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Article

Study on subclinical endometritis in cattle of Kathmandu Valley by using WST (WST)

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Abstract:

The current investigation aimed to identify the different levels of nonspecific bacterial infection in the genitalia of repeat-breeding cattle of Kathmandu District using White Side Test (WST). A total of 307 crossbred Holstein Friesian cows were considered for this purpose, out of which 246 repeat-breeding cattle were under treatment and 61 cattle with normal cycle (control group) were artificially inseminated at their first service. Cervical mucus samples were collected 8 to 12 hours after the first signs of behavioral estrus, and subjected to a WST (WST) and bacteriological examination. From the results of WST, it has been inferred that only 9(14.75%) animals in the control group were positive and the remaining 52(85.24%) were negative. However, the majority of repeat-breeding animals were positive 190 (77.22%)) and only 56 (22.76%) of animals were found negative. Microbiological examination of 190 samples revealed *E. coli* 54(28.42%), *Staphylococcus spp.* 39(20.52%), *Streptococcus spp*. 26(13.68%), *Corynebacterium spp*. 10(5.263%), *Pseudomonas spp*. 7(3.68%), *Klebsiella spp.* 9(4.73%), and mix bacterial growth 44(23.15%). The remaining 56 repeat-breeding cattle were negative with WST, possibly due to the absence of bacteria. Other causes may be viruses, placental retention, ovarian cysts, dystocia, etc. The findings of the present study divulge that WST may be utilized in the field to detect subclinical endometritis caused by bacteria.

Keywords: WST, Subclinical endometritis, estrus cervical mucus, Repeat Breeding

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1. Introduction

Uterine inflammation is a serious problem in Nepal's breeding dairy cows, causing considerable financial losses [1]. Uterine infections include endometritis, metritis, mucometra, and pyometra. Among this endometritis is one of the major gynecology problems affecting reproductive efficacy and the economy of milk production in dairy animals [1].

Among different types of endometritis, subclinical or occult endometritis poses a serious threat to fertility since it is extremely difficult to identify by standard clinical-gynecological examination. Subclinical endometritis is currently being considered as a significant factor in dairy cows' lower conception rates [2]. Endometrial inflammation modifies the uterine environment, impairing the ability to conceive or sustain an embryo. In Nepal, subclinical endometritis has been identified as the most frequent reason why bovines are unable to conceive. The occurrence of endometritis has been associated with a weak uterine defense mechanism (UDM) in females [3] mainly due to inadequate husbandry and sanitation practices and exposed of genital organs to microbial invasion either at parturition or during estrus.

Bovine genital tract infections can be specific or non-specific, with the latter being the most important in causing endometritis. Some of the specific pathogens such as *Campylobactor fetus* and *Trichomonas fetus* develop infection without any predisposing cause, whereas non-specific pathogens residing in the genital tract as saprophytes and set infection under favorable conditions. The postpartum bovine uterus contains wide range of bacteria, both Gram-positive and Gram-negative, aerobes and anaerobes [4] Numerous bacteria have also been found and grown from the uterus of cattle with endometritis, including *Streptococci*, *Staphylococci*, *Corynebacterium spp*., *Klebsiella spp*., and *E. coli.*

One of the emerging businesses in Nepal that offers a reliable source of income is livestock farming. Farmers favor more productive cattle over less productive cattle due to the market's rising need for milk, particularly cross Holstein Friesian (with good blood levels) livestock since they produce milk in greater quantities. From India to Nepal, a sizable number of crossbred cattle are imported. Cattle with various diseases and reproductive disorders have been reported to be imported as a result of improper inspection during quarantine or illegally importing cattle from open borders. Some of the most significant issues farmers today are dealing with include the retention of the placenta, repeat breeding, extended calving intervals, infertility, prolapse, dystocia, anestrus, cervicitis, cystic ovary, and endometritis. This research was undertaken with the intention of improvising these issues by addressing all of the aforementioned challenges. Early detection of metritis or endometritis will help to reduce future treatment costs and complications brought on by this condition.

