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Impact Of Hemoglobin On Uterine Infection Of Dairy Cow At Eastern Terai Districts Of Nepal

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Abstract
Attempts have been taken to resolve the impact manifested in connection with low hemoglobin in cow uterine infection and anti-microbial resistance. The research is a global problem to animal and public health. It has drawn the attention of public health experts, stakeholders, and medical science due to the substantial economic loss that it causes to individuals and nation as a whole. Nepal is one of the major contributors to the growing burden in connection with low hemoglobin in cow uterine infection and anti-microbial resistance due to widespread irrational use of antibiotics along with poor health care systems, poor infection control and prevention measures. In Nepal, Morang and Sunsari is eastern terai district of Nepal in Koshi Province where cattle are kept for various purposes like milk, manure, cash income and traditional value. Particularly, Morang and Sunsari has abundance cattle farming from small scale for daily wages earner from daily sales to large scale industries like Dairy Development Corporation which meets large portion of milk demand of Nepal. Therefore, it is considered that Morang and Sunsari district as suitable place for above research. The study looked at hemoglobin concentrations in blood samples from four District in Sunsari locations: Ikrahi, Sahebgunj, Sitagunj, and Debangunj. A total number of 25 sample were investigated from cattle from above locations and sample were presented at Veterinary Laboratory Biratnagar Province.The experiment was conducted with one controlled group and 4 treatment groups where animals were selected on the basis hemoglobin level where the results didn't reveal statistically significant differences in concentrations. The highest concentration was found in Group D at 8.2 g/100cc, followed by Group B at 7.98 g/100cc, followed by Group A and Group C at 7.92 g/100cc where as Group E found with normal Hemoglobin level at 12.78g/100cc.where all the test group sample had the lowest hemoglobin level. Bacterial growth was observed in all the groups samples. All cows with low hemoglobin were found with uterine infection samples that demonstrated sensitivity to Tetracycline, Ciprofloxacin, and Penicillin G. However, there was no sensitivity towards Erythromycin observed. The study also found the presence of bacteria in cows with low hemoglobin. In terms of antibiotic sensitivity, the study found varied results among different antibiotics.

Key Words: *Uterine Infection; cow; hemoglobin; antibiotic sensitivity*

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I. Introduction

Reproductive performance is one of the most essential factors affecting dairy farm profitability and the improvement of national economy, as well as the living standard of rural and urban societies, because it directly or indirectly impacts the yield of milk, reproductive culling rate and the cost for breeding and calf sales *(Plaizieret al., 1998)* to enhance economic efficiency, dairy cows should only calve once per year. Cow fertility has decreased in current decades in cows that have been heavily selected for milk production; however, because cow fertility is a multi-factorial criterion and its degradation has been caused by a network of genetic, environmental, and managerial factors, it is difficult to pinpoint the precise cause of this decline. *(Walsh et al., 2011).* Dairy is one of the most important sectors of livestock in Nepal for maintaining the need of protein and nutrition as well as empowerment. The reproductive performance of dairy herd is an important factor in a cattle farms profitability. Uterine infections have long been recognized as one of the major causes of reduced fertility in dairy cow *(Alam M and Ghosh A (1994)*. Some investigators favor the contention that iron deficiency is not good for immunity. In addition, infection or inflammation generate anemia *(Tomas walteret al.1997)*. Nutritional iron deficiency is often assumed to be the major etiological factor of anemia and also parasitic infections may also be implicated in the etiology of anemia. Hence, the lower level of hemoglobin may also be one of the cause for uterine infection. Cow uterine infection, either specific or non-specific in nature, account for large number of pregnancy failure in cows *(Sirohiet al., 1989)*. Bacterial infection is the most important among the various causes of the subfertility

(Dholakiaet al., 1987) and repeat breeding. These infections affect fertility by altering the uterine environment. It is major factor in reproduction failure, which in turn causes economic loss to the dairy industries *(Rahman et al., 1996)*. The indiscriminate use of broad spectrum antibiotics and corticosteroids for the treatment of reproductive disorders may lead to microbial infections of the uterine environment *(Raghavanet al., 1983)*. Therefore, present study hypothesized that decreased of hemoglobin level increase the growth of bacteria in uterus causes the uterine infection as a result animal shows reproductive infertility.

