An outbreak of sheep pox on a sheep breeding farm in Jammu, India

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Summary

An outbreak of sheep pox occurred in December 2001 on a sheep breeding farm in Jammu, India. The farm maintains three exotic breeds of sheep, i.e. American Merino, Rambouillet and Australian cross. The disease agent was confirmed as sheep pox virus by clinical and post-mortem examination as well as laboratory testing. Typical pock lesions were dispersed over the body of the affected animals with nodular lesions observed in the lung tissue of the dead animals. Sheep pox virus antigen and antibody were detected in infected tissue and convalescent sera, respectively, with serological tests. Viral deoxyribonucleic acid was extracted from the infected tissue and amplified using a diagnostic polymerase chain reaction. Sheep of the Rambouillet breed were found to be most susceptible to infection with morbidity and mortality rates of 26.9% and 8.3%, respectively. Morbidity and mortality rates in the entire flock were 18.4% and 6.3%, respectively. The grazing and migration pattern indicates that the disease was probably introduced to the farm by local sheep.

Keywords

Capripox – Counterimmunoelectrophoresis – Indirect enzyme-linked immunosorbent assay – Morbidity – Mortality – Polymerase chain reaction – Sheep pox.

Introduction

Sheep pox and goat pox are malignant pox diseases of small ruminants characterised by fever and generalised pock lesions. High mortality rates occur in young animals resulting in significant economic losses. Sheep pox is caused by sheep pox virus (SPV), a member of the *Capripoxvirus* genus in the *Poxviridae* family (8). The other members of the genus are goat pox virus (GPV) and lumpy skin disease virus (LSDV) of cattle. Although the geographic distribution of sheep pox, goat pox and lumpy skin disease is different, suggesting that they are caused by distinct viruses, the viruses are indistinguishable by conventional serology and barely distinguishable by restriction endonuclease analysis (3). Strains of SPV and GPV are not considered host-specific and although the majority of strains show a host preference, a single strain may cause disease in both sheep and goats. Goats may become mildly infected with sheep strains, which can then cause severe disease when transmitted back to sheep. Similarly, sheep may become infected with virulent goat strains. It has been proposed that both sheep pox and goat pox be described as a single disease, called capripox (5). However, in the Indian subcontinent, sheep pox and goat pox appear to be caused by separate viruses and no single virus that can cause disease in both sheep and goats in a mixed flock under natural conditions has thus far been identified. Therefore, it may not be justified to refer to sheep pox and goat pox as a single disease.

Sheep pox and goat pox are endemic in Africa, particularly to the north and west of the Sahara, in the Middle East and Far East, and on the Indian subcontinent. Outbreaks of sheep and goat pox occur frequently in India incurring economic losses to the sheep and goat industry. Mortality in young animals can exceed 50% (2, 7, 8). While indigenous sheep and goats exhibit some natural immunity, the European breeds of sheep and goats are more susceptible to infection with capripoxviruses, with mortality occasionally reaching 100% (5). In this paper, the authors describe an outbreak of sheep pox on a sheep breeding farm in Jammu, India, and demonstrate the presence of pox virus antigen and antibody in infected tissue and sera, respectively. Infection with SPV was further confirmed by amplification of viral deoxyribonucleic acid (DNA) using a diagnostic polymerase chain reaction (PCR).

Materials and methods

The disease outbreak

The farm on which the outbreak occurred maintains three exotic breeds of sheep, i.e. American Merino, Rambouillet and Australian cross (Australian × Rambouillet) housed in separate enclosures. The first incidence of disease was noticed at the beginning of December 2001 when two ewes of the Rambouillet breed began to show signs of sickness. Increasing numbers of animals were subsequently infected with the disease, which continued to afflict sheep of all three breeds until the end of February 2002. Sick animals were segregated from apparently healthy animals and housed together in a separate enclosure away from the main stock. Clinical symptoms, mortality and morbidity were recorded throughout the course of the disease. Post-mortem examinations were performed on a select number of dead animals and samples of infected lung tissue and skin lesions were collected for further laboratory testing. Sera samples (n = 20) were collected from recovered animals and ten sera were randomly chosen for serological tests. The affected animals were treated with streptopenicillin and enrofloxacin parenterally. Sulpha ointment was applied to external lesions to control secondary bacterial infections. The treatment was effective in minimising the suffering of the diseased animals.

