

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.708.080>

Serological Characterization in Cattle Serum for *Listeria monocytogenes*

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ABSTRACT

Keywords

Listeria, *Listeria monocytogenes*, Listeriolysin O

Article Info

Accepted:

06 July 2018

Available Online:

10 August 2018

Listeriosis was important bacterial disease public health significance affecting human as well as animals. Determination of the seroprevalance of antibodies to *L. monocytogenes* in cattle was attempted from the different organized cattle farm around the Nagpur region, Maharashtra India. Listeriolysin O is a haemolysin produced by the bacterium *Listeria monocytogenes* for serological characterization. Present studies were targeted for serological detection of listeriolysin O in cattle serum. Serological prevalence in healthy animal was 4.17% and reproductive cases of cattle with zero prevalence. This result was showing seroprevalance in healthy cattle was higher as compare to reproductive disorder cases of cattle.

Introduction

Listeria monocytogenes, an important foodborne pathogen, has a significant impact on public economy worldwide. Though human listeriosis is rare but it has the ability to cause serious and life threatening condition and mainly associated with contaminated foods (Andritsos *et al.*, 2013). In animals poor quality silage is the main source of infection. The disease can occur sporadically or in the form of an epidemic and often leads to fatal forms of encephalitis. The clinical manifestations of listeriosis in animals include rhombencephalitis, septicaemia and abortion especially in sheep, goats and cattle. The rhombencephalitic form is referred to as

‘circling disease’ because of a tendency to circle in one direction and it is the most common manifestation of the disease in ruminants. Abortion occurs usually in late term (after 7 month in cattle and 12 weeks in sheep) (Hird and Genigeorgis, 1990; Walker, 1999). The septicaemic form is relatively uncommon and occurs in the neonates. It is marked by depression, inappetance, fever and death. Bovine and ovine ophthalmitis has also been described (Walker and Morgan, 1993). *Listeria monocytogenes* is Gram-positive facultative intracellular non-spore-forming, small rods bacteria. *Listeria* genus is comprised of 18 approved species including *Listeria monocytogenes*, *Listeria seeligeri*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria*

marthii, *Listeria innocua*, *Listeria grayi*, *Listeria fleischmannii*, *Listeria floridensis*, *Listeria aquatica*, *Listeria newyorkensis*, *Listeria cornellensis*, *Listeria rocourtiae*, *Listeria weihenstephanensis*, *Listeria grandensis*, *Listeria riparia*, *Listeria booriae* (Orshi 2016) and *Listeria thailandensis* (Leclercq *et al.*, 2016). Among all of these, two of species, *L. monocytogenes* and *L. ivanovii*, are considered pathogens because of their species-specific virulence determinants.

Listeriolysin O (LLO) is produced by all pathogenic strain of listeria spp. (Geoffroy *et al.*, 1989) and is an extracellular 58 kDa haemolysin, is a major virulence factor of *L. monocytogenes* (Gaillard *et al.*, 1986). However, conventional methods remain the 'Gold standard' for the isolation when compared with other methods. Serodiagnosis is considered as one of the quickest tool in diagnostic aids for detection of antibodies against the specific antigens of the causative agent which is known to be responsible for disease condition. An indirect ELISA has been used for the detection of antibodies against LLO antigen (ALLO) of the *Listeria monocytogenes* in the serum samples. The objective of this study was to determine the seroprevalance of antibodies to *L. monocytogenes* is in cattle in and around Nagpur region.

Materials and Methods

Serum Sample were collected on the basis of reproductive disorder cases viz. abortion vaginal discharge or vaginitis cases and healthy cattle with no history of reproductive disorder cases from two organized farm. All the samples were collected aseptically in the containers (Himedia, India) and brought to the laboratory in chilled conditions and processed for serological characterization of *Listeria monocytogenes*. The samples were properly labeled and transported on ice to the

laboratory. Serum was separated at the laboratory and stored at -20°C until further use. The strain of *L. monocytogenes* (EGDe) was employed for preparation and purification of Listeriolysin O (LLO) antigen. For studies pertaining to ion exchange chromatography, protein profile studies, indirect Enzyme linked immunosorbent assay (ELISA) and the chemicals, and reagents of analytical grade were procured from BIORAD (UK) Promega (USA), Sigma Aldrich (US), E Merck (India), SRL Chemicals (Mumbai) and S.d.fine Chem (Mumbai). Serodiagnosis of listeriosis by employing Listeriolysin-O (LLO), a virulence factor of *Listeria monocytogenes* has been thought to be a promising test. Therefore the protein (LLO) was purified, characterized and subsequently employed as an antigen for sero-detection of antibodies against LLO (ALLO).

