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Short communication

Mycoplasmal pneumonia in garole sheep

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ABSTRACT

The prevalence of spontaneous pneumonia in Garole sheep was reported based on isolation of *Mycoplasma* and pathological changes. Mycoplasmal isolate was provisionally identified as *Mycoplasma ovipneumoniae* by cultural characters and biochemical changes. Pathological changes featured interstitial pneumonia with peribronchial and perivascular lymphoid cell hyperplasia and thickening of interlobular septa.

Key words: mycoplasmal pneumonia, garole sheep, pathomorphology

Introduction

Pneumonia is a most serious problem in sheep worldwide and can be an important cause of death and reduced productivity. It is estimated that pneumonia alone causes at least 10% mortality in sheep population in India (MARU et al., 1990). The disease is of multifactorial in origin; usually, a combination of infectious, environmental and managerial factors are involved. Among the infectious agents, *Mycoplasma ovipneumoniae*, *Pasteurella haemolytica*, *Parainfluenza* type-3, adenovirus, and reovirus have been established as the prime etiological agents of ovine pneumonia (JONES, 1983; CHATTOPADHYAYA et al., 1986; HAZIROGLU et al., 1996).

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The Garole sheep is a prolific breed of India, mainly distributed in the Sundarban delta (4226 km^2), West Bengal, India, which is situated within 21^0 to 23^0 N latitude and 87^0 to 89^0 E longitudes. Although very limited information exists on disease problems of Garole, except for a report on the prevalence of parasitic diseases in Garole sheep (SINGH and BOHRA, 1996), no literature is currently available on the major disease problems in this microsheep. Since pneumonia is a constant threat to other breeds of sheep, the present study was undertaken to report the spontaneous disease in Garole sheep based on isolation of Mycoplasma and pathomorphological changes.

Materials and methods

Thirteen blocks of the Sundaban delta (4226 km^2), the homeland of Garole sheep, were surveyed for the prevalence of pneumonia during the period from December 2000 to November 2001. The animals were screened for respiratory signs, viz. oculo-nasal discharge, coughing and sneezing, and tracheal rales. Dead animals at farmers' house were necropsied and the lungs examined in detail to identify the disease. Likewise, animals slaughtered in the local markets were also screened and examined. During necropsy, the entire lungs with trachea were removed and examined for their texture, colour, appearance and visible gross lesions, which were kept on record.

Out of a total of 450 lungs examined, 90 showing active nodular lesions were selected for isolation of Mycoplasma. The tracheal and lung swabs were collected aseptically and transferred to 3 ml of 'PPLO' broth containing 20% horse serum. Similarly, aseptically collected 0.2 g of selected pieces of lung tissues were triturated in 1.8 ml PPLO broth (Himedia, Mumbia, India) containing 20% inactivated horse serum, penicillin and thallous acetate. The samples were incubated in a humidified atmosphere of 5% CO_2 at 37 ^{0}C. Blind passages were followed three times and 0.1 ml from each tube was transferred onto solid plates containing 1.2% 'Diflo' 'Bacto' agar with Mycoplasma supplement and incubated in candle jar at 37 ^{0}C for 5-6 days. Agar surface was examined daily under a dissecting microscope for colonies of Mycoplasma. Diene's method of staining was employed for the evidence of growth (ANONYMOUS, 2000). Morphological studies were conducted after treating the smeared slides in vapour of N/1 HCl at 60 ^{0}C for 10 minutes, followed by staining with Giemsa (1:20) overnight. Pieces of agar containing colonies were removed for subculture. Pure culture of the isolates was obtained by cloning the isolates. The isolates were screened by biochemical tests, viz. glucose fermentation containing 1% glucose (initial pH 7.8), arginine hydrolysis(initial pH 7.2) and urea degradation having 0.4% arginine and 0.4% urea (initial pH. 6.4), reduction of tetrazolium chloride (aerobically and anaerobically) digitonin sensitivity, film and spots formation, serum digestion and phosphatase activity, following the methods described earlier (ERNØ and STIPKOVITS, 1973).

Parts of infective lung having active nodular lesion were preserved in 10% formalin saline, processed for paraffin sectioning at 5 µm thickness and stained with heamatoxylin and eosin (H&E) for histopathological examinations. Duplicate sections also were stained with Giemsa(1:20) for 'PPLO' organisms.

Results

Isolation and identification of Mycoplasma. Mycoplasma was isolated from the trachea and lung of 18 infected animals. On 'PPLO' broth culture, flocular material was appeared on the 3rd day post-incubation. The inoculated culture on 'PPLO' agar plates showed small colonies 0.3 mm in diameter on the 5th day post-incubation, and often lacking a well-developed 'central nipple' (Fig. 1). Isolates on solid media took an intense royal blue colour with Diene's stain and they retained the stain at 24 hrs of incubation at 37 ^{0}C. None reverted back to bacterial colonies even after 5 consecutive passages on solid Mycoplasma media, omitting antibiotics and thallous acetate. Giemsa stained colony smear revealed small coccoid organisms with a tendency towards pleomorphism, suggesting the isolates as Mycoplasma. Biochemically, the isolates were sensitive to digitonin, fermented glucose and reduced tetrazolium anaerobically. None of isolates hydrolysed arginine or urea formed 'film and spots' with negative phosphatase activity. These mycoplasmal isolates on the basis of their cultural characters, staining morphology and biochemical changes, were indistinguishable from *Mycoplasma ovipneumoniae*.