WST has been described as a test for the diagnosis of subclinical endometritis [5]. The normal oestrus discharge has too low number of leucocytes to bring about a positive color change on doing WST [5]. The subclinical discharges have moderate number of leucocytes causing a moderate color change compared to the clinical discharge having very high number causing intense color reaction. The relative efficacy of Whiteside test is 77.5% [6].

Therefore, it was intended for the current study to assess the validity of the WST in detecting genital infection in repeat-breeding cattle raised in the Kathmandu District. Repeat breeders are cows with apparently healthy genitalia, normal estrous, has no abnormal vaginal discharge but failed to conceive in three or more consecutive inseminations [7]. To further demonstrate that the results of the WST for endometritis are accurate under field conditions, bacterial culture was performed.

WST is use for diagnosis of subclinical uterine infection in repeat breeding cows [8]. The investigators classify the positive samples as slight, moderate, and intense subclinical infections based on the color reaction, such as light yellow, yellow, and dark yellow. The test revealed 92.85%of cervical mucus positive from repeat breeding cows indicating subclinical uterine infection. Out of the total, 44.75% were slightly positive, 42.50% were moderate, whereas, 15-38 % were detected to have an intense uterine infection.

WST is interpreted as negative, mild, moderate, and severe as recorded 16.67, 66.67, 09.26, and 7.41 percent color pattern of no color, light yellow, yellow, and dark yellow type, respectively in repeat breeder crossbred Jersey cows. On culture 60 (75.00 %) of the 80 repeat breeding animals were found positive and 20 (25.00 %) were free of bacteria. The different bacterial isolates from repeat breeding cows were *Staphylococcus spp.* 16 (21.05%), *E. coli* 14 (18.42%), *Bacillus spp.* 10 (13.16%), Corynebacterium spp. 10 (13.16%), Pseudomonas spp. 8 (10.53%), *Proteus spp.* 8 (10.53%), *Klebsiella spp.* 6 (7.89%), and *Streptococcus spp.* 4 (5.26%). [9]

2. Materials and Methods

2.1 Site of Study

The study was conducted in a Kathmandu district located in Kathmandu Valley, Bagmati Province of Nepal. It covers an area of 413.69 km2 (159.73 sq mi) with 11 municipalities [10]. Total Population of cattle in Kathmandu District is about 52,470 [11].

The Kathmandu district is surrounded by:

East: Bhaktapur District and Kavrepalanchok District

West: Dhading District and Nuwakot District

North: Nuwakot District and Sindhupalchok District

South: Lalitpur District and Makwanpur District

2.2 Sample size

For estimating the minimum sample size for an unknown population, the formula estimated by ***Cochran*** was [12]

$$\boldsymbol{N}_0 = \frac{z^2 \times p(1-p)}{e^2}$$

z = critical value of the desired level of confidence (i.e. 95%) its z-value is 1.96

e = margin of error i.e., is 5%

p = estimated proportion of an attribute that is present in the population (that can be a maximum of 50%)

n_0 = sample size/proportion of unknown population

now,

$$\boldsymbol{N}_0 = \frac{z^2 \times p(1-p)}{e^2}$$

$$= \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2} = \sim 385 \text{ cattle's}$$

First, calculate the proportion and then use the formulation for the population correction factor to calculate the exact sample size (formula)

$$\boldsymbol{N} = \frac{n_0 \times N}{n_0 + (N-1)}$$

n = sample size of known population

n_0 = proportion of the unknown population

N = known population

Total Population of cattle in Kathmandu District is about 52,470

Now,

$$\boldsymbol{n} = \frac{n_0 \times N}{n_0 + (N-1)}$$

$$= \frac{385 \times 52470}{385 + (52470-1)} = \sim 383 \text{ cattle's}$$

Therefore, the total sample size is 383 but due to the pandemic of Lumpy Skin Disease (LSD), only 307 samples were collected.

