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# LEPTOSPIROSIS ASSOCIATED ABORTION IN COWS AT NATIONAL JERSEY BREEDING CENTRE, SAMTSE

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**ABSTRACT:** Leptospirosis is an acute zoonotic infection caused by spirochete bacteria belonging to genus *Leptospira*. Typical signs of leptospirosis in cattle are abortion, stillbirth, weak new born calf, sudden milk drop and infertility. A cow can abort as early as fourth month of gestation but abortion during third trimester is more common. We conducted a study with the objective to describe the findings of investigation on abortion cases of cows at the National Jersey Breeding Center in Samtse district. Paired sera samples were collected 14 days apart. First set of sera samples were collected soon after the abortion (n=6) and subjected to microscopic agglutination test (MAT) against the panel of *Hardjo, Pomona* and *Lai like* serovars. Semi-structured questionnaire was used to interview farm workers. The highest rate of abortion was 15.8%. Paired sera test revealed over fourfold rise in Microscopic Agglutination Test (MAT) titre against at least one serovar of Leptospira. Leptospirosis can significantly reduce farm profitability due to loss of calves and drop in milk production. Findings on the exposure of farm worker clearly suggest importance of farm biosecurity and occupational health safety. Isolation of organism from clinical sample will provide more information on infecting serovars in Bhutan.

**Keywords:** Abortion; cows; infection; leptospirosis; serovar.

## 1. INTRODUCTION

Leptospirosis is an acute infection caused by spirochete bacteria belonging to genus Leptospira. The two most common genomic species are *Leptospira biflexa* and *Leptospira interrogans*, the latter being the pathogenic Leptospira (Adler and de la Pena Moctezuma 2010). Vast majority of the studies available are from *L. interrogans*. Currently, there are 25 serogroups and 300 pathogenic serovars recognized. Leptospirosis was first identified as spirochete infection as early as 1917 by Naguchi in Japan. Rodents and insectivores are reservoir hosts for a number of Leptospira serovars and act as source for cross-species infection. In India, the first case of Leptospirosis was reported in 1926 from Andaman

and Nicobar Islands. Only in 1940s, Leptospirosis was identified as an important veterinary problem. It has also been recognized as one of the neglected bacterial zoonoses.

Typical signs of leptospirosis in cattle are abortion, stillbirth, weak calf, sudden milk drop and infertility. Abortion causes majority of the losses. A cow can abort as early as fourth month of gestation but is more common during third trimester (Prescott et al. 1988; Langoni et al. 1999). Unlike abortion, milk drop syndrome occurs in the early stage of infection. A sudden drop in milk yield accompanied by rise in temperature and inappetance in milking cows should be suspected of recent exposure. Abortion due to serovar *hardjo* (Kirkbride and Johnson 1989; Ellis et al. 1985) tends to be sporadic as opposed to abortion storm associated with *Pomona* or *grippotyphosa* serovars (Grooms 2006; Genc et al. 2005).

In Bhutan, recently conducted risk based sero-surveillance in cattle reported Leptospirosis sero-prevalence as high as 51% in some of the districts (Tenzin, unpublished data). The report suggested that large proportions of Bhutanese cattle are exposed to Leptospirosis. Higher prevalence rate was reported from Punakha, Wangdue and Trashigang compared to other districts.

In the late spring of 2015, National Jersey Breeding Centre, Samtse, a nucleus breeding farm, reported an unusual abortion in Jersey and Karan Fries cows. Subsequently, the National Centre for Animal Health, Serbithang investigated the abortion cases in NJBC cows. The investigation confirmed abortion as associated with Leptospira infection. This study describes the detail findings.

## 2. MATERIALS AND METHODS

### 2.1 Veterinary inspection of aborted cows

The investigation team visited the farm and inspected all the cows that aborted recently. Cows were examined for general health condition, management and gynecological status, including uterine infection.

### 2.2 Interview of farm staff and data collection

All relevant information such as history of abortion and details of affected animals; clinical manifestation in affected animals; associated risk factors of abortion; and public health were collected using semi-structured questionnaire. The major part of the information collected was for the last eleven months (July 2014-May 2015).

### 2.3 Sample

Samples were collected from cows that aborted recently. Types of sample included vaginal swab, uterine discharge and urine collected around the time of abortion. Serum samples were collected at three different time points (Time A: March 26, 2015; Time B: May 24, 2015 around abortion time and Time C: June 6, 2015, 2 weeks post Time B). Time interval between Time A and B was two months. Similarly, the time interval between Time B and C was two weeks. Additionally, abortion products such as placenta, foetal organs, foetal intestinal and stomach content were also collected.

