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Seroprevalence of Leptospirosis in Dairy Cows with Reproductive Disorders

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ABSTRACT

The present investigation was carried out to study the prevalence of leptospirosis in dairy cows with reproductive disorders in and around Kamrup district of Assam. A total of 130 sera collected from dairy cows with reproductive disorders in different localities of Assam were screened for leptospirosis by MAT. The seroprevalence of leptospirosis in dairy cows with reproductive disorders was found to be 14.62 per cent. Twelve *Leptospira* antigen serovars: *L. Australis*, *L. Autumnalis, L. Ballum, L. Bataviae, L. Canicola, L. Grippotyphosa, L. Hebdomedis, L. Pomona, L. Pyrogenes, L. Sejroe, L. Icterohaemorrhagaie*, and *L. Javanica* were used, to screen for leptospirosis. *Leptospira* antibodies were detected in four serovars: *Australis, Autumnalis, Ballum* and *Bataviae*. Australis was found to be the most circulating serovar with 47.37 per cent. The reproductive disorders reported in the present study were: repeat breeding 41 (31.54%), abortion 34 (26.15%), endometritis 25 (19.23%), post-partum anoestrus 14 (10.77%), dystocia 5 (3.85%), retention of foetal membrane 4 (3.07%), anovulation 2 (1.53%), uterine prolapse 2 (1.53%), silent oestrus 2 (1.53%) and cystic ovaries 1 (0.77%). Repeat breeding was found to be the most prevalent reproductive disorder (31.54%) followed by cases of abortion (26.15%) in different localities of Assam.

HIGHLIGHTS

- Australis, Autumnalis, Ballum and Bataviae serovars of Leptospira were found in dairy cattle in a flood affected areas of Assam
- Leptospirosis was found to be common in dairy cows with history of abortion mostly during the third trimester.

Keywords: Incidence, Prevalence, Microscopic Agglutination Test, Abortion

Leptospirosis is a common global zoonotic disease in all warm-blooded animals, mostly in hot and humid areas. In India, Leptospirosis have been reported to be endemic in Southern states like Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, and other coastal states like Gujarat, Maharashtra including Andaman and Nicobar Islands (Balamurugan *et al.,* 2018). However, works on prevalence of leptospirosis in animals in North Eastern states of India are very scanty. Among the specific causes of reproductive disorders Leptospirosis is a well-known cause of reproductive losses in cattle including abortion, infertility still birth etc. (Mughini grass *et al*., 2014) Typically, the disease occurs through bacterial exposure to mucous membranes and generally results in occult form or relatively mild acute clinical signs (Anwar *et al.*, 2013). *Leptospira* infection had been reported as a source of great economic losses in dairy farms causing reproductive problems (Ijaz *et al*., 2018).

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Bearing in mind that leptospirosis is a zoonotic disease and common to all warm-blooded mammals and the fact that, its presence had been established in other species, but no systematic study has been carried out in this region regarding bovine leptospirosis. Further, no work has so far been conducted on leptospirosis on animals with reproductive disorders. Hence, the findings of the present study would provide a baseline data on leptospirosis in cows with reproductive disorders.

MATERIALS AND METHODS

All work in the present study was conducted with the formal approval of Institutional animal ethics committee, Assam Agricultural University, Khanapara, Guwahati-781022 bearing the approval number 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/18-19/635dated 28.12.2018. A total of 130 crossbred cows, after clinicogynaecological examination and with history of reproductive disorders were selected from different localities in and around Kamrup district of Assam (Fig. 1). Kamrup district is situated between 25.46 and 26.49 North latitude and between 90.48 and 91.50 east latitude in a sub-tropical climatic condition with semi dry in summer and cold in winter. The district is separated into north and south part by the mighty river Brahmaputra. Due to heavy flood (almost 1/3 rd of the district) during rainy season the livestock population is badly affected in this region. Blood samples were collected aseptically by vein puncture from the jugular vein in clot activator vial as shown in the Fig. 1 and the sera were separated by centrifugation which were stored at -20°C till when Microscopic Agglutination Test (MAT) was carried out to study the seroprevalence of leptospirosis in dairy cows. Serum samples were later screened against twelve *Leptospira* antigen serovars: *L. Australis, L. Autumnalis, L. Ballum, L. Bataviae, L. Canicola, L. Grippotyphosa, L. Hebdomedis, L. Pomona, L. Pyrogenes, L. Sejroe, L. Icterohaemorrhagaie*, and *L. Javanica* as shown in the Fig 3 at a titre of 1:20 and highest dilution being 1:160 for leptospirosis by Microscopic Agglutination Test (MAT), following the standard procedure (W.H.O 2011; OIE, 2013).