Counterimmunoelectrophoresis

Counterimmunoelectrophoresis (CIE) was performed to detect pox virus antigens in infected lung tissue and scab/skin samples collected from diseased animals during the outbreak, as per the method described by Ramyar (9). A 10% suspension of tissue material was prepared in phosphate buffered saline (PBS) (pH 7.2). The presence of pox virus antigens in tissue material was detected using sheep pox hyperimmune sera (HIS) produced, according to standard methods of hyperimmunisation, using sheep that were sero-negative for the Jaipur strain of SPV (12). The titre of HIS used in the agar gel immunodiffusion was 1:16. Electrophoresis was carried out on 1% agar gel in 0.04 M barbitone buffer (pH 8.6), at 20 v/cm for 45 min, on glass microscope slides. The gel was dried and stained with Coomassie blue to visualise the precipitin bands.

A positive antigen sample was also prepared from a 10% suspension of scab materials collected from sheep experimentally infected with the Jaipur strain of SPV maintained by needle passage (14). The positive antigen was used to detect pox virus antibody in the sera collected during the outbreak.

Indirect enzyme-linked immunosorbent assay

Serum antibody against pox virus was demonstrated with an indirect enzyme-linked immunosorbent assay (ELISA) according to the method described by Hosamani *et al*. (6). Briefly, SPV infected Vero cells were sonicated and the supernatant (containing the SPV antigens) used to coat a flat-bottom 96-well plate, which was then incubated for 1 h at 37°C. To block the unbound sites, a blocking solution containing gelatin and skimmed milk powder was added to the wells and incubated for 1 h at 37°C. Test sera were diluted (1:150) and added to the wells which were then incubated for 1 h at 37°C. Anti-sheep horseradish peroxidase conjugate was then added to each well and incubated for an additional hour at 37°C. The plates were washed three times with PBS, containing 0.05% tween-20, after each incubation step. *O*-phenylenediamine dihydrochloride substrate was added to each well and the intensity of colour was recorded at 492 nm using an ELISA reader. Positive and negative control sera were also included in the test.

Polymerase chain reaction

The capripoxvirus p32 antigen is a structural protein present in all strains of capripoxvirus (1). The p32 gene has been identified as a homolog of the vaccinia virus H3L gene which encodes a major immunodominant antigen located on the membrane surface of the mature intracellular virion particle (11). Based on the nucleotide sequence of the p32 gene, a capripoxvirus detection PCR was developed and used in the present study to detect SPV nucleic acid in the infected tissue samples (4). Viral DNA was extracted by adding 100 µl of 10% SDS and 20 µl of Protinase K (10 mg/ml) to 0.9 ml of a 10% suspension (in PBS) of scab or lung nodules (4). The mixture was

incubated at 37°C for 2 h and DNA was extracted by adding phenol, followed by equal volumes of phenol:chloroform and then chloroform. One-tenth volume of 3 M sodium acetate (pH 5.2) and 2.5 volume of 100% ethanol were added to the final aqueous phase to precipitate the DNA. The DNA pellet was washed one time in 70% ethanol, air dried, and suspended in 20 µl of Tris-EDTA buffer. The primers used were B68 (5'-CTAAAATTAGAGAGCTATACTTCTT-3') and B69 (5'-CGATTTCCATAAA CTAAAGTG-3') as previously published by Heine *et al*. (5). The PCR was performed in a 50 µl reaction volume containing 5 µl of DNA, 0.2 µM of each primer, 1.5 mM of $MgCl_2$, 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 0.1% Triton X-100, 200 µM of each dNTP and 2 units of *Taq* DNA polymerase. The cyclic conditions for DNA amplification were set at 95°C for 2 min, followed by 35 cycles at 95°C for 30, 47°C for 1 min and 72°C for 1 min. The amplified DNA product was resolved by agarose gel electrophoresis, stained with ethidium bromide and visualised under an ultraviolet transilluminator.