### 2.4 Serovar culture and antigen preparation

Special media for Leptospira was prepared. Commonly prevalent serovars of Leptospira (Pomona, Hardjo and Lai like) were cultured at Human Public Health Laboratory, Thimphu. Culture was enumerated and antigen prepared.

### 2.5 Laboratory analysis

*2.5.1 Serology*

Serology was performed using Microscopic Agglutination Test (MAT), the World Health Organization (WHO) and World Animal Health Organization (OIE) reference method for detection of Leptospirosis in human and animals, respectively. In microtitre plate (Nunc MaxiSorp, Thermo Scientific, USA), serovar specific titrated antigen was reacted with serially diluted serum samples to allow agglutination between antibody (serum sample) and antigen (serovar). The MAT was read by dark-field microscopy. The end point titre was recorded as the highest dilution of serum at which 50% agglutination occurred due to antigen and serum antibody reactivity. The four fold (1/40) and 10 fold (1/100) increase in end point titre in samples collected 2 weeks apart (Time point B and C) were considered the past and recent exposure, respectively. Similarly, the serum samples (paired sera) were also tested using enzyme linked immunosorbent assay (ELISA) (IDEXX, Montpelier, France) for antibody against Brucella exposure. A sample-to-positive (SP) value ≥110% was considered positive to antibody against Brucella. A significant increase ($p<0.05$) in SP% for samples collected 2 weeks apart (Time point B and C) was considered recent exposure.

*2.5.2 Molecular test*

Real time-polymerase chain reaction (RT-PCR) was performed for samples (placenta and vaginal swab), using *Brucella abortus* and *Leptospira interrogans* specific primers and probes. Amplification signal cut-off value of positive control was set at 23 cycle threshold (Ct) for the presence of genomic material belonging to the pathogen of interest.

### 2.6 Data analysis

Statistical analyses and illustrations were performed using Prism 5 (GraphPad Prism Software, Sandiego California, USA). Sero-reactivity between the time points was compared using Mann-Whitney test.

## 3. RESULTS

### 3.1 Abortion trend at NJBC, Samtse for last 2 years

Abortion data for the period 2013-14 (July-June) and 2014-15 (July-May) were compared (Figure 1). For the period 2013-14, the mean percentage of abortion was 2.2±0.8: 95% CI (0-7.7: maximum-minimum). The mean abortion percentage for the period 2014-15, that included current abortion, was 5.7±1.7: 95% CI (0–15.8: maximum-minimum). The rate of abortion for the period 2014-15 was about 2.6 times higher than that of 2013-14. Interestingly, the highest rates of abortion (7.7% and 15.8%) for both the periods were in the month of April.

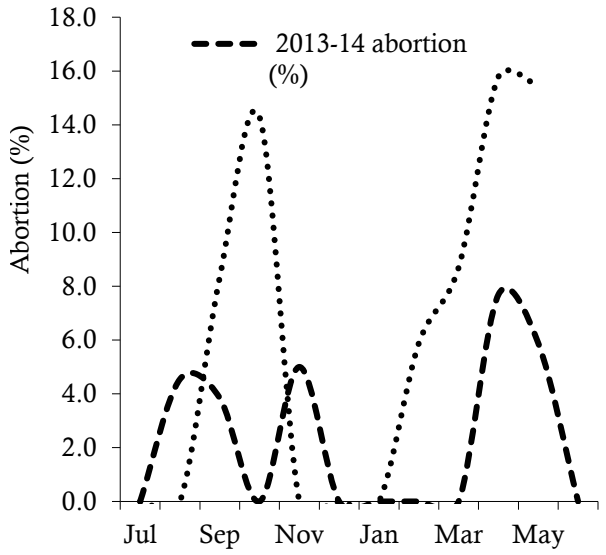


**Figure 1:** Abortion trend at NJBC, Samtse.

### 3.2 Gestation period at abortion

Average age of the cows affected by abortion was 4.3 years. Proportions of animals that aborted in second and third trimester of gestation were 37.5% and 62.5%, respectively. Abortion was not recorded in the first trimester of gestation (Table 1). Majority of abortions were observed in Trimester 3 or in the beginning of Trimester 3 (Figure 2). Trimester was calculated as proportion of gestation days at abortion to gestation period (270 days) multiplied by 3 and the values categorized into three trimesters: 0–1 (Trimester 1); 1 – 2 (Trimester 2) and 2 – 3 (Trimester 3).