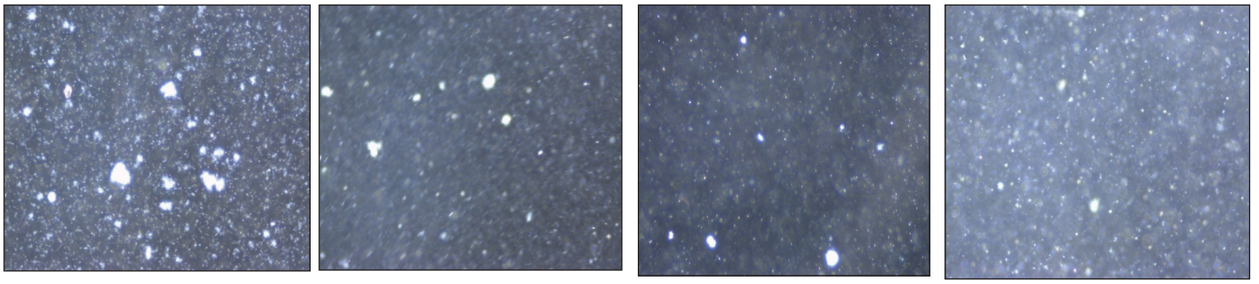
The test samples were examined under the dark field microscope (Leica DM750) with camera (Leica MC190 HD) manufactured by Leica Microsystems (Schweiz) AG Industry Division CH-9435 Heerbrugg. The slides were observed under 20X objective, without coverslip for agglutination. The highest dilution (the one that gives 50% agglutination, leaving 50% of the cells free) was checked and then compared with a control suspension of leptospires diluted in PBS without serum.

Data were analyzed using Microsoft Excel (Microsoft Office 2007, Microsoft corporation, Redmond, Washington, USA).

RESULTS AND DISCUSSION

Incidence of reproductive disorders in dairy Cows

Out of a total of 130 samples collected from dairy cows in the present study, the reproductive disorders repeat breeding 41 (31.54%) being the highest, abortion 34 (26.15%), endometritis 25 (19.23%), post-partum anoestrus 14 (10.77%), dystocia 5 (3.85%), retention of foetal membrane 4 (3.08%), anovulation 2 (1.54%), silent



(a) **(b)** **(c)** **(d)**

Fig. 1: (a) MAT titre at (1:20) **(b)** MAT titre at (1:40) **(c)** MAT titre at (1:80) **(d)** MAT titre at (1:160)

oestrus 2 (1.54%), uterine prolapse 2 (1.54%) and cystic ovaries 1 (0.77%).

This finding corresponds to work done by Ahmed *et al.* (1992) who reported cases of anoestrus, retained foetal membrane, endometritis, cervico-vaginal prolapse, uterine prolapse, dystocia and metritis in cattle in Assam. The present study was in agreement with the work of Dinka (2013), who recorded repeat breeding and abortion, (26.80 and 14.6 per cent respectively) as the major reproductive disorders of dairy cow. The result of the present study, differed from the work of Singh (2003) who recorded incidences of anoestrus, endometritis, retained foetal membrane, metritis, dystocia, abortion, cervicitis, prolapse, pyometra and stillbirth as 21.42, 13.26, 11.22, 6.12, 5.10, 5.10, 4.08, 2.04, 1.02 and 1.02 per cent respectively in cattle in Assam. The result of his study showed a higher percentage of anoestrus (21.42%) which could be due to breeds and management pattern. The present study differed with the work of Satyapal (2003), who reported incidences of abortion, stillbirth, dystocia, prolapse, retention of foetal membrane, and metritis in Karan Fries to be 7.85, 2.87, 3.05, 0.12, 27.65 and 29.70 per cent respectively.

Seroprevalence of Leptospira infection in dairy cows with history of reproductive disorders

In the present study, out of 130 dairy cows with history of reproductive disorders, the highest incidence of positive reactors was recorded in animal with history of abortion (35.29%). The overall seroprevalence of leptospirosis in the study area was found to be 14.62 per cent and is presented in the Table 1.

Table 1: Seroprevalence of *Leptospira* infection in dairy cows with history of reproductive disorders

Reproductive disorders	Total no. of samples tested	No. of Positive	Prevalence (%)
Abortion	34	12	35.29
Repeat breeding	41	3	7.31
Endometritis	25	2	8.00
Post-partum anoestrus	14	1	7.14
Silent oestrus	2	—	—
Retention of foetal membrane	4	1	25
Uterine prolapse	2	—	—
Anovulation	2	—	—
Cystic ovaries	1	—	—
Dystocia	5	—	—
Total	**130**	**19**	**14.62**

Kader (2018), recorded 17.89 per cent seroprevalence of leptospirosis in cows in 10 districts of Assam. Malakar *et al.* (2016) reported 2.13 per cent seroprevalence of leptospirosis among human population. A higher seropositivity reported in the present study and Kader (2018) in cattle, might be due to regular exposure of animals and presence of prevailing risk factors in the farm premises.

