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ScienceDirect
Research in Veterinary Science 85 (2008) 617–621
www.elsevier.com/locate/rvsc

Sheep pox disease outbreaks in Madras Red and Mechery breeds of indigenous sheep in Tamilnadu, India

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Accepted 10 March 2008

Abstract

Sheep pox disease outbreaks were recorded among Madras Red ($n = 145$) and Mechery ($n = 80$) breeds of indigenous sheep on three farms in Tamilnadu. Over both breeds, adult mortality rate ranged from 2.66% to 37.5% and lamb mortality ranged from 10% to 17.33%. However, mortality was more in Mechery sheep (50% overall; 37.5% adults, 12.5% lambs) than in Madras Red sheep (24.28% overall; 10.34% adults, 13.79% lambs). The clinical signs observed were high fever, anorexia, respiratory distress, mucopurulent nasal discharge and in a few cases diarrhoea. Cutaneous lesions were mainly observed around nostrils, eyes, lips, ears and in the abdomen. Most of the lesions were covered with purulent materials and on cleaning with sterile swabs, fresh wounds were observed. Dry scabs were also observed over the oral commissure and maxillary areas, which on removal exposed fresh wounds. Important observations on necropsy were severe nodular lesions in the lungs and intestine. The disease was diagnosed as sheep pox by agar gel immunodiffusion test, isolation of virus and its neutralization in BHK_{21} cells by specific antiserum and by electron microscopy.

Keywords: Sheep pox; Clinical signs; Necropsy lesions; Morbidity; Mortality; Agar gel immunodiffusion test; Cell culture; Cytopathogenic effects; Electron microscopy

1. Introduction

Sheep pox is a malignant cutaneous disease of sheep caused by a virus, a member of the genus Capripox virus (Murphy et al., 1995). Capripox virus infections in sheep, goats and cattle cause major economic losses and disturb both international trade and introduction of exotic breeds to endemic areas (Davies, 1981). Sheep pox was apparently present in Asia and Europe as early as the second century A.D. (Hutyra et al., 1946). In India the disease was first reported from Tamilnadu, Bombay and United provinces (Sathe, 1931) and despite the availability of efficient vaccines, the disease continues to be recorded. The severity of the disease depends on the strain of virus and the breed of host (Davies, 1981). Indigenous sheep and goats exhibit some natural immunity and European breeds are more susceptible to infection with Capripox viruses (Heine et al., 1999). The disease causes skin defects, abortion, mastitis in ewes and high mortality in lambs (Singh et al., 1979). The present paper describes natural outbreaks of sheep pox affecting Madras Red and Mechery breeds of indigenous sheep and identification of the etiological agent by agar gel immunodiffusion test, electro microscopy and virus neutralization with specific antiserum.

2. Materials and methods

2.1. The disease outbreaks

Disease outbreaks occurred in three different private farms housing indigenous breeds of sheep in December

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doi:10.1016/j.rvsc.2008.03.011

2005 (1st and 2nd farms) and February 2006 (3rd farm). The 1st and 2nd farms housed 70 and 75 Madras Red sheep, respectively, and both the farms were located at Mavalurkuppam village in Kanchipuram district of Tamilnadu. The 3rd farm housed 80 Mechery sheep and the farm was located at Mechery village in Salem district of Tamilnadu. The affected sheep showed clinical signs of sickness and pock lesions on the skin. Disease investigation was undertaken and necropsy was performed on selected 2 Madras Red and 4 Mechery sheep breed of adult sheep. Samples collected for laboratory investigation included scabs, pieces of lung and lymph nodes. The affected animals were segregated and treated with Strepto-penicillin parenterally and neem oil was applied locally to skin lesions. Animals in nearby farms were vaccinated with sheep pox vaccine. Outbreaks were controlled after about one and half months of treatment.

2.2. Agar gel immunodiffusion test

Agar gel immunodiffusion (AGID) test was performed to detect sheep poxvirus specific antigen in scab, lung and lymph node samples collected from the affected sheep. The test was performed as described earlier (Rao and Negi, 1997). A 10% suspension of tissue samples was prepared in phosphate buffered saline (PBS, pH 7.2). Anti-sheep poxvirus antiserum used in the test was obtained from the Institute of Veterinary Preventive Medicine (IVPM), Ranipet, India. The test was performed in 1% agarose (low EEO, SISCO) on micro slides. Antiserum was applied to the central well and different samples (supernatants of tissue homogenates) applied to peripheral wells at a volume of 25 µl. The diameter of the wells and the distance between them were 4 mm. For positive control antigen, known sheep poxvirus positive tissue homogenate available in this laboratory was used. Similarly for negative control, known negative tissue homogenate was used. The micro slides were left at room temperature (25 °C) in a humidity box and results were read after 24 h, 48 h and 72 h.

2.3. Electron microscopy

Supernatants (the suspensions spun in a centrifuge) of 10% suspension of tissue samples in PBS were coated on electron microscopic grid, stained with 1% phosphotungstic acid and viewed under Technai-10 transmission electron microscope (Holland) at 80 KV.

2.4. Virus isolation

BHK_{21} cells were grown to confluence in tissue culture flasks (25 cm^2) with minimum essential medium (MEM) containing Earle's salts, L-glutamine and non essential amino acids (GIBCO-BRL, USA) supplemented with 10% heat inactivated fetal bovine serum and antibiotics (500 IU Penicillin/ml and 0.25 mg Streptomycin/ml). Growth medium with a reduced level of serum (2%) served as a maintenance medium. To confluent monolayers were added 300 µl of sample (10% tissue homogenate filtered through membrane filter of 0.45 µm porosity). After incubation at 37 °C for one hour to allow any virus to adsorb, the inoculum was decanted and fresh maintenance medium was added. The cells were incubated at 37 °C and examined periodically for any cytopathological effects (CPEs). After two blind passages, cells were harvested three days after 3rd passage and clear supernatant was inoculated to the confluent monolayer as described above and examined periodically for CPE. Similarly, corresponding healthy cells (to which samples had not been added) were maintained as controls. Cover slip cultures were also prepared for infection and controls. To cells on cover slips, 0.1 ml of sample was added. Infected and corresponding control cover slips were fixed in acetone and stained with haematoxylene and eosin for microscopic examination and photography.

2.5. Neutralization test

When CPE was consistent at 3rd passage, cells were harvested after 72 h of culture and subjected to repeated freeze and thaw. The supernatant was mixed with sheep pox antiserum in equal volume (1:1) in varying dilution of supernatant and keeping the dilution of antiserum as constant. They were incubated at 37 °C for 1 h. The mixture was inoculated onto BHK_{21} monolayer and CPE observed at different time intervals.

3. Results

3.1. Clinical and necropsy findings

The clinical signs observed in diseased animals were high fever (up to 106 °C) respiratory distress and the majority of

Fig. 1. Madras Red sheep: pock lesions (arrow) are seen in ears, around eyes and over the maxillary region.