II. Materials And Methods

Study area and period:

The research study was conducted at different small-holder dairy farms in Biratnagar and Sunsari districts of Nepal. It is situated at 25° 37" N latitude and 88°39" E longitude on the eastern bank of the river Punarvhaba. It is located in the Terai region, which is a flat, lowland area in the southern part of Nepal.

Sample

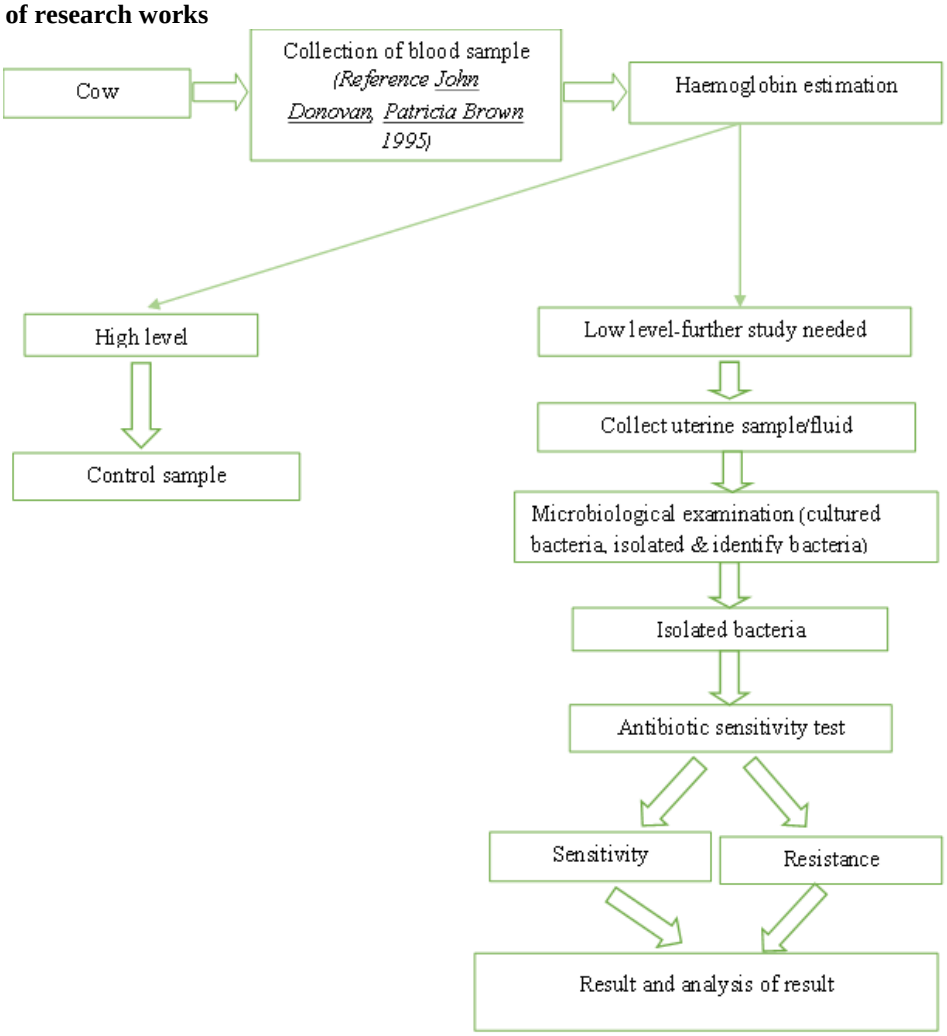
Selection of households and donor cows

Dairy cows were selected based on showing weak heat signs and symptoms, thin vaginal mucous that are usually referred as sub fertile animal and repeat breeding animals. A total of 4 farms and 25 animals were selected for final study to conduct the research.