Results

Clinical and post-mortem findings

The clinical syndrome observed in diseased animals, i.e. pyrexia (up to 106.7°C), mucous discharge from the nostrils, and decreased appetite, is typical of infection with SPV. Cutaneous nodules, approximately 1 cm in diameter, were widely distributed over the body. These lesions were most obvious in areas where there is less hair/wool, such as around the mouth and eyes, along the abdomen, under the tail and around the inguinal region (Fig. 1a). The nodules progressed into pustules in the later stages of the disease. Death generally occurred between seven and ten days after the onset of illness, although in some cases infected animals survived for more than twenty days after the onset of illness. Post-mortem examination revealed severe nodular lesions in the lungs of the dead animals (Fig. 1b). These types of lung lesions are a characteristic feature of generalised sheep pox.

There was significant morbidity and mortality in the Rambouillet and Australian cross sheep breeds. Lambs under one year of age were most severely affected. Sheep of the Rambouillet breed were the most susceptible, with the highest morbidity and mortality rate of all three breeds (26.9% and 8.3%, respectively). Australian cross sheep were also affected, but the morbidity and mortality rates (8.3% and 4.5%, respectively) were less than the morbidity and mortality rates observed in the Rambouillet breed. There were no deaths (0% mortality) in the American Merino breed, although two sheep were infected and subsequently recovered (morbidity 1.5%). Of the 1,369 sheep maintained on the farm, 252 were affected (and subsequently recovered) and 86 died of sheep pox. The overall morbidity and mortality rates in the sheep flock were 18.4% and 6.3%, respectively (Table I).

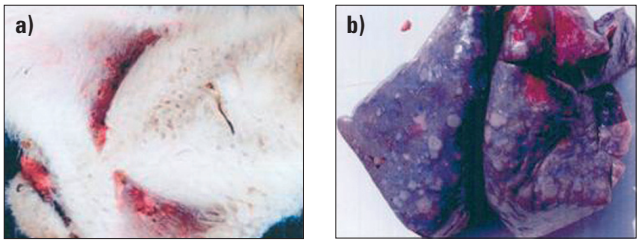


Fig. 1
Clinical and post-mortem lesions in a sheep infected with sheep pox virus
Pock lesions observed in the inguinal region (a) and lungs (b) of an infected animal

Counterimmunoelectrophoresis

The scab and lung tissues of the dead animals were found positive for precipitating pox virus antigens. Precipitin bands were observed against sheep pox HIS using CIE (Fig. 2). Clinical sera samples were also positive for poxvirus antibody (data not shown).

Indirect enzyme-linked immunosorbent assay

Ten sera samples randomly chosen for indirect ELISA were all found positive for poxvirus antibody (Fig. 3). The antibody titres of the test sera were comparable with the positive control, except for one serum sample (Number 6) in which the titre was quite low but still well above the value of the negative control.

Table I
Morbidity and mortality rates following an outbreak of sheep pox on a sheep breeding farm in Jammu, India

Rates	American Merino	Rambouillet	Australian cross	Total
Number of animals	133	792	444	1369
Morbidity	1.5% (2)	26.9% (213)	8.3% (37)	18.4% (252)
Mortality	0	8.3% (66)	4.5% (20)	6.3% (86)

Note: The value in parenthesis refers to the number of sick (morbidity) or dead (mortality) animals

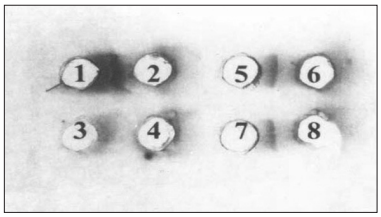


Fig. 2
Detection of sheep pox antigen in infected tissue by counterimmunoelectrophoresis (CIE)
The appearance of precipitin bands using CIE demonstrates the presence of SPV in scab (precipitin band between wells 5 and 6) and lung (precipitin band between wells 7 and 8) lesions of a diseased animal. Wells 5 and 7 contain scab and lung material, respectively, from a dead animal. Wells 1 and 3 contain positive SPV antigen (Jaipur strain) and negative antigen (healthy skin), respectively. Wells 2, 4, 6 and 8 contain sheep pox hyperimmune serum