2.3 Sampling Technique

Simple random sampling was adapted as it favors the unbiased selection of animals [12] and a total of 307 samples were collected from cattle at the heat from different farms in the Kathmandu district.

Out of total 307 samples, 12 (Budhanilkantha Municipality), 18 (Chandragiri Municipality), 10 (Dakshinkali Urban Municipality), 35 (Kageshwori Manahara Municipality), 6 (Kathmandu Metropolitan City), 123 (Kirtipur Municipality), 10 (Nagarjun Municipality), 9 (Shankharapur Urban Municipality), 11(Tarkeshwor Municipality), 56 (Tokha Municipality), were collected. (Table 1)

Table 1: No. of sample collection from different municipalities of Kathmandu District

Municipality of Kathmandu District	Total number of samples collected
Budhanilkantha Municipality	12
Chandragiri Municipality	18
Dakshinkali Municipality	10
Gokarneshwor Municipality	35
Kageshwori Manahara Municipality	17
Kathmandu Metropolitan City	6
Kirtipur Municipality	123
Nagarjun Municipality	10
Shankharapur Municipality	9
Tarkeshwor Municipality	11
Tokha Municipality	56
Total	307

2.4 Experimental Design

The present study was conducted at different farms of Kathmandu District during the period from May 1 to September 1 2023. Several 307 crossbred Holstein Friesian cattle of 246 repeat breeding cows presented for treatment and 61 clinically normal cows presented for artificial insemination at their first service, were selected for sample collection (shown in supplementary figure S1).

All of the animal's estrus cervical mucus was collected 8 to 12 hours after the first signs of behavioral estrus. Animals were properly restrained for this purpose. For rectal feces evacuation, a full-sleeve gloved left hand was inserted per rectum and lubricated (with sterile liquid paraffin/oil). The vulva and the perineum were both properly cleaned with soap and water, dried with soft absorbent cotton, and then disinfected with 70% alcohol. The vagina had been penetrated with a 10 mL sterilized pipette and a 20 mL disposable syringe was linked to the pipette's exterior pointed end.

The pipette was guided through the vaginal canal to the cervical os or mid cervix and was grabbed by the left hand already introduced per rectum then cervical mucus was aspirated from the cervical os or mid cervix and then transferred to a sterilized test tube.

For WST, 1 mL of estrus cervical mucus was heated with an equal volume of 5% sodium hydroxide up to boiling point (shown in supplementary figure S2) and after cooling the intensity of color changes was studied and graded as normal (no color), mild infection (light yellow color), moderate infection (yellow color) and severe infection (dark yellow color) [13]. (shown in supplementary figure S3)

In animals that were positive for the WST, the cervical mucus was sent for bacteriological culture, and isolation and identification of the organisms were carried out based on cultural, morphological, colony characteristics, motility, and biochemical reactions.

3. Results

From the WST out of 307 samples there are 246 repeat breeding cattle, 56(22.76%) showed no color, 147(59.75%) showed light yellow, 29(11.78%) showed Yellow, and 14(5.69%) showed Dark Yellow color with a normal, mild, moderate, and severe grade of infection. The control group (normal cyclic cattle) shows 52(85.24%) with no color change and 9(14.75%) shows light yellow with normal and mild grades of infection. (Table 2)

Table 2: Results of WST showing grades of infection based on color intensity between repeat breeders and normal cyclic (Control group) animals

Color Intensity	Grade of Infection	Repeat Breeding cattle		Control group	
		N	%	N	%
No color	(0) Normal	56	22.76%	52	85.24%
Light Yellow	+ Mild	147	59.75%	9	14.75%
Yellow	++ Moderate	29	11.78%	-	-
Dark Yellow	+++ Severe	14	5.69%	-	-
Overall		246	100	61	100