Examination of reproductive disorder

Rectal palpation of the ovary and uterus was performed on the treatment group of cows, and the results were recorded to diagnose uterine infection. Exploration of reproductive disorders was carried out and data was collected using the current questionnaire through direct interviews with the farm's owner and/or workers. Closed inspection was performed on the dairy cattle to look for any clinical sign.

Flow chart of research works



Grouping of the animals

25 Animals were grouped into two categories, each group consisted of 5 animals. These were the Infected group and Controlled group. Animal were selected as mentioned below:

- Infected Group: (Group A, Group B, Group C, Group D)
- Controlled Group: (Group E)

Blood collection

Blood collection is done from the site of jugular vein. The collected blood samples is stored in EDTA vial appropriately to maintain their integrity and transported for hematological examination at veterinary laboratory Biratnagar.

Hemoglobin Analysis

Measuring hemoglobin levels in blood is made more precise and effective with the use of an automatic hemoglobin analyzer. This method reduces the chance of human error while ensuring precision by automating the process and using state-of-the-art laboratory apparatus. The automated analyzer delivers a digital readout of the blood sample's haemoglobin concentration. Typically, the findings are given in grams per deciliter (g/dL) or grams per liter (g/L) of blood. This data is compared to the typical haemoglobin level of cattle, which is 10.9±0.86 grams of hemoglobin per 100cc of blood.Cattle with low hemoglobin levels are chosen for further testing.

Uterine sample collection

A cow's uterus is usually sampled for diagnostic purposes, such as evaluating the health of the cow's reproductive system, identifying infections, or keeping an eye on the estrous cycle.The cow is clean in the genital area, the vulva and the surrounding area with warm, soapy water and rinsed thoroughly.A lubricated Sterile gloves were used to maintain aseptic conditions.Gently and carefully insert a clean, sterile, and flexible sheath into the cow's vagina with the help of an AI gun.The instrument was passed through the cervix into the uterine body.Once in the uterus, the desired sample was collected with the help of a syringe by aspirating it for the specific diagnostic goals.The collected sample is transferred into a clean and sterile specimen container for analysis.The specimen container was labeled with relevant information, such as the cow's identification, date, and the type of sample collected.

Bacteriological investigation

Preparation of culture media

Different media was made to make an environment for the growth of the bacteria. Selection of media should be done according to the requirement e.g. EMB agar media is used as a selective media for *E.coli* growth, MHA for AST etc.Required amount of distilled water was taken on conical flask according to the requirement of number of media to be made. Agar was weighed according to recommendation of manufacturer and mixed in distilled water.Mixture was boiled until the agar gets totally dissolved.Media was autoclave at 121^0 C at 15 lb. pressure for 15 minutes.The content was kept for cooling to 50^0C and pour into petri dish (20-22 ml in 90 mm petri dish)Petri dish was kept in incubator for 24 day and observed for any of bacterial colonies growth due to contamination. contaminated petri dish were stored in refrigerator and used as a culture media for bacterial growth

Primary culture of bacteria from the suspected specimen

A microbiological culture, or microbial culture, is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions. Microbial cultures are foundational and basic diagnostic methods used extensively as a research tool in molecular biology.Burner was turned on and all the work was carried around the sterile zone of burner.Uterine Specimen was diluted on sterile distilled water of equal amount and swab of diluted sample was taken using sterile inoculating loop.For culture of the organ surface of sample organ was seared by hot spatula, and then sterile microbiological loop was introduced deeply in the affected organs and then loop full was taken.Inoculating loop was streaked over the agar surface.The media was incubated aerobically at 37^0C for 24 hours.

Secondary culture

Growth of various bacteria resulting in different characteristic colony in the primary media. The specific bacterial colony is again inoculated into another media carefully to separate the bacteria for further examination.

Gram staining

Grease free glass slide was taken. Smear of bacterial colony was made and heat fixed by passing slide through flame. Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the

staining procedure.The smear was than washed and few drop of iodide was poured and left for 1 minute.Some drop of rapid decolorizing chemical ethanol or acetone was poured and left for 10-20second.Counterstained with safranin for 1 minute and dried.Immersion oil is dropped on the smear and observed under microscope**.**Gram positive bacteria are distinctive purple appearance after gram staining examples include all staphylococci, all streptococci and some listeria species .Gram negative bacteria are pale reddish color after gram staining examples include enterobacter species, salmonella species and pseudomonas species. (Karen Steward 2019).

