



## RESEARCH ARTICLE

### Sero-Epidemiology of Bovine Leptospirosis and Associated Risk Factors in a Flood Affected Zone of Pakistan

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#### ABSTRACT

Bovine leptospirosis is an emerging zoonotic disease that compromises farm economy. Keeping in view limited studies on bovine leptospirosis, this project was designed to estimate its sero-prevalence in flood-hit zone of Pakistan, along with its associated risk factors. A total of 385 serum samples were randomly collected from tehsils Rajanpur, Jampur and Rojhan of district Rajanpur with necessary information captured in data form for risk factors analysis. Chi-square test, univariable and multivariable logistic regression analyses were applied to check association of hypothesized risk factors with disease. The study showed 23.12% of the bovine samples positive for *Leptospira* antibodies with non-significant difference ( $P > 0.05$ ) between cattle (25.52%) and buffaloes (20.72%). The non-significant difference ( $P > 0.05$ ) of bovine leptospirosis among tehsils of study area presented 26.56, 21.88 and 20.93% in tehsils Jampur, Rajanpur and Rohan, respectively. Chi-square test analysis depicted body temperature and socio-economic status of worker positively associated ( $P < 0.05$ ) with the disease. The univariable analysis based on Mantel Haentzel test and Wald statistics found early age, confined system, fodder type, jaundice status, and socio-economic status of the animal's owner as significant candidates (Odds ratio  $OR > 1$ ) while, multivariable logistic regression showed bovine specie, gender of animal, fodder type, jaundice status, and socio-economic status of the animal's owner as key risk factors for leptospirosis dynamics (Odds Ratio  $> 1$ ). The study concluded that, the flood hit area is having considerable prevalence of bovine leptospirosis and is positively associated with presence or absence of certain animal, management and environmental factors.

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#### INTRODUCTION

The floodwater becomes stagnant and eventually, appears as potential bacterial contamination source (Christopher and Portier, 2009) that has been reported as a call for disease outbreaks (Anonymous, 2015). Apart from diseases to livestock the fodder shortages put animals to the verge of selling at cheaper rates to other areas, which may also become an additional source of zoonosis to the areas where they are sold (Anonymous, 2015). Among various zoonotic diseases leptospirosis is known as disease strongly linked with floodwater (Sharma *et al.*, 2006) due to bacterial shedding through urine of animal (Adler and Moctezuma, 2010). The infected animals are mostly silent shedders of bacteria, which become a danger

to the humans exposed to urine directly or urine-contaminated water indirectly (Levett, 2010).

*Leptospira*, a spirochete, has vast specie vulnerability having 20 species with more than 200 serovar (Vinetz, 2001) jeopardizing smallholder's entrepreneurship with especial context to bovine leptospirosis. The disease is important both in terms of losses to the wealth and health of animals and humans. In Pakistan 2-10% prevalence of bovine leptospirosis has been reported in early nineties (Mahmood, 1992) that tuned to 85% at the end of 20<sup>th</sup> century (Bharti *et al.*, 2003). Few studies have been conducted on bovine leptospirosis in Pakistan. The rural areas are prone to this disease because of dearth lack of quarantine and precautionary measures especially, during and after raining/ flood seasons. The developed countries

are known to observe this disease due to outdoor recreational activities involving water (Sharma *et al.*, 2006). The identification of leptospirosis involves actual isolation of organism through microbiological growth and serological examination. The former technique is laborious, time consuming and associated with growth constraints. However, later technique is quick and globally obliged technique.