### 3.3 Rainfall data

Leptospirosis is predominantly encountered in tropical areas with abundant rainfall. Previous studies (ref) reported that rainfall, along with availability of rodent reservoir, plays an important role in the spread of infection. Two-year rainfall data did not appear to have played a role in spurring the current spread of infection. The rainfall data for 2014-15 was similar to that of 2013-14 when abortion case was not reported in the farm (Figure 3).

### 3.4 Milk production trend

Daily milk production trends for the period July 2014 to May 2015 for both Jersey (n=74) and Karan Fries (KF) (n=27) cows were compiled and analysed (Figure 2). For both breeds, about 50% drop in milk was observed at the end of January 2015. The drop in milk yield continued for subsequent two months for KF but recovered from the beginning of April 2015. However, unlike in KF, the drop in milk yield for Jersey cows did not recover in the subsequent months (Figure 4).

**Table 1:** Temporal data of abortion.

| Cow* | Age ** | Successful AI date | Abortion date | Gestation stages at abortion | | |
|---|---|---|---|---|---|---|
| | | | | Day | Month | Trimester*** |
| 509KF | 3.0 | 18.11.2014 | 23.04.2015 | 156 | 5.2 | 1.73 |
| 204J | 7.3 | 18.10.2014 | 17.05.2015 | 211 | 7.0 | 2.34 |
| 503KF | 3.1 | 10.11.2014 | 10.05.2015 | 181 | 6.0 | 2.01 |
| 363J | 4.0 | 15.10.2014 | 06.04.2015 | 173 | 5.8 | 1.92 |
| 521KF | 4.9 | 25.08.2014 | 07.03.2015 | 194 | 6.5 | 2.16 |
| 388J | 3.6 | 14.11.2014 | 21.05.2015 | 188 | 6.3 | 2.09 |
| 324J | 4.8 | 18.10.2014 | 06.04.2015 | 170 | 5.7 | 1.89 |
| 373J | 3.6 | 23.10.2014 | 30.05.2015 | 221 | 7.4 | 2.46 |

*Karan Fries (KF), Jersey (J); **Years; Artificial insemination (AI); ***0<*Trimester 1*≤1, 1<*Trimester 2*≤ 2, 2<*Trimester 3*≤3.

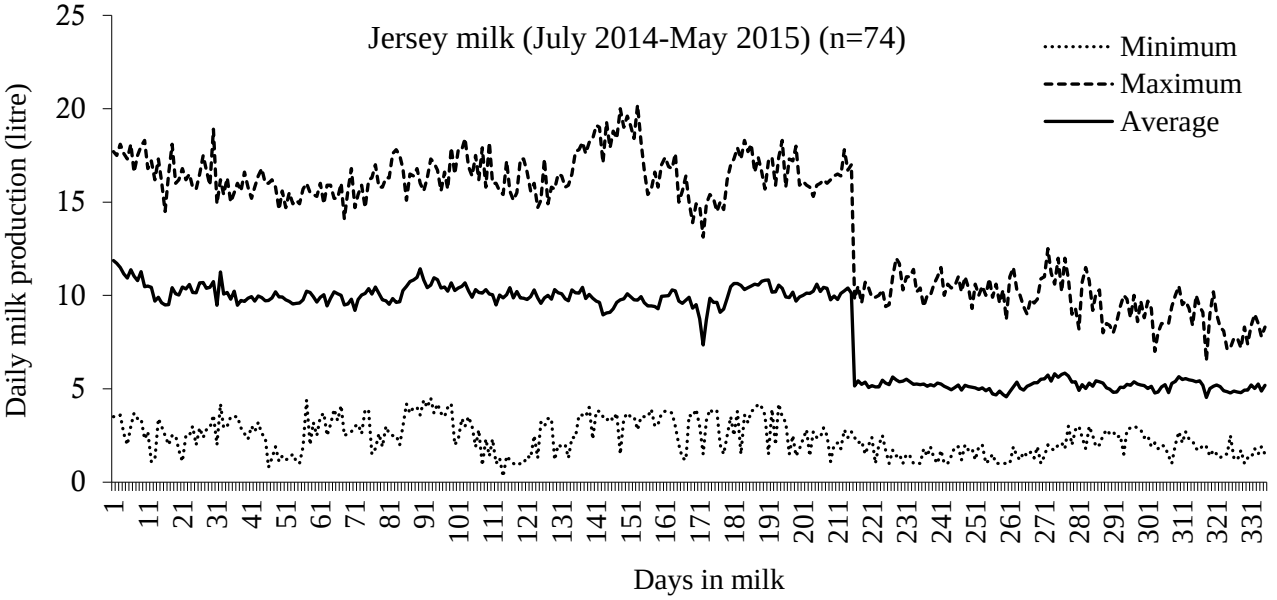


**Figure 2:** Timeline of abortion. Each colored cell is the date of successful artificial insemination, gestation and abortion. First date of successful AI was 25.8.2014. Gestation is divided into three trimesters and terminates at abortion.