Preparation of the virulence factor Listeriolysin-O (LLO)

Listeriolysin O (LLO) was extracted from the cell free supernatant and purified by ion-exchange chromatography from standard strain of *L. monocytogenes* (EGDe) in accordance with the method of Lhopital *et al.*, (1993) with some important modification. Protein estimation was carried out as per the method suggested by Lowry *et al.*, (1951). Diethylaminoethyl (DEAE) Cellulose Chromatography was done as per the method suggested by Kundzicz (2010). After then the peak values of optical density was pooled together for further characterization. Polyethylene glycol (PEG- 20,000) was used to concentrate the pooled fractions (protein) at 4°C. The concentrated protein was further subjected to peak values of optical density was done to estimation protein concentration. Characterization of purified protein by Sodium-dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) was used for estimation of molecular weight of purified protein as per Lammeli (1970) and then it was

processed for calculations of Haemolytic Unit (HU) as stated by Kohda *et al.*, (2002). The protein profiles were visualized by employing silver staining technique as per method suggested by Rosenberg, (1996). The haemolytic activity of purified LLO was screened as per the method described by Kohda *et al.*, (2002).

Detection of antibodies against Listeriolysin O (ALLO)

The indirect plate ELISA was performed as per the method of Low *et al.*, (1992). The ELISA was standardized by Checker board analysis. purified LLO as an antigen is used in a concentration of 1 µg and 2 µg per well the hyper-immune serum as well as healthy (zero day) serum was diluted in range of 1:100 to 1:800 and added with anti-species HRPO conjugate (Merck, India) in the range of 1:1000 to 1:4000 at the rate of 100 µl/well after that addition with 1 mg/ml solution of O-phenylene-diaminedihydrochloride (OPD) (Sigma Aldrich, US) as substrate @ 100 µl per well. The plates were incubated for 15 min in dark for development of colour and OD was measured at 492 nm in ELISA reader (MultiskanGo, Thermofisher Scientific, Finland). The serum sample at the dilution of 1:200 with the positive to negative P/N ratio of ≥ 2 was considered positive for listeriosis in standardized ELISA employing purified LLO.

Results and Discussion

Sera samples of 07 cattle from reproductive disorder as well as 24 cattle from apparently healthy cases were screened for detection of antibodies against LLO (ALLO) by employing LLO based indirect ELISA. The results are given below (Table 1).

In the present study 31 sera samples comprising of reproductive cases (seven) and healthy (24) cattles were processed by LLO

based indirect ELISA for detection of antibodies against LLO from the clinical sera samples none of the sample turned positive exhibiting zero seroprevalence whereas 4.17 per cent seroprevalence was recorded in healthy animals (Table 1).

Our result was showing 3.22% (31) seroprevalence of antibody to listeria monocytogenes in reproductive and healthy cattle. The result of present study among healthy cattle is in lower side with Boerlin *et al.*, (2002) who reported 48 percent seroprevalence among dairy cattle. The seroprevalence lower side may be due number of sampling, properly sampled, area of sampling, applied method of seroprevalence, It is lower seroprevalence when it is as compared with researcher result, it was found in 48.3% (101) of the 209 cattle tested (Kennerman 2005). Researcher concluded that silage feeding is an important factor in the epidemiology of listeriosis in Bursa province of Turkey. Cattle sera sample were collected from organized farm there was no silage feeding system. They were fed on open range system so might be it was not a contributing factor for listeria monocytogenes infection. Since then, Most listeriosis outbreaks in livestock have been linked to contaminated silage (Wiedmann *et al.*, 1994, 1999, 2002). *L. monocytogenes* numbers in poorly fermented silage can be as high as 10^8 colony-forming units (CFU)/g wet weight of silage (Wiedmann *et al.*, 2002). The interpretation of serological tests for antibodies against *L. monocytogenes* is made difficult by the presence of positive reactions of up to 1:200 in clinically normal animals. The *L. monocytogenes* is widespread in the environment and a significant proportion of animals sporadically shed the organisms in their feces or milk (Skovgaard and Morgen, 1988). Thus, many animals in the dairy cattle population are expected to present antibodies towards *L. monocytogenes*.

Table.1 Details of seroprevalence in cattle

Animal	No. of sera samples from reproductive disorder cases	No. of positive samples (ALLO positivity)	No. of sera samples from healthy animals	No. of positive samples (ALLO positivity)
Cattle	07	Nil (0 per cent)	24	1 (4.17 per cent)

No seroprevalance were reported in our reproductive cases of cattle may be because of other persistent infection which are responsible for abortion in cattle. The healthy animal were showing seropositivity this may be because of *Listeria monocytogenes* have reported fecal shedding in dairy cattle shows high levels with day-to-day variation and includes outbreaks and sporadic cases of shedding of specific *L. monocytogenes* subtypes(Ho *et al.*, 2007).

Our results were showing antibodies of *Listeria monocytogenes* in apparently healthy animal. This animal might be responsible for shedding of *Listeria monocytogenes* in milk and fecal which can cause serious illness in immunocompromise animals as well humans also.

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How to cite this article:

Rashmi Kulesh, S.V. Shinde, P.K. Kawareti and Arti Salame. 2018. Serological Characterization in Cattle Serum for *Listeria monocytogenes*. *Int.J.Curr.Microbiol.App.Sci.* 7(08): 730-734. doi: <https://doi.org/10.20546/ijcmas.2018.708.080>