specific and non-specific infections of reproductive tract. ✓ Predisolone incorporated in Mastalone-U acts as an antinflammatory agent. It is evident that the pathogenic bacterial population in the uterus induces inflammatory conditions there. Prednisolone is a gluco-corticoid which exerts a suppressive effect on the basic process of inflammation (Mc Donald, 1969). Vascular permeability is decreased and exudation is diminished. It has also been reported that corticosteroids enhance the antibacterial activity of antibiotics when combined together. Stowe (1969) reported that histamine is always present in any damaged tissue or in any decomposing tissue extract. He found that in cases of retained placenta and puerperal metritis histamine was liberated



from decomposed uterine tissues and developed the symptoms of depression, dullness, reduction in milk yield and inappetance which were immediately relieved after the start of antihistamine therapy. The chlorpheniramine maleate, an antihistaminic, which is incorporated in Mastalone-U, may have also contributed partly for the overall effectiveness of this chemotherapeutic agent. It seems possible that the denuded, cast off and damaged endometrial cells may have liberated histamine to some extent causing local oedema on the mucous membrane of the uterus. The induction of oedema by the endogenous histamine thus liberated which may have aggravated the endometrial injury seems to have been fruitfully counteracted by the antihistaminic ingredient incorporated in Mastalone-U.

#### In-vitro and in-vivo antibiotic sensitivity of causative organisms :

An study on the isolation, identification of the micro-organisms associated with cases of endometritis and their sensitivity to antibiotics was carried out with the view to increase the efficacy of treatment. As such, sensitivity of a particular organism to particular antibiotic in-vitro was taken as a guide for in-vivo trial in the particular cow from where the organisms were isolated.

A total of 19 cases of second degree endometritis were chosen for this purpose. The isolation and identification of the organisms isolated from swabs taken from uterine discharge were conducted.



During the present investigation, a total of 28 isolates were recovered. They comprised of 5 different types of organisms such as Streptococci, Staphylococci, Pseudomonas, Salmonella and E. coli. Mixed type of cultures were isolated from 9 of cases (47.4%). The maximum number of organisms isolated from a single case was two. Baurle (1950), Merkt (1953), Ohm (1955) and Genegal (1955) opined that mixed cultures are generally found and they obtained on an average two genera per sample. Kiesel and Dacres (1959) found upto seven organisms in one sample. This depends undoubtedly on the laboratory technique and method employed for the isolation of organisms. In the present investigation every possible precautions were taken, so that the material does not contaminate with the organisms of vaginal flora. This may be reason for getting more cases of endometritis caused by a single organism in comparison to mixed infection.

The percentage of isolation of different organisms were in decreasing order, Staphylococci (63.2%), E. coli (36.8%), Streptococci (26.3%), Pseudomonas (15.8%) and Salmonella (5.3%). The recovery of Staphylococci and E. coli in higher percentage is in consonance with several other workers as reviewed by Dawson (1960). Pseudomonas and Salmonella has been recovered in comparatively lower percentage during this study. Wunner (1950) and Corris and Molinari (1959) concluded that Pseudomonas is not infrequently a facultative pathogen spread venereally.

Salmonella was isolated from only one cow (5.3%)



during this study. Vanwaveren (1962) has reported the isolation of S. dublin from a cow having mucopurulent uterine discharge. Earlier Plazas-Morales in 1955 isolated salmonella from a normal cow.

The percentage of recovery of Streptococci was intermediate (26.3%). This organism was isolated for the first time from uterine pus in 1917 by Albrechtsen. Subsequent reports of isolation of Streptococci from diseased genital organs of cows have been made by several workers like Quinlan (1929), Sykora (1932), Bauman (1933), Pietzsch (1958), Van Waveren (1962) and others.

On summa rising the results of the in-vitro sensitivity test it was found that chloramphenicol was most effective and inhibited the growth of 25 organisms (89.3%). The other antibiotics in their effectiveness in decreasing order were Terramycin (78.6%), Streptomycin (71.4%), Iedermycin (50%) and Penicillin (28.6%). All the 12 strains of Staphylococci were sensitive to chloromycetin and Terramycin except one. 8 strains were sensitive to streptomycin and 5 to ledermycin and penicillin. Chloromycetin and Streptomycin were effective on all the 5 strains of Streptococci. Terramycin on 4, Penicillin on 3 and Iedermycin on 2. All the 7 strains of E. coli were sensitive to chloromycetin, 6 to ledermycin, 5 to terramycin, 4 to streptomycin and none were sensitive to penicillin. All the 3 strains of Pseudomonas were sensitive to Streptomycin, 1 to Chloromycetin and Terramycin and none to Iedermycin and Penicillin. The single



strain of salmonella isolated was sensitive to chloromycetin, terramycin and ledermycin and resistant to streptomycin and penicillin.

After in-vitro sensitivity test one antibiotic effective against the respective isolates from the case was chosen arbitrarily and applied through intra uterine route in that particular animal for in-vivo trial. In cases where there was mixed infection that antibiotic was chosen which was found effective to both the organisms.

Out of 19 cases only 16 could be available for in-vivo (drug) trial. The effective antibiotic was chosen and it was applied through intra-uterine route after calculation of proper dose through this route. A maximum of 5 treatment schedule on alternate day was made. The treatment was stopped when the cases showed recovery even if the animal had not completed its 5 treatment schedule. 14 cases recovered and out of that 4 could return for pregnancy diagnosis after 1½-2 months and found to be pregnant. 2 cases could not be cured even after completion of 5 treatments schedule. One case was cured after 2 applications only whereas 3 cases on 4 treatments and 5 treatments each. 7 cases (43.7%) required 3 treatments only. Thus it was concluded that maximum number of recovery took place only after 3 successive dosing of antibiotics. On the whole 87.8% of cases were cured after treating them on the basis of in-vitro sensitivity report.

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## **SUMMARY**



## S U M M A R Y

In the first part of experiment, studies have been made on the cases of second degree endometritis in cattle. A total of 105 cases were taken. Lugol's solution of iodine, Uni-Berbyl, Aureomycin, Steclin and Mastalone-U were tried as remedial measures and the results produced by each of them has been noted.

Among Lugol's solution of iodine and Uni-Berbyl the former has proved better since it was effective in 57.1% of cases whereas the latter could cure 52.4% of cases. Among the antibiotics, Mastalone-U was the most effective agent since it cured 80.9% of cases, Steclin bolus was second in merit having cured 66.7% of cases. Aureomycin was least effective among the three antibiotics as it produced recovery in 33.3% of cases.

In the second part of the experiment 19 cases were taken. Bacterial cultures were made from each of these cases and antibiotic sensitivity test was performed. The following micro-organisms were present in the uterine discharge :

(a) Staphylococci (b) Streptococci (c) E. coli. (d) Pseudomonas and (e) Salmonella. Sensitivity test was performed using the following antibiotics Penicillin, Streptomycin, Iedermycin, Terramycin and Chloromycetin. The results of treatments on the basis of informations gathered after sensitivity test was 87.8%.

Since the results of treatments after the sensitivity test have proved to be better, it is suggested that drug sensitivity test should be performed wherever facility exist.



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