The district Rajanpur is located across Indus River that finds union of all five rivers of Pakistan in its jurisdiction resultantly, observing maximum water pressure. The district faces flood every year due to hill torrents from Suleiman mountain ranges producing flash floods, and riverine of Indus River. The rise in level of water in Indus River is also fostered by rain water and snow melted from mountains. The flash flood (water coming from hills) situation aggravates due to over flooded Naalas (local terms for lower water source channels) hence accounting for 80% damage to the area while disturbing 60% population in flood hit area (Anonymous, 2015). Tehsil Jampur is affected both by flash and riverine floods while tehsil Rajanpur and tehsil Rojhan is usually affected by riverine flood only (Anonymous, 2015). The leptospiral infection is arising as common malady in developing countries due to favorable transmission circumstances (Ahmad, 2000). The infection goes directly thorough contact with infected urine, uterine discharges, infected placenta, and sexual contact as direct sources of infection. The more important transmission mean is the indirect way that involves contact with contaminated environment infective of farm animals (Anon, 2003).

So, for only one study has been conducted on bovine leptospirosis in Pakistan (Ijaz *et al.*, 2018). However, status of bovine leptospirosis is still not explored in district Rajanpur which is among the major flood hit regions of the country. Hence the objective of this study was to investigate cases positive for bovine anti-leptospira antibodies in this flood-affected areas.

## MATERIALS AND METHODS

**Study area:** The sampling for current study was executed from flood affected Rajanpur district situated in south Punjab at 29.1018°N, 70.3245°E comprising of three tehsils (Rajanpur, Jampur and Rojhan). The district coincides with Dera Ghazi Khan in north, Muzafar Garh and Raim Yar Khan districts in east in such a way that it spreads across River Indus, and Jaccobabad of Sindh province in south, while Dera Bugti and Barkan of Balochistan in west occupying 12,318 Km<sup>2</sup> with 90 persons per Km<sup>2</sup> population density.

**Sampling plan:** There are no previous records on sero-prevalence of leptospirosis in Rajanpur district of Punjab, thus 50% prevalence was assumed at 95% confidence interval which gave value of 384 serum samples using information given in table described by (Thrushfield, 2005). The samples were randomly collected from each tehsil with further randomization in such a way that four union councils were selected from each tehsil and two villages from each union council. The animals were considered as primary sampling units, villages as secondary sampling units while union council as tertiary

sampling unit in this study. A 16 bovine (n=8 cattle, n=8 buffalo) samples from each village were collected aseptically by drawing blood from jugular vein in such a way that it added up to 32 samples from union council level (n=16 cattle, n=16 buffalo), and 128 at tehsil level (n=64 cattle, n=64=buffalo). One additional buffalo sample was collected from Rojhan.

**Sample collection:** The blood samples were aseptically drawn from jugular vein into non-EDTA vacutainer that were left at room temperature for half an hour to be centrifuged eventually getting serum decanted aseptically in 1.5ml Eppendorf's tube. The samples from field were shifted to Medicine Laboratory, University of Veterinary and Animal Sciences, Lahore maintaining the cold chain in iceboxes. The serum samples were preserved at -20°C till further processing. The information pertinent to animal, farm management and farmer workers' involvement with animal care were captured in piloted questionnaire after formal and informal testing in order to find regression between disease response and its predictors.

**Samples processing:** The serum samples were put to estimation of bovine anti-leptospiral antibody prevalence following indirect ELISA protocol using "RecomBac Bovine Anti-Leptospira LipL32 IgG kit" (Alpha Diagnostic, international, USA). The plates were read using ELISA reader at Medicine laboratory. The cut off values were adjusted for each experimental run.

**Statistical analysis:** Sero-prevalence was estimated for one-year period without peculiarity between former and new cases as "No. of existing diseased cases/No. of overall animal examined" as per Thrushfield, (2005) formula. The data recorded on data capture form was uploaded to excel sheet and was analyzed using chi-square test, univariable and multivariable logistic regression. The P-value less than "0.05" and odds ratio (OR) greater than "1" was considered significant.

## RESULTS

**Sero-prevalence of bovine leptospirosis:** The overall prevalence of bovine leptospirosis in district Rajanpur was found 23.12% with non-significant difference (P>0.05) of bovine leptospirosis among different tehsils (Table 1). Tehsil Jampur presented highest bovine prevalence with 26.56% bovine leptospirosis followed by Rajanpur and Rojhan revealing 21.88 and 20.93% prevalence of bovine leptospirosis, respectively. The study showed non-significant difference between bovine species for leptospirosis however, there was 25.52% of leptospirosis in cattle while 20.72% in buffaloes from study area (Table 2). Similar non-significant (P>0.05) response in case of gender was noticed with 24.44 of females and 17.57% of males positive for leptospirosis.