Out of 246 repeat breeding cattle, 147 show light yellow color, 29 show yellow color, 14 show dark yellow color, and 56 show no color change whereas, in the control group 52 show no color change, and 9 show light yellow color. (Figure 2)

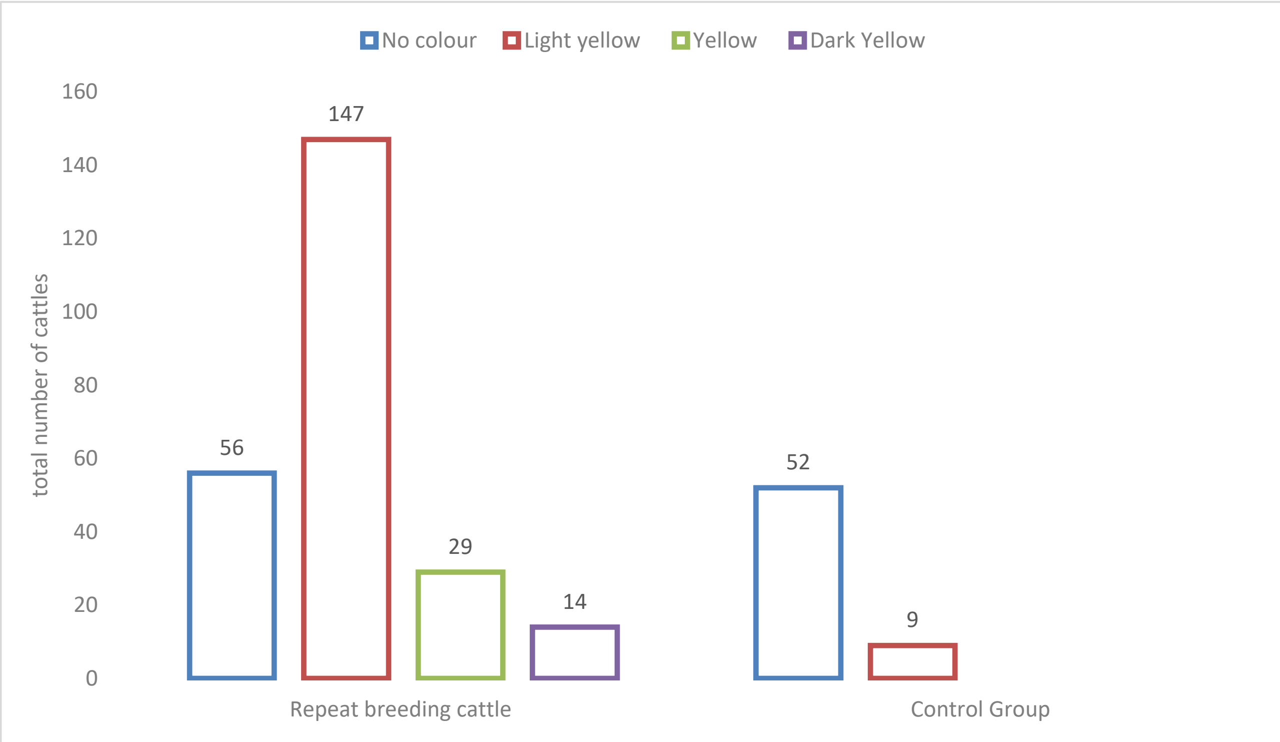

Figure 2: The above bar diagram represents the repeat breeding and control group of cattle about color intensity according to WST

A total number of positive cases in Budhanilkantha Urban Municipality 9(3.65%), Chandragiri Urban Municipality 14(5.69%), Dakshinkali Urban Municipality 7(2.84%), Dakshinkali Urban Municipality 7(2.84%), Gokarneshwor Urban Municipality 28(11.38%), Kirtipur Urban Municipality 105(42.68%), Kageshwori Manahara Urban Municipality 12(4.47%), Kathmandu Metropolitan City 5(2.03%), Nagarjun Urban Municipality 5(2.03%), Shankharapur Urban Municipality 9(3.65%), Tarkeshwor Urban Municipality 7(2.84%), and Tokha Urban Municipality 45(18.29%). (Table 3)

