Prevalence and Antibiogram of *Listeria monocytogenes* in Cases of Abortion and Stillbirths in Sheep of Kashmir

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**ABSTRACT**

Prevalence of listeric abortions in organized and unorganized sheep farms of Kashmir valley was studied during 2008-2009. A total 52 cases of abortions/stillbirths were screened for isolation of *Listeria* species. *Listeria monocytogenes* was the only species isolated from the cases of abortions/stillbirths under study. An overall prevalence of 15.38% of listeriosis was recorded in these farms. The prevalence was higher in organized (18.51%) than in the un-organized farms (12.0%). Out of a total 8 isolates recovered, 4 were isolated from only brain tissues, 2 from both brain and livers and one each from liver and stomach contents. All the isolates under study were sensitive to gentamicin, doxycycline, ampicillin, tetracycline and pencillin G and resistant to nalidixic acid, co-trimoxazole, ciprofloxacin, chloramphenicol, erythromycin and lemoloxacin.

**Keywords**: Abortion, *Listeria monocytogenes*, prevalence, still births

Listeriosis is an acute infectious disease of animals and humans caused by bacteria belonging to the genus *Listeria*, which presently includes eight species. Among these *L. monocytogenes* and *L. ivanovii* are regarded as pathogenic species, whereas *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. marthii*, *L. rocourtiae* and *L. grayi* are non pathogenic species (Seeliger and Jones, 1986; McLuachlin, 1987; Graves et al., 2009; Leclercq et al., 2009). Disease occurs mainly in temperate climates and is much less common in tropical and sub tropical areas. Global occurrence of listeriosis has been reported from almost all species of domestic animals as well as from many species of poultry, fish, wild animals and rodents. The disease is of great economic importance in sheep, goats and cattle and less so in pigs and poultry. The role of *L. monocytogenes* in causing meningoencephalitis, septicemia, endocarditis, abortion, cervicitis, diarrhoea, mastitis, keratoconjunctivitis has been established beyond doubt (Gitter et al., 1986). In pregnant animals, placenta and fetuses are affected resulting in abortions and stillbirths. Listeriosis is a significant public health problem (Roucort and Berche, 1987). The present investigation was conducted to study the prevalence of *Listeria* spp. in sheep of Kashmir valley based on clinical manifestation, isolation and identification of *L. monocytogenes*.

A total number of 52 cases of abortions/stillbirths in sheep from both organized (27 cases) and unorganized (25 cases) farms of Kashmir valley were selected for the present study. The organized farms under the study included Sheep Breeding Farm Dachigam, Kralpathri, Zawoora, Gaobal and Sheep Research Station, Shuhama. The samples from unorganized farms were...
collected from local breeders from different districts of the valley viz., Pulwama, Budgam, Anantnag, Ganderbal, Baramulla and Srinagar. Heart blood, liver, stomach contents, lungs, brain and kidneys samples were collected aseptically from each case of the aborted/stillborn lambs in ice packs and processed for isolation of *Listeria* spp. within 6 h of collection. Twenty five grams of each sample (triturated in case of liver, lungs, and kidneys) were inoculated into 100 ml of brain heart infusion broth and incubated at 37°C for 24-48 h. A loopfull of culture was streaked on *Listeria* selective agar (Hi media) and incubated at 37°C for 48 h. Greenish-yellow colonies typical of *Listeria* spp. were considered positive. These colonies were also streaked on PALCAM agar and incubated at 37°C for 48 h for confirmation of *L. monocytogenes*. Greyish colonies with black zone of aesculin hydrolysis were considered to be confirmatory for *L. monocytogenes*. The isolates were further subjected to different biochemical tests (Cowan and Steel, 1993), hemolysis on 10% sheep blood agar and intra-ocular instillation (Anton Test) in rabbits. Antibiotic sensitivity was done as per the standard method (Bauer et al., 1966). The antibiotic discs used were gentamicin, doxycycline, tetracycline, ampicillin, penicillin G, amoxycillin, cephalothine, amikacin, lemofoxacin, erythromycin, chloromphenicol, ciprofloxacin, nalidixic acid and cotrimaxazole.

Out of the 52 cases of abortions/stillbirths in ewes, the *L. monocytogenes* was isolated from 8 (Table 1) with a prevalence of 15.38%. The prevalence was higher in the organized (18.51%) than in the unorganized farms (12%). Listeriosis was first reported from Jammu and Kashmir by Vishwanathan and Uppal (1981) in cases of abortions in organised sheep farms. Since its first reporting, no work was carried out to study the prevalence of this disease in the state till 2005, when Willayat et al. (2005) reported 26.99% cases of abortions in an organised sheep farm of Kashmir valley. The lower prevalence observed in the present study could be due to the better hygienic conditions having been followed subsequent to the reporting by previous workers (Willayat et al., 2005). Higher prevalence was recorded in the organised farms compared to the unorganised sector which could partly be due to more confinement of the livestock in the winter months in the organised farms leading to a more stressed condition. As a general practice, animals are let loose in the unorganised farms even in the