**Table 1:** Sero-prevalence of bovine leptospirosis in different tehsils of district Rajanpur

| Tehsil    | Samples tested | Positive | Prevalence | P-value |
|-----------|----------------|----------|------------|---------|
| Rajan Pur | 128            | 28       | 21.88      | 0.535   |
| Jam Pur   | 128            | 34       | 26.56      |         |
| Rojhan    | 129            | 27       | 20.93      |         |
| Total     | 385            | 89       | 23.12      |         |

P<0.05 indicate significant difference.

**Table 2:** Chi square analyses of data on prevalence of leptospirosis in district Rajanpur

| Variables                           | Variable levels          | Positive/<br>Total | Prevalence | *P-<br>value |
|-------------------------------------|--------------------------|--------------------|------------|--------------|
| Age                                 | 1-2 years                | 13/058             | 22.41      | 0.972        |
|                                     | 3-5 years                | 57/248             | 22.98      |              |
|                                     | >5 years                 | 19/079             | 24.05      |              |
| Bovine species                      | Cattle                   | 49/192             | 25.52      | 0.160        |
|                                     | Buffalo                  | 40/193             | 20.72      |              |
| Breed                               | Nili Ravi                | 25/116             | 21.55      | 0.520        |
|                                     | Kundi                    | 15/77              | 19.48      |              |
|                                     | Sahiwal                  | 08/28              | 28.57      |              |
|                                     | Dhajal                   | 19/78              | 24.36      |              |
|                                     | Cholistan                | 00/02              | 00.00      |              |
|                                     | Fresian                  | 00/04              | 00.00      |              |
|                                     | Dhani                    | 00/03              | 00.00      |              |
|                                     | Cross                    | 21/73              | 28.77      |              |
|                                     | Non-descript             | 01/04              | 25.00      |              |
| Gender                              | Female                   | 76/311             | 24.44      | 0.208        |
|                                     | Male                     | 13/74              | 17.57      |              |
|                                     | Open                     | 50/235             | 21.28      |              |
| Rearing system                      | Confined                 | 09/043             | 20.93      | 0.364        |
|                                     | Both                     | 30/107             | 28.04      |              |
| Grazing status                      | No                       | 04/014             | 28.57      | 0.22         |
|                                     | Grazing alone            | 25/118             | 21.19      |              |
|                                     | With ovine               | 46/170             | 27.06      |              |
|                                     | With equine              | 14/083             | 16.87      |              |
| Water Source                        | Pond/Lake                | 11/044             | 25.00      | 0.924        |
|                                     | Canal                    | 13/060             | 21.67      |              |
| Fodder type                         | Fresh                    | 65/281             | 23.13      | 0.75         |
|                                     | Mixed with previous      | 17/078             | 21.80      |              |
|                                     | Good                     | 82/354             | 23.16      |              |
| Body condition                      | Thin                     | 03/016             | 18.75      | 0.98         |
|                                     | Emaciated                | 04/015             | 26.67      |              |
| Body temperature                    | Normal                   | 74/341             | 21.70      | 0.049        |
|                                     | High                     | 15/044             | 34.09      |              |
| Immunization status                 | Yes                      | 13/079             | 16.46      | 0.11         |
|                                     | No                       | 76/306             | 24.84      |              |
| Jaundice status                     | Yes                      | 85/365             | 23.29      | 0.73         |
|                                     | No                       | 04/020             | 20.00      |              |
| Urine color                         | Normal                   | 66/285             | 23.16      | 0.97         |
|                                     | Blood in Urine           | 23/100             | 23.00      |              |
| Socio-economic status               | Literate                 | 37/147             | 25.17      | 0.029        |
|                                     | Primary                  | 19/087             | 21.84      |              |
|                                     | Higher Secondary         | 24/113             | 21.24      |              |
| Call for Vet. Professionals         | Graduate/More            | 09/038             | 23.68      | 0.48         |
|                                     | Only Emergency           | 38/155             | 24.52      |              |
| Veterinary Professional Consultancy | Few days' post treatment | 27/113             | 23.89      | 0.93         |
|                                     | Always on call           | 08/024             | 33.33      |              |
|                                     | Self-treatment           | 04/024             | 16.67      |              |
|                                     | Always veterinarian      | 12/069             | 17.39      |              |
| Veterinary Professional Consultancy | Only vet Assistant       | 38/158             | 24.05      | 0.93         |
|                                     | Only vet officer         | 24/106             | 22.64      |              |
| Consultancy                         | Both                     | 27/121             | 22.31      |              |