Table 3: Total number of positive cases from 11 different municipalities

Municipality of Kathmandu District	Total number of positive cases	Percentage of positive cases
Budhanilkantha Urban Municipality	9	3.65%
Chandragiri Urban Municipality	14	5.69%
Dakshinkali Urban Municipality	7	2.84%
Gokarneshwor Urban Municipality	28	11.38%
Kageshwori Manahara Urban Municipality	12	4.47%
Kathmandu Metropolitan City	5	2.03%
Kirtipur Urban Municipality	105	42.68%

Nagarjun Urban Municipality	5	2.03%
Shankharapur Urban Municipality	9	3.65%
Tarkeshwor Urban Municipality	7	2.84%
Tokha Urban Municipality	45	18.29%
Total	246	100

Out of 307 samples collected, Kirtipur Municipalities show the highest percentage of positive cases 105 (42.68%), followed by Tokha Municipalities 45 (18.29%), and Gokarneshwor Municipalities 28 (11.38%), while Kathmandu Metropolitan City shows the lowest percentage of positive cases 5(2.03%), followed by Nagarjun Municipalities 5(2.03%). (Figure 3)

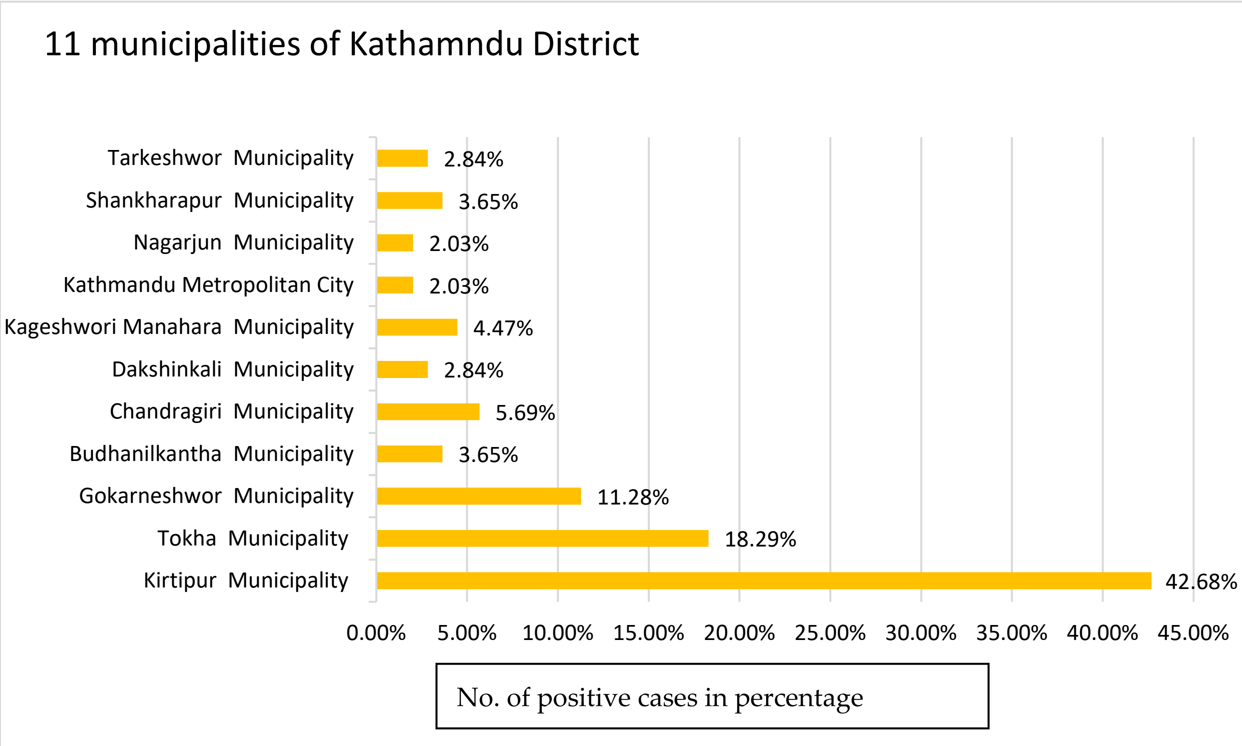

Figure 3: Total percentage of positive cases in Repeat breeding cattle of different Municipalities of Kathmandu