Antibiotics sensitivity test (AST)

AST is the test carried out for measuring the sensitivity of the bacteria to an antibiotic for appropriate and accurate selection of the antibiotics for treatment of the disease of bacterial origin. Various antibiotic discs are used for this method for finding best antibiotic sensitive to that bacteria causing disease. Antibiotic sensitivity test was performed by disc diffusion method *(Kirby-Bauer's technique)* using commercially available discs(Tetracycline 30 µg,Ciprofloxacin 5µg,Penicillin G 10 µg,Erythromycin 15µg,Gentamycin 10 µg).All the work was carried around the sterile zone around the glowing burner.Inoculating loop was made red hot to sterilize and cooled down.Loop full of colony was taken from the primarily cultured media petri dish.Sterilized media according to the requirement was taken.Approximately one-third of the agar surface (at the edge) was streaked using the infected inoculating loop (without scratching the agar).Quadrant streaking method was followed for inoculating loop full of colony in media. Petri plate was incubated at 37^0C for 24 hours. streaking is done throughout media. Antibiotics disc was placed on the petridish containing MHA media making certain gap (6 disc in 90mm petridish).Media was observed after incubation for 24hrs at 37^oC.Antibiotic disc with the larger area of zone of inhibition indicates more sensitivity of the bacteria to that antibiotic.

Table 1: Table showing the standard sensitivity level of selected 5 antibiotics (J. C. Gould *et al*).

Antibiotics	Sensitive	Indication	Resistant
Tetracycline 30 µg	≥19	15-18	≤14
Ciprofloxacin 5µg	≥21	16-20	≤15
Penicillin G 10 µg	≥15		≤14
Erythromycin 15µg	≥23	14-22	≤13
Gentamycin 10 µg	≥15	13-14	≤12

Statistical analysis

The results of various parameters were expressed as ± SDM. Statistical data analyses were done by using Mini Tab Statistical Software and Microsoft Excel. Statistically significant differences were determined by one way Analysis of Variance (ANOVA) for hemoglobin.

III. Result

Fig. The bar graph showing the concentration of hemoglobin according to the location.

Figure illustrates the hemoglobin concentration in blood samples collected from various locations of Sunsari District (Ikrahi, Sahebgunj, Sitagunj, Debangunj). The statistical analysis revealed no significant differences (P = 0.931) among the five groups within the municipality. Among the infected group Group D showed the highest concentration numerically at 8.2 g/100 cc, followed by Group B with 7.98 g/100 cc.

Conversely, Group A and Group C both displayed the lowest concentration numerically at 7.92 g/100 cc .All the groups concentration shows the lower level than that of normal hemoglobin level of cattle 10.9 ±0.86 whereas controlled group Group E showed highest concentration numerically at 12.78g/100cc which showed the value similar to normal hemoglobin .