P-value is based on Wald Statistic

**Risk factors:** The chi-square analysis of different assumed determinants with bovine leptospirosis showed significant association of body temperature and socio-economic status of worker with this disease (Table 2). The factors including age, bovine species, breed, gender, rearing system, grazing status, water source, fodder type, body condition, immunization status, jaundice status, urine color, call for veterinary professionals and veterinary professional consultancy presented non-significant association ( $P>0.05$ ) with bovine leptospirosis.

The univariable analysis of assumed risk factors based on Mantel Haentzel test and Wald statistics (Table 3) explored the potential risk factors for leptospirosis in bovine herds of Rajanpur district based on odd ratios ( $OR>1$ ). The age was found to be a potential risk factor

$OR=1.48$  ( $CI=0.54-4.07$ ) for leptospirosis sero-prevalence. Bovine specie although non-significantly associated on chi-square bases ( $P>0.05$ ) was found a potential risk factor upon univariate analysis  $OR=1.31$  ( $CI=0.81-2.109$ ). In rearing system the confined system proved to be a potential risk factor for leptospirosis  $OR=2.37$  ( $CI=0.23-24.48$ ). Among grazing system, no grazing and grazing with ovine showed higher effects on disease dynamics [ $OR=1.70$  ( $CI=-0.062-47.0$ );  $OR=2.01$  ( $CI=0.038-105.6$ )]. The fodder type proved to be a potential risk factor  $OR=1.09$  ( $CI=0.60-2.00$ ) affecting the disease dynamics. Another factor associated with leptospirosis was the jaundice status  $OR=1.21$  ( $CI=0.39-3.73$ ). Urine color was also found positively associated  $OR=1.00$  ( $CI=0.58-1.73$ ) with sero-prevalence of leptospirosis. Mastitis status of farm animals also proved to be a potential risk factor for the sero-prevalence  $OR=7.22$  ( $CI=0.08-73.53$ ). Beside these disposal of aborted fetus, services per conception and socio-economic status of the animal's owner all proved to be potential risk factors (Odd ratios  $>1$ ) affecting the disease dynamics.

Multivariable models (Table 4) were later on developed using the elimination technique of backward manual stepwise process removing the variables with greater  $p$ -value respectively. The variables, which were found no longer statistically associated after adjustment for the other variables, were removed ( $p$ -value=0.05). Variables were eliminated or retained from the model considering the  $p$ -value of 0.05 based on Wald statistic. The confounding presence in data was countered by monitoring the calculated coefficient values and by checking that the values did not changes by  $>10\%$  after dropping the non-significant variables from the model. The bovine specie, gender of animal, fodder type, jaundice status and socio-economic status of the animal's owner were found key risk factors for leptospirosis sero-prevalence dynamics ( $OR>1$ ).