The sample which shows mild (+), moderate (++), and severe (+++) grades of infection from the WST were cultured. i.e., 190 samples were cultured. In bacterial culture, different bacteria were isolated i.e., *E.coli* 55(28.94%), *Pseudomonas spp.* 7(3.68%), *Staphylococcus spp.* 39(20.52%), *Streptococcus spp.* 26(13.68%), *Corynebacterium spp.* 10(5.26%), *Klebsiella spp.* 9(4.73%), and mix bacterial growth were 44(23.15%).*Pseudomonas spp.* 7(3.68%), *Klebsiella spp.* 9(4.73%), *Corynebacterium spp.* 10(5.26%), *E. coli* 55 (28.94%), *Staphylococcus spp.* 39(20.52%), *Streptococcus spp.* 26(13.68%), and mix bacterial growth were 44(23.15%) seen in (Figure 4).

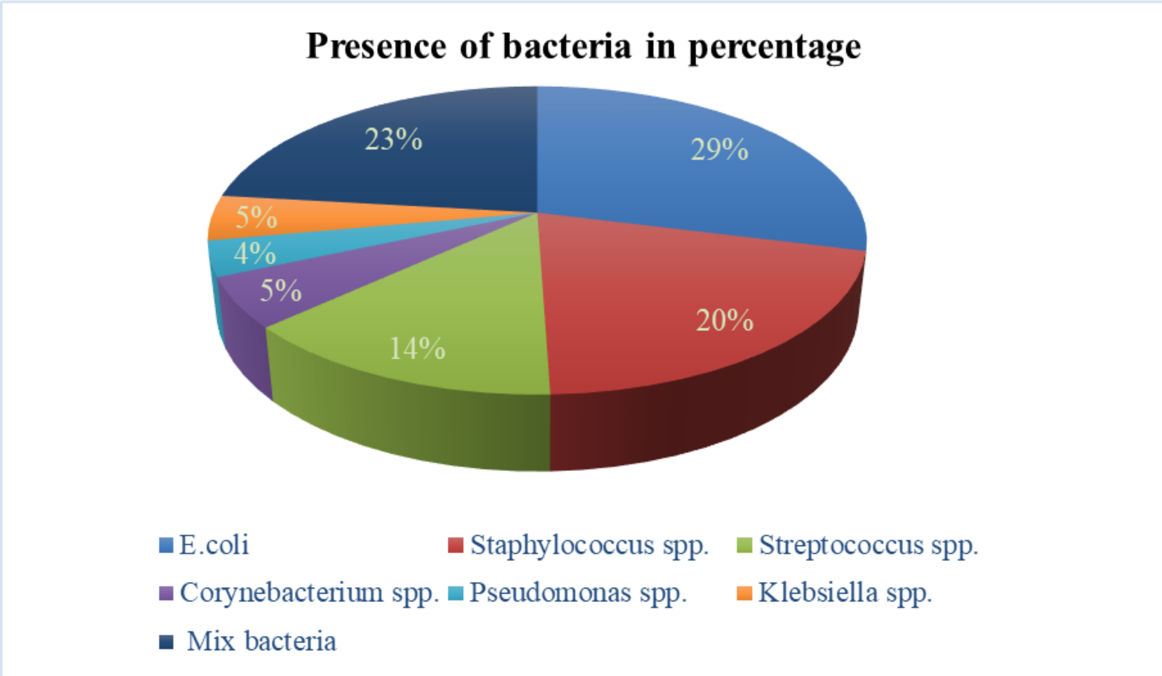

Figure 4: Total percentage of bacteria present in the culture of repeat-breeding cattle