Pathological changes. Grossly, trachea revealed red, frothy exudates and congestion of its mucosal lining. In the lungs, the most striking lesions were clearly demarcated palm-coloured or greyish pink consolidated areas distributed throughout the apical, cardiac and anterior diaphragmatic lobes with presence of frothy exudates. The areas measured from 2.5 cm × 2.0 cm to 1.5 cm × 1.0 cm on the visible lung surface and extended to 1.0 cm

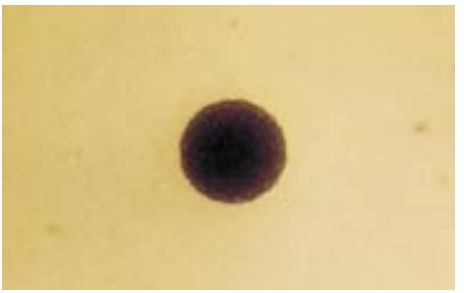

Fig. 1. Small colony, lacking the development of central'nipple' shaped in 'PPLO' agar plate, suggestive of *Mycoplasma.* ×20

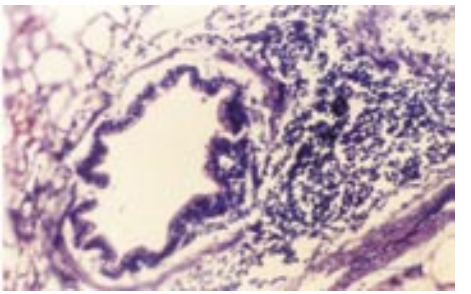

Fig. 2. Peribronchial lymphoid cells hyperplasia in Mycoplasmal pneumonia. H&E, ×250.

to 1.5 cm into the lung parenchyma. Cut surfaces showed grey- white nodules protruding from the surface. Most of the consolidated areas exhibited atelectasis.

Histologically, the lesions consisted of congestion of pulmonary capillaries, serofibrinous exudation, infiltration of mononuclear cells in alveolar lumina, thickening of alveolar walls and interlobular septa, with presence of mononuclear cells and proliferation of type II pneumocytes in interlobular septa. In the infected lungs, there were massive infiltrations of lymphocyte and macrophages around in bronchi, bronchioles and blood vessels. A frank lymphoid cell hyperplasia was highly evident in the peribronchial and perivascular areas (Fig. 2). Hyperplasia of bronchiolar epithelial cells was also a prominent lung lesion in all 18 sheep. The bronchial and mediastinal lymph nodes also had congestion and lymphoid cell hyperplasia.

Giemsa stained sections revealed the presence of minute coccoid mycoplasmal organisms in the lumina of alveoli and bronchioles.

Discussion

In this study, Mycoplasma was isolated in culture media in 18 pneumonic lungs of Garole sheep, indicating that the organism may be regarded as an important pathogen causing pneumonia in this microsheep. It is possible that the sheep with lowered resistance might pick up the infection from the pasture, as the organism is widespread in nature. It is reported that mycoplasmal pneumonia is most frequently observed in the grazing lambs which manifest a mild form of the disease, with signs of coughing in many of the infected lambs over a period of many weeks or months and which eventually spreads the infection to other healthy animals (MARTIN, 1996). The mycoplasmal isolates, on the basis of their cultural characters, staining morphology and biochemical changes, were indistinguishable from *M. ovipneumoniae*. The appearance of small colonies, 0.3- 0.4 mm in diameter, often lacking the typical central nipple, on solid media, glucose breakdown and digitonin sensitivity, negative of arginin, serum digestion and phosphatase urease activities are characteristics of *M. ovipneumoniae*. However, it is necessary to obtain known antiserum and to identify the agents serologically, or to produce the typical disease in experimental sheep.

Pathologically, damage to the lungs of infected animals is highly appreciated from a diagnostic point of view. The gross lesions comprising palm-coloured consolidation in apical, cardiac and diaphragmatic lobes with atelactasis and nodule formation are considered pathognomic in ovine mycoplasmal pneumonia, as also reported by GILMOUR et al. (1982) and MARTIN (1996). In this study, lymphoid cell hyperplasia in the peribronchial and perivascular areas, and bronchiolar epithelial hyperplasia, were inconsistent in all the cases, which may be considered as specific histopathological lesions of ovine mycoplasmal pneumonia, as also reported by STAMP and NISBET (1963), GILMOUR et al. (1982), MARTIN

(1996). In addition, histopathological changes featuring the interstitial pneumonia in the infected animals clearly suggested a proliferative exudative lesion produced by *M. ovipnuemoniae*, which confirmed the reports of GILMOUR et al. (1982) and SREERAMULU et al. (1987).

It is concluded that the lung lesions in mycoplasmal pneumonia are likely to persist over a period of months from its early-consolidated lesions through the regression phase.

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SAŽETAK

Pri pojavi pneumonije u Garole ovaca izdvojene su mikoplazme te prikazane patološke promjene. Izdvojeni izolat je na osnovi kulturelnih i biokemijskih značajki identificiran kao *Mycoplasma ovipneumoniae*. Patološke promjene očitovale su se intersticijskom pneumonijom s peribronhalnom i perivaskularnom hiperplazijom limfoidnih stanica te zadebljanjem interlobularnih septi.

Ključne riječi: mikoplazmalna pneumonija, Garole ovca, patomorfologija