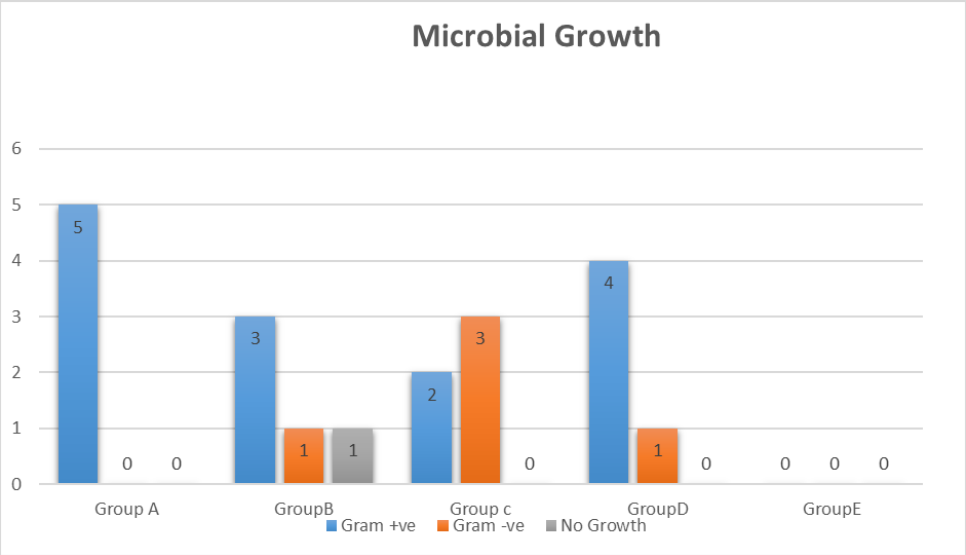


Fig. : The bar graph showing the microbial growth in different cow samples.

Figure depicts the aggregate count of uterine samples of cattle having low hemoglobin acquired from 5 different groups and their subsequent growth on nutrient agar for microbiological assessments. Specifically, in Group A, all 5 samples exhibited growth exclusively of gram-positive bacteria. In Group B, three cows tested positive for gram-positive bacteria, while one cow showed no growth, and another showed growth of gram-negative bacteria. Contrastingly, in Group C and Group D, all samples displayed gram-positive bacterial growth. Notably, among those 5 samples in Group B, three were also tested for gram-negative bacteria, and in Group D, one sample tested for gram-negative bacteria. whereas all the samples from Group E exhibited no growth.

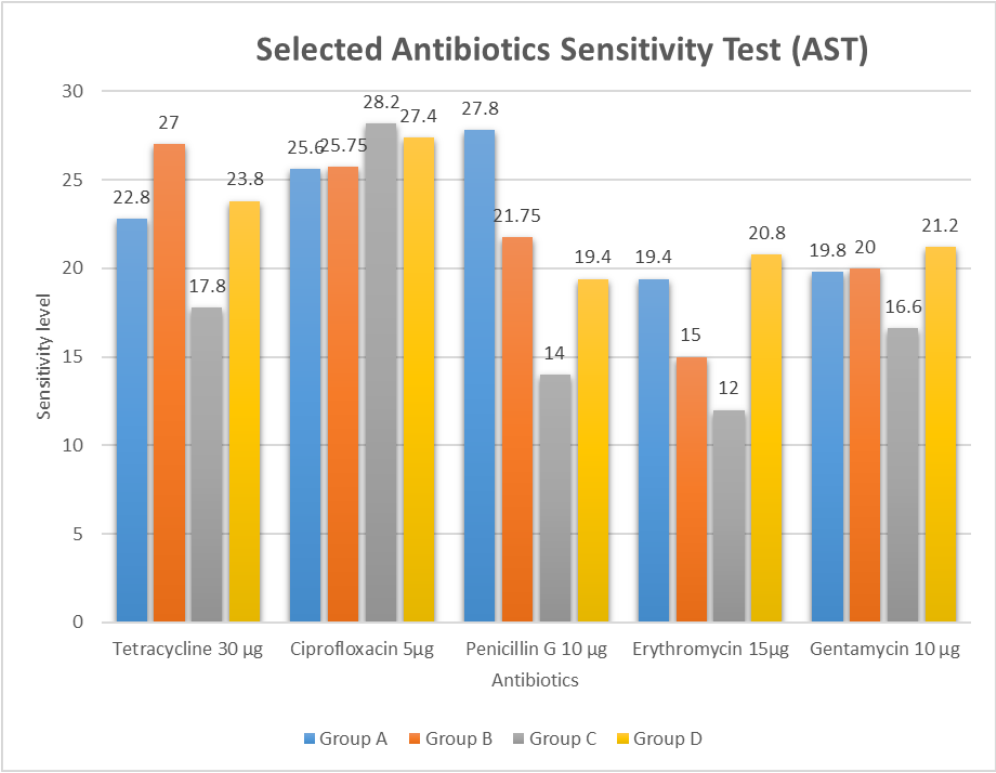


Fig.: The bar graph showing the antibiotics sensitivity level of 5 selected antibiotics.