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the farm</th>
<th>No. of abortions/stillbirths</th>
<th>Cases positive for <em>Listeria</em> spp.</th>
<th>Per cent positive for <em>Listeria</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sheep Breeding Farm, Dachigam</td>
<td>12</td>
<td>3</td>
<td>25.00</td>
</tr>
<tr>
<td>2.</td>
<td>Sheep Breeding Farm, Gaobal</td>
<td>4</td>
<td>1</td>
<td>25.00</td>
</tr>
<tr>
<td>3.</td>
<td>Sheep Breeding Farm, Kralpathri</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>Sheep Breeding Farm, Zawoora</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Sheep Research Station, Shuhama</td>
<td>9</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>6.</td>
<td>Unorganised Sheep farms,District Pulwama</td>
<td>7</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td>7.</td>
<td>Unorganized Sheep Farms, District Budgam</td>
<td>7</td>
<td>2</td>
<td>28.57</td>
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<tr>
<td>8.</td>
<td>Unorganized Sheep Farms, District Baramulla</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.</td>
<td>Unorganized Sheep Farms, District Anantnag</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10.</td>
<td>Unorganized sheep farms, District Ganderbal</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<td>11.</td>
<td>Unorganized sheep farm, District Srinagar</td>
<td>1</td>
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<td>0</td>
</tr>
</tbody>
</table>
winter months. Variable reports are available on the prevalence of listeriosis in sheep. Broadbent (1972) recorded an abortion rate of 20% due to \textit{L. monocytogenes} from 23 Victorian flocks in Australia. Pasa \textit{et al.} (2003) reported a higher prevalence of 35% in sheep from France. A lower prevalence rate has also been reported by some workers (Nigam \textit{et al.}, 1996). In the present study, higher prevalence of 25% each was recorded in sheep breeding farms, Dachigam and Gaobal compared to Sheep Research Station, Shuhama where the prevalence was as low as 11.11%. \textit{L. monocytogenes} could not be isolated from some organised farms possibly because of very less number of samples available (Table 1). The variability in the prevalence rates could be due to the variability in the local sanitary and hygienic conditions in these farms. Similar justifications may hold true for the variability of the prevalence rates in the unorganised farms of the valley. Kashmir valley, as such, seems to be no exception to the sustenance of \textit{L. monocytogenes} as reported elsewhere with similar topography and agro-climatic conditions.

None of the aborted ewes presented any clinical manifestations of listeriosis prior to or following abortions/still-births which is in agreement with the findings of other workers (Kennedy and Miller, 1993). Half of the still-born lambs positive for listeriosis were mummified, while others were oedematous and had clear or blood tinged fluid in their abdominal and thoracic cavities. Similar findings were observed by McDonald (1967), Jeleff and Djurov (1969), while studying 24 spontaneously aborted ovine foetuses, from which \textit{L. monocytogenes} was isolated, noticed that subcutaneous oedema was most pronounced in area of abdominal wall and head. In the present study, haemorrhages in the sub-cutis, skin, lungs, epicardium and in the kidneys and the mesenteric lymph nodes of aborted/stillborn foetuses were also observed. Yellowish white necrotic foci on the lobes of the livers were seen in 37.5% of the still-born lambs, while others presented focal or multifocal haemorrhages on the surface of the livers. Jeleff and Djurov (1969) found miliary and sub-miliary grey white foci on the livers of aborted or still-born lambs in the listeric infections. Focal necrosis was observed in livers of 75% of \textit{Listeria} infected lambs by Dennis (1966). The livers of foetuses observed in the present study had rounded borders, were friable in consistency and were yellowish brown in colour. This is in accordance with the observations made by previous workers (Jeleff \textit{et al.}, 1964; Ladds, 1970). Moderate to severe congestion of brain was also observed in 6 of the 8 positive cases. In some cases, however, the medullary meninges were thickened by a gelatinous oedema. Most of the brains had an increased amount of cerebro-spinal fluid, being cloudy in some cases. These findings are in agreement with the findings of Gray and Killinger (1966) and Chand and Sadana (1999). Abomasal ulcers, subcutaneous oedema with clear or blood tinged fluid in the abdominal and thoracic cavities have also been reported by some workers (Ladds, 1970; Njoku \textit{et al.}, 1972). In the present study, some still-born lambs also showed deformities of limbs as observed by previous workers (Willayat \textit{et al.}, 2005).

All the isolates in the present study produced marked degree of beta haemolysis on 10% sheep blood agar plates in 24-36 h depicting the extent of their pathogenicity in the host species. An 18 h old broth culture produced kerato-conjunctivitis and monocytes in healthy rabbits within 2-6 d by intra-ocular route. Similar observations on experimental inoculation in rabbits have been made previously (Dutta and Malik, 1978; Radostitis \textit{et al.}, 1994).

Isolates of \textit{L. monocytogenes} under study were highly sensitive to gentamicin (100%), doxycycline (87.5%), tetracycline (75%) and ampicillin (75%). However, all the isolates showed a high degree of resistance towards co-trimoxazole, nalidixic acid, ciprofloxacin, chloramphenicol, erythromycin and lemofoxacin. Sensitivity to gentamicin and resistance to chloramphenicol and nalidixic acid has also been reported by Willayat \textit{et al.} (2005).

The study revealed an overall prevalence of 15.38% of listeric abortions was recorded in
sheep of Kashmir. The prevalence was higher in organised (18.51%) than in unorganised farms (12%). All the isolates were virulent in the host species as they produced marked degree of beta haemolysis on 10% sheep blood agar plates as well as produced keratoconjunctivitis and monocytosis in healthy rabbits within 2-6 d of instillation via intraocular route. The isolates were sensitive to gentamicin, doxycycline, ampicillin, tetracycline and penicillin G and resistant to nalidixic acid, co-trimoxazole, ciprofloxacin, chloramphenicol, erythromycin and lemoloxacin.

References