4. Discussion

From the results of WST, it can be inferred that only 9(14.75%) animals in the control group showed infection and the remaining 52(85.24%) animals were free of infection; however, the majority of repeat breeding animals showed infection 190(77.22%) and only 56(22.76%) of animals were free of infection. Furthermore, on bacterial culture, we found *E.coli* 55 (28.947%), *Staphylococcus spp.* 39(20.526%), *Streptococcus spp.* 26(13.684%), *Corynebacterium spp.* 10(5.263%), *Pseudomonas spp.* 7(3.684%), *Klebsiella spp.* 9(4.736%), and mix bacterial growth were 44(23.157%).

This result is consistent with the results of the White Side Test in repeat breeder crossbred Jersey cows, which were recorded as 16.67, 66.67, 09.26, and 7.41 percent color pattern of no color, light yellow, yellow, and dark yellow type, respectively. These results were interpreted as negative, mild, moderate, and severe. The various bacteria that were isolated and cultured from repeat breeding cattle included *Staphylococcus spp*. 16 (21.05%), *E. coli* 14 (18.42%), *Bacillus spp*. 10 (13.16%), *Corynebacterium spp.* 10 (13.16%), *Pseudo-monas spp.* 8 (10.53%), *Proteus spp*. 8 (10.53%), *Klebsiella spp*. 6 (7.89%), and *Streptococcus spp*. 4 (5.26%). Of the 46 (76.67%) repeat-breeding cows, a single organism was identified, whereas mixed infections involving many types of organisms were found in 14 (23.33%) [9]

The results of the present study were more or less in agreement where the majority of aerobic bacteria (84.72%) are present in the cervical mucus of repeat-breeding cows with subclinical endometritis. *Staphylococcus aureus* isolates have been shown to make up the biggest proportion of all facultative anaerobic bacterial species isolates, followed by *E. coli*, *Streptococcus spp.*, *Enterobacter spp*., *Proteus spp.*, and *Pseudomonas spp.* [14].

The reported color reaction on WST in 92.85% of cervical mucus samples of normal animals indicating subclinical uterine infections which differ from our result where 77.22% were positive for WST in repeat breeding cattle [8]. The recorded color changes in 100% samples of cervical mucus in repeat breeding cows suffering from endometritis which differ from our result [15]. The variance in the infection rate between research may be attributable to the severity of the infections being studied, sample size and demographic variation, and the agro-climatic conditions in the regions where the studies were conducted.

The proportion of positive cases was highest in Kirtipur Municipalities, where it was 105 (42.68%), and lowest in Kathmandu Metropolitan City and Nagarjun Municipalities, where it was 5(2.03%) and 5(2.03%), respectively. Less sample collection and a smaller number of cattle being raised may be to reasons for the disparity in the results.

5. Conclusions

The findings from this study underscore the significant prevalence of nonspecific bacterial genital infections in repeat breeding cattle, as evidenced by the WST. With 77.22% of repeat breeders testing positive for infection, compared to a mere 14.75% in the control group, it is clear that these infections pose a major concern for cattle reproductive health. The predominance of specific bacterial pathogens, particularly E. coli and Staphylococcus spp., highlights the urgent need for targeted management strategies to mitigate their impact on fertility. Geographical analysis reveals notable disparities in infection rates across different municipalities, suggesting that local management practices, environmental conditions, and herd health protocols may significantly influence the occurrence of these infections. This variability emphasizes the importance of region-specific interventions and monitoring systems to enhance reproductive performance in cattle. The WST proves to be a valuable diagnostic tool for identifying subclinical endometritis in cattle, facilitating early intervention and potentially improving reproductive outcomes. By employing such a rapid and cost-effective test, farmers can make informed decisions regarding treatment and management, ultimately leading to better herd health and productivity.