## DISCUSSION

**Seroprevalence of bovine leptospirosis:** The prevalence of current study was in agreement with findings of (Gamage *et al.*, 2011) and (Subharat *et al.*, 2011) from Sri Lanka and Australia who reported 20.3 and 27.4% of bovine leptospirosis in their countries, respectively. The bovine leptospirosis slightly higher to the finding of this study was noted as 30.3, 31.3, and 31.3% in studies conducted by Schoonman and Swai, (2010), Tabatabaeizadeh, (2011) and Dos *et al.* (2012) from Iran, Tanzania and Brazil, respectively. Contrary to the results of current study very high prevalence of leptospirosis was previously recorded in Bangladesh (Parvez *et al.*, 2015), India (Natarajaseenivasan *et al.*, 2011), Poland (Czopowicz *et al.*, 2011), and Mexico (Joel *et al.*, 2011) with 47.27, 87.0, 89.9 and 82.2% of prevalence, respectively. The discrepancies might have appeared because of variation in geographical areas, climatic conditions, and management aspects in conjunction with resistance showed by different breeds. It has been noticed that infections by *Leptospira spp*s remains usually silent with respect to clinical examination while serological assays give positive reports. This could have resulted in

**Table 3:** Univariable analysis based on Mantel Haentzel test and Wald statistics

| Variables                           | Categories               | Positive/Odd ratio<br>Total (95% C.I) | *P-<br>Value |
|-------------------------------------|--------------------------|---------------------------------------|--------------|
| Tehsil                              | Rajan Pur                | 28/128 0.95 (0.47-1.91)               | 0.535        |
|                                     | Jam Pur                  | 34/128 0.56 (0.09-3.2)                |              |
|                                     | Rojhan                   | 27/129                                |              |
|                                     |                          |                                       |              |
| Age                                 | 1-2 years                | 13/058 1.48 (0.54-4.07)               | 0.956        |
|                                     | 3-5 years                | 57/248 1.10 (0.53-2.28)               |              |
|                                     | >5 years                 | 19/079                                |              |
|                                     |                          |                                       |              |
| Bovine species                      | Cattle                   | 49/192 1.31 (0.81-2.109)              | 0.160        |
|                                     | Buffalo                  | 40/193                                |              |
| Gender                              | Female                   | 76/311 1.51 (0.79-2.91)               | 0.208        |
|                                     | Male                     | 13/074                                |              |
| Rearing sys                         | Open                     | 50/235 0.97 (0.18-5.08)               | 0.76         |
|                                     | Confined                 | 09/043 2.37 (0.23-24.48)              |              |
|                                     | Both                     | 30/107                                |              |
| Grazing status                      | No                       | 04/014 1.70 (-0.062-47.0)             | 0.22         |
|                                     | Grazing alone            | 25/118 0.85 (0.01-42.2)               |              |
|                                     | With ovine               | 46/170 2.01 (0.038-105.6)             |              |
|                                     | With equine              | 14/083                                |              |
| Water Source                        | Pond/Lake                | 11/044 0.52 (0.026-10.43)             | 0.67         |
|                                     | Canal                    | 13/060 0.684 (0.10-4.32)              |              |
| Fodder type                         | Fresh/tap                | 65/281                                | 0.75         |
|                                     | Fresh                    | 72/307 1.09 (0.60-2.00)               |              |
|                                     | Mixed previous           | 17/78                                 |              |
| Body condition                      | Good                     | 82/354 0.881 (0.14-5.22)              | 0.98         |
|                                     | Thin                     | 03/016 0.789 (0.05-10.81)             |              |
|                                     | Emaciated                | 04/015                                |              |
| Body temperature                    | Normal                   | 74/341 0.53 (0.27-1.05)               | 0.06         |
|                                     | High                     | 15/044                                |              |
| Immunization status                 | Yes                      | 13/079 0.59 (0.31-1.14)               | 0.11         |
|                                     | No                       | 76/306                                |              |
| Jaundice status                     | Yes                      | 85/365 1.21 (0.39-3.73)               | 0.73         |
|                                     | No                       | 04/020                                |              |
| Urine color                         | Normal                   | 66/285 1.00 (0.58-1.73)               | 0.97         |
|                                     | Blood in Urine           | 23/100                                |              |
| Mastitis status                     | No                       | 49/176 1.47 (0.05-41.52)              | 0.04         |
|                                     | Acute                    | 14/081 2.24 (0.075-67.54)             |              |