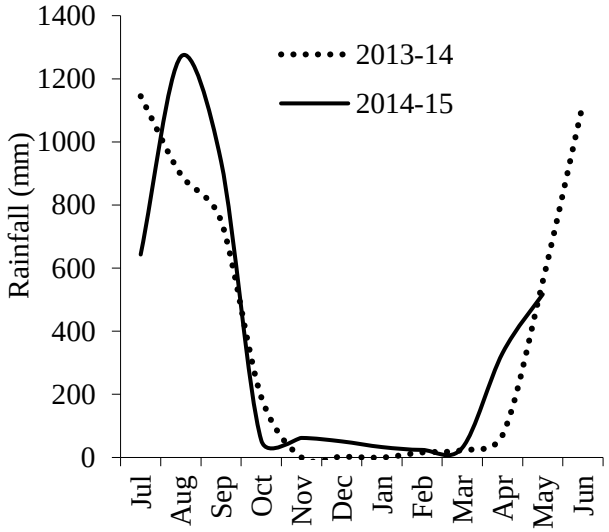


**Figure 3:** Rainfall data (2013-14 and 2014-15) of NJBC, Samtse.

Myriad of farm activities were reported to have taken place in and around the time of this milk drop. These activities were: all the animals in the farm received Foot and Mouth Disease vaccine on Jan 7, 2015; silage feeding began on Jan 16, 2015; and animals were vaccinated against Haemorrhagic Septicemia on March 20, 2015. From the end of January to first week of February 2015, the veterinary health record also showed several cases of mastitis and wound treatment.

### 3.5 Serology

**MAT:** In March, April and May 2015, there were nine abortions in total. Since these abortions were spread over the period of three months, serum samples for all three different time points were available only from six animals that aborted. All the samples collected two weeks post abortion

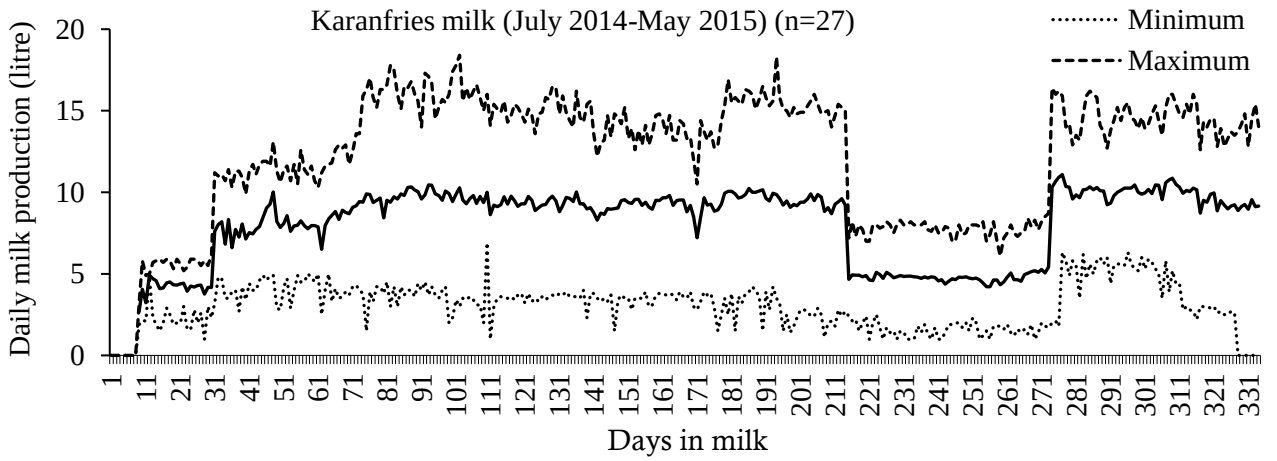


**Figure 4:** Milk production trend at NJBC, Samtse

(Time C) showed MAT titre of greater than fourfold when compared with that collected around the time of abortion (Figure 5). Majority of the titre values were greater than 100, indicating recent infection. Serovar Hardjo showed the highest reactivity followed by Lai like and Pomona. Thus, the MAT titre of Time C was significantly higher than Time points B and A.