Figure displays the antibiotic sensitivity profiles of the samples assessed against five distinct antibiotics: Tetracycline 30 µg, Ciprofloxacin 5 µg, Penicillin G 10 µg, Erythromycin 15 µg, and Gentamycin 10 µg. According to the standard reference table (table number1) outlined in the methodology section, all samples from Group A, Group B, and Group D demonstrated sensitivity to Tetracycline and Penicillin G. Conversely, samples from Group C exhibited lower sensitivity to the same Tetracycline and Penicillin G. Additionally, all samples, irrespective of location, displayed sensitivity to Ciprofloxacin and Gentamycin. However, for Erythromycin, none of the samples (across all locations) demonstrated sensitivity; instead, they fell into the category of low sensitivity.

IV. Discussion

The hemoglobin concentration in blood samples collected from various locations of Sunsari District (Ikrahi, sahebgunj, sitagunj, Debangunj). The relation between hemoglobin level (gm%) and animals selected are in 5 groups each groups contain 5 samples and it have been divided into two categories 4 groups as infected group and one group having normal hemoglobin level as controlled group. The statistical analysis revealed no significant differences (P = 0.931) among the five groups of animal with in the municipality. Group D cow showed the highest concentration numerically at 8.2 g/100 cc, followed by Group B cow with 7.98 g/100 cc. Conversely, Group A and Group C cows both displayed the lowest concentration numerically at 7.92 g/100 cc.) whereas Group E controlled group cows showed the normal level of hemoglobin. Hemoglobin level significantly related to the clinical symptoms of dairy cow. Results indicate that anestrus, repeat breeding, conception failure and normal cyclic animals contain (7.98,7.92,7.92 and8.2)g/100cc hemoglobin (Hb) level respectively. Hemoglobin is the iron containing oxygen-transport metalloid protein present in red blood cells.

The study found that the animals with low hemoglobin level were found with uterine infection. The aggregate count of blood samples acquired from 5 different group samples of different locations and their subsequent growth on nutrient agar for microbiological assessments. Specifically, in Group A, all 5 samples exhibited growth exclusively of gram-positive bacteria. In Group B, three cows tested positive for gram-positive bacteria, while one cow showed no growth, and another showed growth of gram-negative bacteria. Contrastingly, in Group C and Group D, all samples displayed gram-positive bacterial growth. Notably, among those 5 samples in Group C, three were also tested for gram-negative bacteria, and in Group D, one sample tested for gram-negative bacteria.

(Sarkar et al. (2016) study showed that a significant decrease in hemoglobin levels in animals with uterine infections when compared to the control group. The mean hemoglobin levels in the experimental, control, non-descriptive, and crossbred groups were 8.28 ± 0.11 g/dl, 9.84 ± 0.201 g/dl, 8.85 ± 0.20 g/dl, and 9.27 ± 0.11 g/dl, respectively. This suggests that a uterine infection could result in anemia due to a low hemoglobin percentage.

The present result was in agreement with the finding of *Sarkar et al. (2016)* there is significant decrease in hemoglobin level in uterine infected animal in compared to the control group; there is also appeared low hemoglobin level in uterine infected animal of repeat breeding cattle. Similarly, agreement with *Ahmed et al. (2003) and Parizaet al. (2009)* there I found that normal cycling cows had higher hemoglobin levels than cows with uterine infections.

In the present study hemoglobin level decreased with the uterine infection which was in agreement with the findings of *(Sarkar et al. (2016)Ahmed et al. (2003) and Parizaet al. (2009).*

Studies show that 89 of 100 uterine samples contained different strains of bacteria. Bacillus subtilis (8.99%), Corynebacteriumpyogenes (19.10%), Escherichia coli (29.21%), Neisseria meningitides (3.37%), Staphylococcus aureus (23.60%), Streptococcus pneumonia (3.37%) and Streptococcus pyogenes (12.36%) were isolated from these samples *(Idrees Ali Zahidet al).*