|                                     | Sub-clinical             | 04/012 7.22 (0.08-73.53)              |              |
|                                     | Chronic                  | 08/040 2.3 (0.072-66.31)              |              |
|                                     | NA                       | 14/076                                |              |
| Breeding system                     | AI                       | 71/296 0.28 (0.005-16.95)             | 0.66         |
|                                     | Natural                  | 03/014 0.62 (0.01-32.84)              |              |
|                                     | NA                       | 01/005 4.10 (0.067-50.39)             |              |
|                                     | No                       | 14/070                                |              |
| Abortions History                   | Yes                      | 16/086 0.069 (NA)                     | 0.16         |
|                                     | No                       | 60/226 3.65 (NA)                      |              |
|                                     | NA                       | 13/073                                |              |
| Disposal of Aborted Fetus           | Buried                   | 02/034 5.10 (NA)                      | 0.03         |
|                                     | Thrown in water          | 05/012 1.27 (NA)                      |              |
|                                     | Thrown in field          | 09/041 6.6 (NA)                       |              |
| History of stillbirth               | Yes                      | 73/298                                | 0.48         |
|                                     | No                       | 03/013 0.157 (0.004-6.48)             |              |
|                                     | No                       | 73/299 0.537 (0.026-10.90)            |              |
|                                     | NA                       | 13/073                                |              |
| Services Per Conception             | 1                        | 34/145 6.19 (0.35-107.5)              | 0.82         |
|                                     | 2                        | 26/113 5.86 (0.30-111.7)              |              |
|                                     | 3                        | 09/031 5.18 (0.27-96.90)              |              |
|                                     | 4                        | 05/018 5.16 (0.25-105.33)             |              |
|                                     | NA                       | 15/078                                |              |
| Socio-economic status               | Literate                 | 37/147 8.50 (0.74-97.0)               | 0.029        |
|                                     | Primary                  | 19/087 3.47 (0.15-76.44)              |              |
|                                     | Higher secondary         | 24/113 14.84 (1.32-166.78)            |              |
|                                     | Graduate/More            | 09/038                                |              |
| Call for Vet. Professionals         | Only emergency           | 38/155 0.07 (0.006-0.92)              | 0.48         |
|                                     | Few days' post treatment | 27/113 0.073 (0.009-0.603)            |              |
|                                     | Always o call            | 08/024 0.203 (0.016-2.58)             |              |
|                                     | Self-treatment           | 04/024 0.034 (0.001-1.070)            |              |
|                                     | Always veterinarian      | 12/069                                |              |
| Veterinary Professional Consultancy | Only vet assistant       | 38/158 0.941 (0.114-7.76)             | 0.93         |
|                                     | Only vet officer         | 24/106 0.08 (0.007-1.14)              |              |
|                                     | Both                     | 27/121                                |              |

P-value is based on Wald Statistic.

**Table 4:** Multinomial logistic regression

| Variables             | Categories       | B     | SE (b) | Odd ratio (95% C.I)  | *P-<br>Value |
|-----------------------|------------------|-------|--------|----------------------|--------------|
| Age                   | 1-2 years        | -     | -      |                      | -            |
|                       | 3-5 years        | -0.28 | 0.43   | 0.75 (0.32-1.75)     | 0.50         |
|                       | >5 years         | -0.23 | 0.31   | 0.78 (0.42-1.48)     | 0.45         |
| Bovine species        | Cattle           | -     | -      |                      | -            |
|                       | Buffalo          | 0.30  | 1.32   | 1.36 (0.80-2.30)     | 0.25         |
| Gender                | Female           | -     | -      |                      | -            |
|                       | Male             | 15.52 | 1.56   | 5.51E6 (2.5E5-1.1E8) | 0.000        |
| Fodder type           | Fresh            | -     | -      |                      | -            |
|                       | Mixed            | 0.133 | 0.320  | 1.14 (0.61-2.14)     | 0.67         |
| Jaundice status       | Yes              | -     | -      |                      | -            |
|                       | No               | 0.405 | 0.601  | 1.50 (0.46-4.87)     | 0.500        |
| Socio-economic status | Literate         | -     | -      |                      | -            |
|                       | Primary          | 0.12  | 0.43   | 1.13 (0.48-2.65)     | 0.77         |
|                       | Higher secondary | -0.08 | 0.460  | 0.91 (0.37-2.65)     | 0.85         |
| Graduate/More         | Graduate/        | -0.11 | 0.468  | 0.88 (0.35-2.22)     | 0.79         |
|                       | More             |       |        |                      |              |