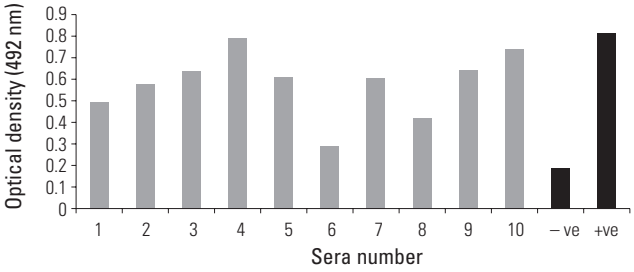


Fig. 3
Titres of sheep pox virus antibody in the sera of diseased animals
The titre was measured by indirect enzyme-linked immunosorbent assay. Ten sera (1 to 10) were chosen randomly from a pool of 20 sera collected from recovered animals. Serum from healthy sheep and serum from sheep experimentally infected with Jaipur SPV were used as negative (— ve) and positive (+ve) controls, respectively. Sera samples showing an optical density value double the value of the negative control were considered positive

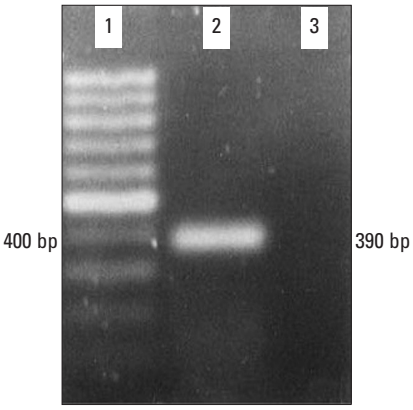
Polymerase chain reaction

Infected tissues and scabs were examined by a capripox diagnostic PCR for the detection of an SPV specific DNA sequence. A DNA fragment of expected size (390 bp) was observed on gel electrophoresis (Fig. 4). Nine samples of scab and lung tissues were tested by PCR. Amplification of specific DNA was evident in all the samples. As DNA amplification was not observed when parapox virus DNA was used as a template, the PCR was therefore verified as specific for SPV.

Discussion

A severe outbreak of sheep pox virus was investigated in the present study. Clinical and post-mortem findings characteristic for sheep pox were highly suggestive of SPV as the causative agent. The results of CIE, indirect ELISA and PCR further confirmed that the outbreak was due to infection with SPV. Exotic sheep are considered highly susceptible to sheep pox. In the present outbreak the severity of the disease (especially in young animals) was high, although the mortality was moderate. The large number of lesions dispersed over the body surface and throughout the lungs of diseased animals was probably the major cause of mortality.

Fig. 4
Detection of a sheep pox virus (SPV) specific deoxyribonucleic acid (DNA) fragment by polymerase chain reaction.



A DNA fragment (390 bp) of the size appropriate for SPV was amplified (lane 2) from infected scab material. No amplification (lane 3) was observed when parapoxvirus DNA was used as a template. Lane 1 contains a 100 bp ladder molecular weight marker

The presence of viral antigens in infected tissues and viral antibodies in the sera of infected animals was demonstrated through the use of CIE and indirect ELISA, respectively. Since capripoxviruses share common precipitating antigens with parapoxviruses (13), the CIE (using HIS) or the indirect ELISA (using polyclonal antibody) may also detect parapoxvirus antigen or antibody. However, severe generalised pock lesions observed over the cutaneous surface and, particularly, in the lungs of affected animals, are characteristic of sheep pox while not of parapoxvirus infections. Moreover, the capripox diagnostic PCR used in this study amplified a DNA fragment of the size that is expected for SPV (390 bp), but failed to amplify parapoxvirus DNA, confirming the specificity of the diagnostic PCR used in this investigation.