**Brucellosis ELISA:** The sera samples collected at Time B and Time C (paired sera) showed high SP%. However, SP% of sera from Time C did not show significant increase from that of Time B.

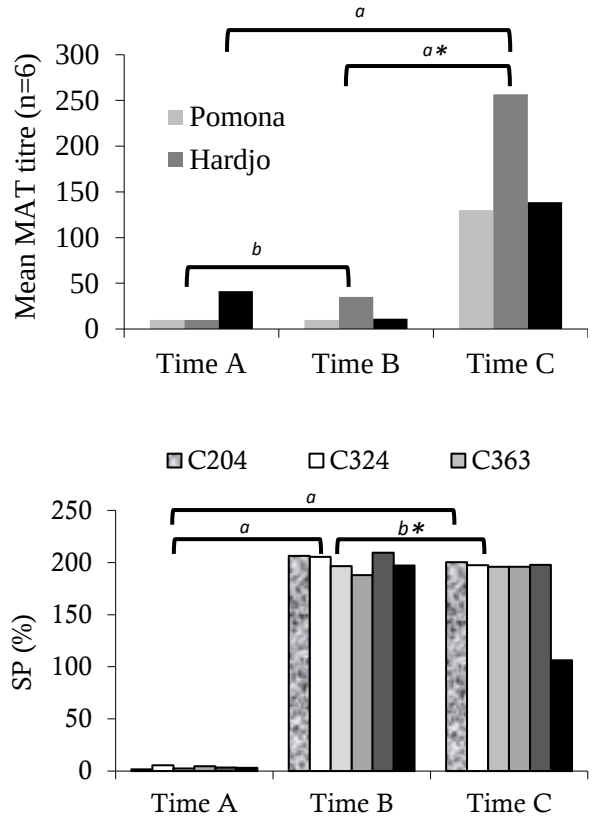


**Figure 5:** MAT titre and Brucellosis ELISA.
[a]significant ($p<0.05$); [b]not significant ($p>0.05$);
*paired sera.

**RT-PCR**

Placenta sample from recently aborted cow was tested positive for Leptospira with detectable signals at cut-off point Ct value of 23. All other samples tested negative for Leptospira. Similarly, all samples were tested negative for Brucella by RT-PCR.

Based on the findings from serological and molecular investigation, the abortion storm in cows of NJBC, Samtse may be associated with Leptospirosis. Additionally, majority of the abortions occurred in the third trimester of gestation, which is typical of leptospirosis.

## 4. DISCUSSION

Leptospirosis is a spirochete infection that has significant zoonotic importance. Exposure to Leptospira is influenced primarily by the presence of pathogen in the environment, persistent herd mate shedder as potential source, rodent population in the premises, rainfall, and stress conditions in the host species. Abortion rate in cattle is dependent on the time of exposure during gestation. Generally, clinical signs are manifested seven days after the exposure. Clinical signs in cows are characterized by fever, inappetance, conjunctivitis and bleeding. In milking cows, these signs are accompanied by sudden drop in milk yield, soft and flabby udder and mastitis. In pregnant cows, abortion occurs approximately after ten weeks, following exposure to infective dose (Grooms 2006). Majority of the abortion occurs at third trimester of gestation. A detectable antibody titre is developed by eleventh week post infection.

In this study, the sudden drop in milk yield at the end January 2015 appeared to have been the point of exposure that led to sudden increase in abortion rate at NJBC. The disease chronology conformed to all the events that transpired through the period of exposure till abortion. The rainfall data during the year of abortion and the preceding year did not show significant difference. Although studies have shown that rainfall plays important role in the spread of Leptospira infection (Simoes et al. 1969; Estavoyer et al. 1980; Chen 1985), it may not have influenced this outbreak. However, there is a possibility that myriad of activities taking place at the farm between January and February 2015 may have put susceptible animals under considerable level of stress, thus predisposing to exposure. Therefore, it is important that the farm management considers spreading out activities to avoid such incidence in future.

Infected animals are known to shed organism in urine. This investigation tested only placenta and uterine discharge. Low detection of Leptospira genomic materials by molecular analysis may be attributed to inappropriate sampling. Future investigation should focus on testing urine sample from aborted animals for detection of Leptospira.