Also, cows fail to conceive on one or more cycles in the same season, bacterial pathogens are a possible cause. S. pyogenes, E. coli, and Streptococcus spp. were isolated and identified as the most frequently associated bacteria with fertility problems *Nibretet al. (2013). Sulakeet al. (2013).*

The present result in agreement with *(Idrees Ali Zahidet al, Nibretet al. (2013). Sulakeet al. (2013)Nibretet al. (2013). Sulakeet al. (2013)*the aggregate count of Uterine samples of cattle having low haemoglobin acquired from 4 different locations and their subsequent growth on nutrient agar for microbiological assessments. Specifically, in Group A, all 5 samples exhibited growth exclusively of gram-positive bacteria. In Group B, three cows tested positive for gram-positive bacteria, while one cow showed no growth, and another showed growth of gram-negative bacteria.

According to *(suborton 2007)*blood parasites can cause losses in the form of stunted growth, weight loss, decreased work power, decreased reproductive power, decreased milk production, and abortion *(Kocanet al., 2004)*.According to *(Anandaet al., 2016)* it causes anaemia, hides damage, reduces milk production and poor reproductive performance and increased mortality.

The present study in agreement with Subroton, kocanet al and anandaet at the cattle with lower hemoglobin level shows the poor reproductive performance and poor productivity.

In previous study was conducted on dairy cows to investigate the prevalence of bacterial flora in repeat breeding syndrome and uterine infections. The study discovered that the presence of bacteria was higher in repeat breeding cases compared to normal fertile cows. Staphylococcus, Bacillus, Escherichia coli, Pseudomonas, and Gram-negative minute rod-shaped bacteria were the most commonly isolated bacteria. The study also looked at antibiotic sensitivity and discovered that the majority of bacterial isolates were moderately to highly sensitivetoamoxicillin, oxytetracycline, and ciprofloxacin *(Himdedia, Mumbai).*

In present study the antibiotic sensitivity profiles of the samples assessed against five distinct antibiotics: Tetracycline 30 µg, Ciprofloxacin 5 µg, Penicillin G 10 µg, Erythromycin 15 µg, and Gentamycin 10 µg. According to the standard reference table (table number1) outlined in the methodology section, all samples from Group A, Group B, and Group D demonstrated sensitivity to Tetracycline and Penicillin G. Conversely, samples from Group C exhibited lower sensitivity to the same Tetracycline and Penicillin G. Additionally, all samples, irrespective of location, displayed sensitivity to Ciprofloxacin and Gentamycin. However, for Erythromycin, none of the samples (across all locations) demonstrated sensitivity; instead, they fell into the category of low sensitivity. (Figure 4.3).

V. Conclusion

The study reveals that Low hemoglobin level favors the growth of bacteria in cow uterus which causes infertility in dairy cow. It will generate a new diagnosis for the infertility problem of dairy cows in a short time and provide specific antibiotic therapy for the treatment of specific diseases associated with the infertility problem of dairy cows. Analysis of hemoglobin is cheaper and requires less time. If a low hemoglobin level is found, it can be treated with a specific antibiotic after the microbial examination of the uterine sample. Veterinarians can detect the cause and treatment of the infertility problem by analyzing hemoglobin levels (automatic blood analyzer) and sorting the low hemoglobin status which can be further forwarded for antimicrobial testing.

The study set out to evaluate the blood by a complete blood cell test (hemoglobin) of repeat breeder cattle and its relationship to uterine infection in cattle, which leads to reduced productivity and performance. The study also reveals that 80% of cattle were classified as repeat breeders and 20% showed conception failure and a low hemoglobin level. It also found that there is the presence of bacteria in cow uterine sample with low hemoglobin levels showed a positive response for antibiotic sensitivity test, there were different results among different antibiotics demonstrated sensitivity but Erythromycin was observed resistance. The highest concentration of hemoglobin was found in Group D at 8.2 g/100 cc, followed by Group B at 7.98 g/100 Group A and Group C at 7.92 g/100 cc. Group A and Group C had the lowest concentration due to geographical location, feeding habits, disease conditions and parasitic conditions. There was variation in hemoglobin levels. Many diagnostic procedures are required in cases of infertility in dairy cows

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