It is a routine practice on this farm to graze the sheep in high altitude pasture land during the summer months and return the sheep to the farm at the beginning of the winter. Indigenous sheep from the local areas also graze in the same pastures. In May 2001, four months prior to the disease outbreak on the farm, the sheep began migrating to the upper pastures, taking approximately one month to reach the final destination. Australian cross and

Rambouillet sheep migrated up to and down from the upper pastures by foot while the American Merino sheep were transported by truck. All three breeds of sheep were grazed together on the same pasture land. In mid-November, after more than five months in pasture, the sheep returned to the farm with the first signs of infection occurring in the beginning of December. The same route of migration was also used by local shepherds who often leave behind the ailing or dead animals, creating potential sources of sheep pox infection. As only the Australian cross and Rambouillet breeds were involved in the outbreak, with the first signs of disease occurring approximately two weeks after returning to the farm (sufficient time for incubation of virus), these two breeds probably became infected en route while returning from the upper pastures. Sheep of the American Merino breed were not involved in the disease outbreak with the exception of two sheep that may have become infected on the farm from diseased sheep of the other two breeds. If the Australian cross and Rambouillet sheep had become infected in the upper pastures, it would be expected that the American Merino sheep would also have become infected since all three breeds were grazed together for several months in the upper pastures.

Based on the results of the present study and the observed grazing pattern of the sheep, it is rational to conclude that SPV was the cause of the outbreak on the farm and that the infection was introduced from the local sheep during the time of migration from the upper pastures.

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Épisode de clavelée dans un élevage ovin à Jammu, Inde

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Résumé

Un foyer de clavelée est apparu en décembre 2001 dans un élevage ovin de Jammu, Inde. Trois lignées ovines exotiques sont élevées dans cette exploitation, à savoir Mérinos américain, Rambouillet et croisés Australiens. L'agent de la maladie a été confirmé comme étant le virus de la clavelée d'après les examens cliniques et post-mortem, ainsi que par les tests de laboratoire. Des lésions pustuleuses typiques ont été observées sur le corps des animaux infectés et des lésions nodulaires dans le tissu pulmonaire des animaux morts. Les antigènes et les anticorps du virus de la clavelée ont été détectés respectivement dans les tissus infectés et dans les sérums prélevés pendant la convalescence par des tests sérologiques. L'acide désoxyribonucléique viral a été extrait de tissus infectés et amplifié en utilisant une réaction d'amplification en chaîne par polymérase. On a constaté que les ovins de la race Rambouillet étaient les plus sensibles à l'infection, leurs taux de morbidité et de mortalité étant respectivement de 26,9 % et 8,3 %. Les taux de morbidité et de mortalité pour l'ensemble du troupeau étaient respectivement de 18,4 % et 6,3 %. Le mode de pâturage et de déplacement des troupeaux indique que la maladie a été probablement introduite dans l'élevage par des animaux de la même localité.

Mots-clés

Amplification en chaîne par polymérase – Clavelée – Contre-immunoélectrophorèse – Méthode de dosage immuno-enzymatique indirecte – Morbidité – Mortalité – Variole caprine.

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Brote de viruela ovina en una explotación de cría de ovejas de Jammu, India

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Resumen

En diciembre de 2001 se produjo un brote de viruela ovina en una explotación de Jammu (India) que se dedica a la cría de tres razas de ovejas: merino americana, Rambouillet y *cruce* australiano. Los exámenes clínicos y postmortem, así como las pruebas de laboratorio, confirmaron que el agente etiológico era el virus de la viruela ovina. Los animales afectados presentaban las típicas pústulas diseminadas por todo el cuerpo, y en los ejemplares muertos se observaban lesiones nodulares en el tejido pulmonar. Al aplicar pruebas serológicas a tejidos infectados y al suero de animales convalecientes se detectó la presencia de antígeno vírico, en el primer caso, y de anticuerpos, en el segundo. A partir de tejidos infectados se extrajeron también muestras de ácido desoxirribonucleico viral, que después se amplificaron con la técnica de reacción en cadena de la polimerasa. Se observó que las ovejas Rambouillet eran las más sensibles a la infección, con índices de morbilidad y mortalidad de un 26,9% y un 8,3% respectivamente. Tomando el rebaño en su conjunto, dichos índices resultaban de un 18,4% y un 6,3%. Los patrones de pastoreo y migración llevan a pensar que la enfermedad fue probablemente introducida en la granja por ovejas locales.

Palabras clave

Contrainmunoelectroforesis – Ensayo inmunoenzimático – Morbilidad – Mortalidad – Reacción en cadena de la polimerasa – Viruela caprina – Viruela ovina.

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