One interesting finding from this study was the pattern of milk yield over a period of eleven months. Sudden drop in milk yield was observed towards the end of January 2015 in both the breeds. The drop continued for two months in Karan Fries breed and regained to fit into normal lactation curve. However, the drop in Jersey breed failed to recover. This difference was not well understood but one possible explanation could be in the stages of lactation that this investigation captured. Milk data collected for eleven months revealed that

majority of Jersey cows had attained peak production by July 2014 and steadily decreased over the period of eleven months' record as opposed to KF breed milk data that steadily increased for the same period of time. Perhaps, this steadily increasing trend of milk yield in KF breed was able to regain yield, despite a drop for two months in February and March, 2015.

During the entire sequence of events from exposure to abortion, many farm personnel were involved in direct handling of infected animals. Occupational health safety needs to be considered to protect farm workers from being exposed to such zoonotic infection. Through the questionnaire survey, it was learnt that the farm workers did not experience any possible signs of Leptospirosis, but it is important to screen them for any cases of sero-positivity. In humans, the response to exposure to leptospirosis can be easily mistaken with the signs of common flu.

Leptospirosis in animals can be cured if detected in time. There is a range of antibiotics that are effective against Leptospira species. Top of the order of choice of antibiotics is dihydrostreptomycin/streptomycin, followed by doxycycline, penicillin G and tetracycline. In many countries, antibiotic therapy complemented with vaccination has proven to be effective in control of Leptospirosis in cattle (Russell 1958; McClain et al. 1984; Guidugli et al. 2000). To some extent, streptomycin and dihydrostreptomycin are also effective against brucellosis. In absence of test that differentiates infected from vaccinated animals (DIVA) for Brucellosis, this investigation could not conclude whether the observed sero-reactivity was from infection or vaccination.

Unpublished data from previous study on sero-prevalence in Bhutan revealed past exposure of cattle to numerous serovars of Leptospira species. This is the first study undertaken to confirm active infection and its impact on production. However, further investigation is required to determine the extent of infection and to examine if agents other than Leptospira are involved.

## 5. CONCLUSIONS AND RECOMMENDATIONS

For the immediate control of abortion cases at NJBC, Samtse, following precautions must be noted and recommendations implemented:

### Precautions

- Care should be taken by individuals handling streptomycin for injection to avoid skin sensitivity reactions. As with all intramuscular preparations, streptomycin sulfate injection should be injected well within the body of a relatively large muscle and care should be taken to minimize the possibility of damage to peripheral nerves.
- Streptomycin may impair fetal development when administered to a pregnant animal because streptomycin readily crosses the placental barrier. Caution in use of this drug is important to prevent ototoxicity in the fetus.
- Treatment with streptomycin may not produce expected result especially when leptospirosis has already done damage to the endometrium. Under such circumstances, abortion will take place inadvertently.
- Withholding period of milk should be observed based on the use of specific antibiotic. In the case of use of streptomycin, a withholding period of 72 hours should be observed before it is declared safe for human consumption.

### Use of antibiotics

- All clinically affected animals should be treated with antibiotic (dihydro-streptomycin or streptomycin), following dose rate for veterinary use. If the choice of antibiotic is not available, the farm management in liaison with the National Centre for Animal Health, Serbithang should discuss and source for immediate procurement.

### 5.3 Public Health

- NCAH should notify DoPH, MoH and Samtse Hospital, regarding the current outbreak of leptospirosis in the NJBC, Samtse.
- Until the antibiotic therapy is initiated, the farm management should notify all the consumers to boil the farm supplied milk properly before consumption.
- Leptospirosis being one of the important zoonoses, all staffs that are regularly in contact with affected animals are advised to contact Samtse Hospital to screen for exposure to leptospirosis.

### 5.4 Sampling

- All the herd mates to be sampled to understand the actual extent of infection in the population. Serum samples collected at two time points two weeks apart and tested by MAT to examine the rising antibody titre.
- Cattle in the areas of close proximity to the farm needs to be sampled to establish the endemicity of leptospirosis.
- The samples (serum/urine) from all the animals should be collected at regular interval, based on standard protocol to assess the control program.
- Rodents within the farm premises should be trapped and samples collected to ascertain the reservoir status.

**5.5 Environment management**

- The farm should place top priority to control the rodents in the farm structures such as feed stores, silage pit and shed.
- The farm management should ensure to clean the barn/manger before and after feeding by applying power pressure water hose. This is to clean rodent urine deposits in the feeding manger/shed and remove leftover feed to discourage rodents visiting such areas.

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