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#### **Short Communication**

# SEROPREVALENCE OF *LEPTOSPIRA* HARDJO IN THE MEGHALAYA CATTLE POPULATION: A PILOT STUDY

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ABSTRACT: Leptospirosis is a seemingly unexplored disease among cattle population of Meghalaya. Prior to the present study, the seroprevalence of Leptospirosis in cattle population of Meghalaya was unknown. This pilot study was designed to find out serological evidence of Leptospirosis in dairy cattle. For this purpose, 276 bovine serum samples were collected from two cattle dense districts of Meghalaya. Serological testing was performed employing a commercially available double sandwich ELISA and the seroprevalence was found as 8.33% (23/276). Therefore, it could be stated that the cattle population of Meghalaya is maintaining *Leptospira* Hardjo, which is one of the most important host specific (cattle) serovar. This warrants further studies, intensive surveillance and control strategies in the state in order to reduce the disease burden and economic loss.

Key words: Leptospirosis, Hardjo, Seroprevalence, ELISA, Cattle, Meghalaya.

Leptospirosis is a well-recognized but a neglected disease of cattle worldwide (Ellis 1984, Langoni et al. 1999). The organism has many serovars consisting of both pathogenic and non-pathogenic. There are more than 250 and 60 different serovars under pathogenic and nonpathogenic leptospira group, respectively (Cerqueira and Picardeau 2009). In cattle, two species are mainly associated in causing leptospirosis: Leptospira interrogans serovar Hardjo and Leptospira borgpetersenii serovar Hardjo. Although they have some pathological and genetic differences, they are indistinguishable by serological assays. So, they can be collectively referred to as L. Hardjo (Ellis 1990, Ryan et al. 2012). Rodents are the main reservoir of infection while cattle serve as maintenance hosts of L. Hardjo. Cattle, being colonized with these bacteria in renal and genital organs, it continuously sheds the organism in urine and other genital discharges. Transmission of leptospira occurs by direct contact with the infected urine and the infection mostly remains subclinical in cattle (Ellis 1984,

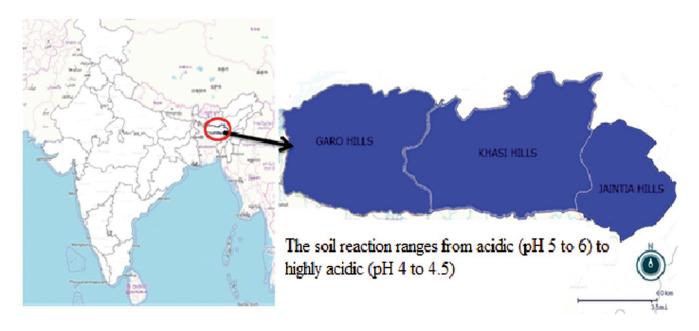
Balamurugan *et al.* 2018). In cattle, Leptospirosis usually causes abortion after the seventh month of pregnancy, whereas in calves it causes clinical infection with hematuria followed by death. In subclinical infection, it induces infertility and other reproductive problems (Balamurugan *et al.* 2018). Leptospirosis is also recognized as an important zoonotic disease among the occupational groups like livestock farmers, agricultural workers, animal handlers, veterinarians etc. (Ryan *et al.* 2012).

In India, leptospirosis affects most of the farm animals *viz.* cattle, buffalo, sheep, goat, pigs thereby causing heavy economic losses to the farmers (Srivastava 2008). Leptospirosis in cattle was first reported in India by Adinarayan *et al.* (1960). Since then a number of reports emerged establishing the prevalence of leptospirosis in bovines in different states of India (Srivastava and Kumar 2003, Natarajaseenivasan *et al.* 2011, Mitra *et al.* 2015). In India, most of the sousveillance works were undertaken in the southern parts, where the disease is widespread in

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nature. However, in Meghalaya, one of the northeastern states of India, the prevalence of leptospirosis in the bovine population is still unknown. In addition, the disease is often under diagnosed, since most of its symptoms are nonspecific and the complicatedness of its culture methods and the reference serological tests (OIE 2013). Hence this study was undertaken in Meghalaya to know the status of *Leptospira* serovar Hardjo in cattle using *Leptospira* Bovine Hardjo ELISA commercial kit to establish the seroprevalence.