a. The reference category is negative; b. This parameter is set to zero because it is redundant; c. Floating-point overflow occurred while computing this statistic. Its value is therefore set to system missing

misdiagnosis on application of other than serological or molecular techniques and might have allowed animals to spread infection due to reporting less than exact prevalence estimation of this disease (Hassanpour *et al.*, 2004). The conventional techniques used in previous studies do have limitations in diagnosis of leptospirosis (Bharti *et al.*, 2003 and Parvez *et al.*, 2015). The conventional techniques do require specific cells/mL ( $10^9$  leptospores/mL) for *Leptospira* to be diagnosed under dark field microscopy (Bharti *et al.*, 2003) in addition to recommendation of early diagnosis in blood. Such limitations produce false estimation of prevalence, which is the result of discrepancies in findings of various studies.

**Risk factors:** The current study was in line with findings of (Bahaman *et al.*, 1987) with respect to positive association of breeds with leptospirosis occurrence but Parvez *et al.* (2015) found contrary to the finding of current study. The specie of animal was a found a significant risk factor based on univariate and multivariate logistic regression in the current study. However, there was a non-significant difference in the prevalence between cattle and buffalo based on chi-square test. These findings are in line with the study conducted by Suwancharoen *et al.* (2013) who also found bovine species as a significant effector of the disease dynamics. In this study age was associated positively with seroprevalence of leptospirosis. These findings are supported by the study conducted by Mazeri *et al.* (2013) exploring the age as a significant risk factor based on logistic regression. In the current study confined system of animal rearing proved as a risk for the spread of leptospirosis. The possible reason could be the retention of animals in infected environment as the confinement can provide a microenvironment for the infection maintenance and transmission as stated by (Bharti *et al.*, 2003). Fodder type also proved to be a key risk factor affecting the disease dynamics. No study have yet been explored this aspect however, the possibility could be harboring of pathogens in the feed over time and if provided mixed with fresh fodder to reduce the feed costs can cause losses in terms of disease transmission. The higher education was considered as predictor of bovine leptospirosis by Parvez

*et al.*, (2015) which is in contradiction to the findings of current study while in agreement with findings of (Dias *et al.*, 2007). However, lower education status was in positive association of spread of this disease. This could be justified with the fact that lack of knowledge for disease control and farm management can result in disease spread (Parvez *et al.*, 2015).

**Conclusions:** The study showed higher and uniform seroprevalence of bovine leptospirosis in different tehsils of flood affected zone. The cattle and buffaloes were equally susceptible to leptospirosis while females were more prone to this disease. Confined rearing system, jaundice status, fodder type, socioeconomic status of farmer, and flooded areas were found as key risk factors for spread of this disease. The study concluded this flood-hit area is having considerable prevalence of bovine leptospirosis with assumed determinants appearing as potential risk factors for leptospirosis. The study demands regular prevalence estimation of this disease and development of comprehensive preventive measures accordingly.

**Authors contribution:** The blood sampling, data collection, processing and interpretation of results were made by SHF, MI, SS and AIA. The data analysis was made by PB, AG and AA. The manuscript was written by SHF, AIA, PB and AA. All the authors read the manuscript and approved the contents.

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