The seroprevalence of leptospirosis in Assam might be said to be lower than the seroprevalence of leptospirosis in places like Andaman Islands where Sunder *et al*. (2016) reported 42.15 per cent (180/427) in cattle. Sharma *et al*. (2006) reported a high seroprevalence of leptospirosis in Andaman Islands recorded as 53.70 per cent (327/611) among human population. Sharma *et al*. (2014) reported a high seroprevalence of leptospirosis in cattle (37.00%) and goat (29.00%) in Andaman Islands compared to seroprevalence of leptospirosis of 14.62 per cent in the present study. Similar higher seroprevalence of 50.85 per cent in cattle with history of infertility, abortion and haemogalactia in Odisha and West Bengal states of Eastern India was reported by Behera *et al*. (2014).

Seroprevalence of Leptospira serovars in dairy cows with reproductive disorders based on Microscopic Agglutination Test (MAT)

Various serovars of *Leptospira* in dairy cows with different reproductive disorders were identified by MAT. Positive agglutination was seen as a glistering woolen ball appearance under the dark field microscope. This pattern indicated that the sera were specific to *Leptospira* serovars. The details of the *Leptospira* serovars identified through MAT and their seroprevalence are presented in Table 2.

Table 2: Seroprevalence of *Leptospira* serovars in dairy cows with reproductive disorders based on MAT

Sl. No.	Serovars	No. of positive serovars	Prevalence (%)
1	Australis	9	47.37
2	Autumnalis	6	31.58
3	Ballum	2	10.53
4	Bataviae	2	10.53
5	Canicola	—	—
6	Grippotyphosa	—	—
7	Hebdomedis	—	—
8	Pomona	—	—
9	Pyrogenes	—	—
10	Sejroe	—	—
11	Icterohaemorrhagiae	—	—
12	Javanica	—	—
	Total	**19**	**100**

Results showed that the most predominant serovars circulating in dairy cows with reproductive disorders in the study area were, *L.* Australis (47.37%). The serum samples were tested for serovars specific antileptospiral antibodies, and were detected for their presence at different titre values of 1:20, 1:40, 1:80 and 1:160 as shown in Fig. 1 and the results have been presented in Table 3. A MAT titre ratio of 1:20 or above has been considered as standard (baseline dilution) for the positive reactor.

Table 3: Serovars specific antileptospire antibodies and their titre in seropositive dairy cows with reproductive disorders

Serovar	1:20	1:40	1:80	1:160
Australis	9	7	2	1
Autumnalis	6	5	1	—
Ballum	2	1	—	—
Bataviae	2	1	—	—
Canicola	—	—	—	—
Grippotyphosa	—	—	—	—
Hebdomedis	—	—	—	—
Pomona	—	—	—	—
Pyrogenes	—	—	—	—
Sejroe	—	—	—	—
Icterohaemorrhagiae	—	—	—	—
Javanica	—	—	—	—

Antibodies against *Leptospira* were detected in dairy cows with the history of abortion, repeat breeding, endometritis, post-partum, anoestrus and retained foetal membrane. The highest seroprevalence of leptospirosis (35.29%) was recorded in dairy cattle with the history of abortion followed by retention of foetal membrane (25.00%).

Antibodies against the serovars Australis, Autumnalis, Ballum and Bataviae were detected in dairy cows with reproductive disorders which corresponded to the report of Kader (2018), who recorded the serovars Autumnalis, Ballum, Bataviae and Javanica in cattle in 10 districts of Assam with low reproductive performance. This work was also in agreement with the work of Guitian *et al*. (2001), who reported suboptimal reproductive efficiency in *Leptospira* seropositive cattle in Galicia-Spain.

Seroprevalence of Leptospira infection in different stages of gestation in dairy cows with history of Abortion

Seroprevalence of *Leptospira* infection in dairy cows with history of abortion in different stages of gestation is shown in Table 4.

Table 4: Seroprevalence of *Leptospira* infection in different stages of gestation in dairy cows with history of abortion

Trimester	No of samples tested	Positive	Prevalence (%)
First	4	1	25
Second	5	1	20
Third	25	10	40

Out of 34 cases of abortion, 12 (35.29%) were positive for leptospirosis and out of 25 cows reported to have abortion at the third trimester, 10 (40%) were positive for leptospirosis. The result of the present study revealed, that abortion due to leptospirosis occurred mostly in the third trimester of gestation. This finding was in agreement with that of earlier worker (Guitian *et al.,* 2001) who reported late abortion, stillbirth and birth of weak calves in leptospirosis seropositive cows.

CONCLUSION

From the above study we can conclude that Australis, Autumnalis, Ballum and Bataviae serovars of Leptospira were found in dairy cattle and the Leptospirosis was found

to be common in dairy cows with history of abortion mostly during the third trimester.

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