Implications for South Asia

These findings are particularly relevant for South Asian nations, such as Nepal and India, where agriculture and dairy production are vital components of the economy. The high prevalence of reproductive infections in cattle can lead to significant economic losses due to reduced milk yield and fertility. By utilizing the WST, farmers can quickly identify infected animals and implement appropriate management strategies, thus enhancing milk production and overall herd health. Improving reproductive health in dairy cattle not only increases milk availability, which is crucial for food security, but also supports the livelihoods of millions of smallholder farmers dependent on dairy for their income. Enhanced reproductive efficiency can contribute to the sustainability of dairy farming in these regions, ensuring that farmers can meet the growing demand for dairy products while maintaining the health of their livestock. Future research should focus on elucidating the relationship between specific bacterial pathogens and reproductive failure, as well as exploring effective management practices to reduce the incidence of genital infections in cattle populations. These efforts will be crucial in advancing cattle health and productivity, ultimately contributing to sustainable livestock management practices that benefit both farmers and the broader agricultural economy in South Asia. By prioritizing the health of livestock, these nations can secure a more resilient agricultural framework capable of withstanding economic and environmental challenges.

Supplementary Materials (Figures)

Figure S1: Collection of cervical mucus for sample

Figure S2: Laboratory Examination of samples

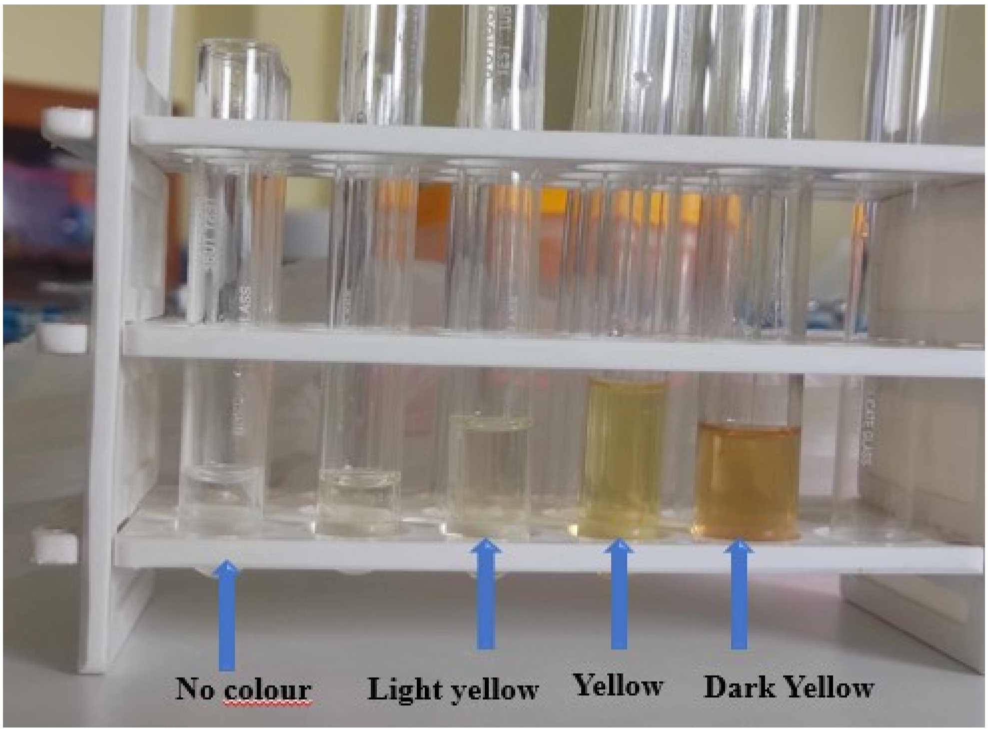

Figure S3: Changes in colour after WST

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