### The Study

Meghalaya is located in the Northeast India between the coordinates 20°1' and 26°5' North latitude and 85°49' and 92°52' East longitude with a geographical area of 22,429 sq. km with an average annual rainfall of 1,200 cm and is the wettest Indian state. Due to high altitude, heavy rainfall and leaching, the soil of the state is acidic (pH 5.0 to 6.0) to strongly acidic (pH 4.5 to 5.0) (http://www.megagriculture.gov.in/PUBLIC/agri\_scenario\_soil.aspx). A total of 276 bovine sera

Table 1. Seroprevalence of Leptospirosis (L. Hardjo) in cattle.

District Name	No. of samples screened	No. of +ve samples	Percentage of positivity
Ri-Bhoi	92	7	7.60%
East Khasi Hills	184	16	8.69%
Total	276	23	8.33%

samples were collected from Ri-Bhoi (n=92) and East Khasi Hills (n = 184) district of Meghalaya from August 2017 to February 2018. Following proper restraining, blood was collected by venipuncture of jugular vein and the site was disinfected with alcohol. Sera were collected following centrifugation at 2500 rpm for 15 min and transferred to the microfuge tube and stored at -80°C until tested. All the serum samples were screened by Bovine Leptospira Hardjo Antibody Test (Linnodee Diagnostics) for detection of Leptospira Hardjo serovar antibody. This Kit employs double sandwich ELISA for the detection of L. Hardjo-specific antibodies in serum. The sensitivity and specificity of the ELISA was mentioned as 94.1% and 94.8%, respectively. The assay was performed following the manufacturers described protocols. Out of the total of 276 sera samples screened, 23 samples were found positive for L. Hardjo antibody. The results were summarized in Table 1.

Perusal of literature revealed that leptospirosis outbreaks have been mostly reported in southern peninsular India and Andaman Islands. Earlier investigations conducted in India since 1995 have revealed that the seroprevalence of leptospirosis in various states has been 7.5% in cattle, 5.4% in buffaloes, 14.6% in horses, 12.5% in sheep and 15.9% in dogs (Srivastava 2008). Various researchers have reported different serogroup-specific antibodies across India viz. 51.4% from Tamil Nadu, 4.6% from Karnataka (Natarajaseenivasan et al. 2011), 22.22% in South Andaman (Mitra et al. 2015), 10.5% from Andhra Pradesh (Srivastava 2008), 4-8% from Uttar Pradesh and 7.3% Maharashtra

(Srivastava and Kumar 2003), 37.74% from Odisha (Balamurugan et al. 2017), 41% from Konkan Region of Maharashtra (Balamurugan et al. 2016), 12.8% from Gujarat (Patel et al. 2014). It is obvious from the earlier reports that, leptospirosis is more prevalent in southern peninsular and coastal regions of India. The damp alkaline soil in this region may favor the survival of the Leptospira for longer periods of time. Because, Leptospira is considered to be injured outside the pH range of 5.8 to 8.4 and neutral to alkaline conditions favor survival of Leptospira spp. (Ellis and McCalla 1978, Horvath and Reid 1984). Extreme weather events such as cyclones and floods, increased rainfall associated with global warming are considered as the factors for the increased incidence of leptospirosis (Lau et al. 2010). However, our study reports 8.33% prevalence of L. Hardjo among cattle. This shows that Leptospira might be adapting and persisting in slightly acidic or acidic soil of Meghalaya. The disease ingrain might have happened through the purchase of unscreened dairy animals from other parts of the country. This could be a prospective peril in spreading the infection to other healthy herds and it may turn into a public health concern. Apart from that, the presence of rodents in the farm premises, close association of healthy and affected animals might have also been contributed to the prevalence of the disease in the state. Moreover, vaccination against leptospirosis in cattle is not regularly practiced in India. Infection with host-adapted serovar (L. Hardjo) has been reported to cause subclinical infection in apparently healthy animals which may serve as chronic carriers and persistent shedders of the Leptospira through their urine and other body fluids. Most importantly they may act as source and pose a potential risk to livestock farmers, agricultural workers, other occupational groups and other animal species etc. (Balamurugan et al. 2018).

The results of the study indicate presence of antibodies against leptospirosis in Meghalaya and warrants further studies with wide range of samples from other animal species and human beings.

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