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CASE REPORT

Pathology and molecular diagnosis of Jaagsiekte in Goat - Case report

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ABSTRACT

A two-years-old goat with anorexia, progressive emaciation and weak body condition were received for post mortem examination in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Himmatnagar from Teaching Veterinary Clinical Complex, CVS & AH, Himmatnagar, Gujarat. Grossly, diffuse pale oedematous lungs were found. Mediastinal lymph nodes revealed mild congestion and oedema. Further, PCR was used for molecular detection of jaagsiekte sheep retrovirus (JSRV). The presence of jaagsiekte was confirmed by PCR results with anticipated sizes of 229 bp and 176 bp for the gag gene and U3 region of the JSRV genome, respectively.

Keywords: Goat, Jaagsiekte, pathology, PCR

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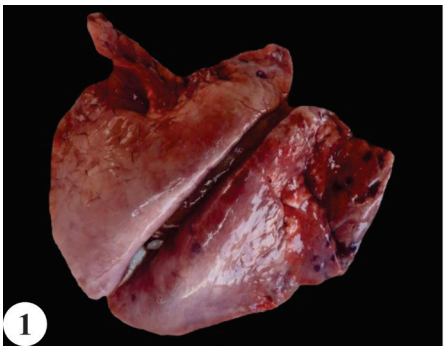
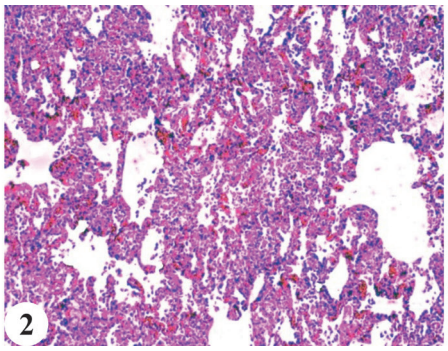
Jaggsiekte is a contagious lung tumor mainly of sheep and it is rarely seen in goats. It is also known as ovine pulmonary adenocarcinoma (OPA) or ovine pulmonary adenomatosis[1]. The natural occurrence rate is low in goats, but experimentally OPA has been transmitted in goats[2]. Jaagsiekte sheep retro virus (JSRV) is the virus that is causative agent for the OPA[3] and is a Beta retrovirus which belongs to the family Retroviridae. In goat, incidence of neoplastic conditions are very few, and it is reported that neoplasms in goats range from 0.8 to 7.6% of total recorded tumors of domestic animals[4]. Transmission of this virus mainly occur through inhalation of infected respiratory secretions and it may also be transmitted by colostrum and milk to nursing animals[5]. Jaggsiekte is an Africans term that means driving sickness, jagmeans chase and siekte means sickness to describe the respiratory distress observed in an animal from being chased especially when the animals are stressed through exercise such as herding[6].

Clinical diagnosis of the disease usually possible at later stage, when animal start to show the clinical signs and symptoms due to longer incubation period of virus. Mainly, JSRV infected animals does not show any clinical signs during their lifespan[6] but once clinical signs become evident it show progressive respiratory distress particularly after exercise and there is accumulation of frothy fluid within the respiratory tract which drains from the nostrils when animal lower down its head. Coughing, fever and inappetence are not common but weight loss is progressive and the disease is terminal within weeks or months. Death may occur due to secondary bacterial pneumonia. It is very difficult to identify JSRV infection in live animals because there is no reliable laboratory method for the ante mortem diagnosis of OPA in individual animals[7] and production of antibody to JSRV due to poor immune response. This virus cannot yet be propagated *in vitro*, therefore routine diagnostic methods such as virus isolation are not available for diagnosis. Farm history, clinical signs and post mortem lesions are the primary method for the diagnosis of disease and other than these histopathology, immunohistochemistry and PCR are also useful methods for confirmatory diagnosis[7].

During necropsy finding, OPA lesions in most cases are confined to the lungs although intra and extrathoracic metastasis to lymph nodes and other tissues may occur[1]. In typical case, affected lungs are enlarged and heavier than normal and there is presence of frothy fluid in respiratory passages. Tumors are solid, grey or light purple with a glossy translucent sheen. By PCR, viral DNA or RNA can be detected in tumor, draining lymph nodes, blood, milk or nasal swabs. Histologically, the lesions are characterized by neoplastic proliferation of the type 2 pneumocytes, a secretory epithelial cell of alveoli and clara cells of bronchioles. Prominent feature is the accumulation of large numbers of alveolar macrophages in the alveoli adjacent to the neoplastic lesions[1].

In the present case, a two-years-old goat with anorexia, progressive emaciation and weak body condition were received





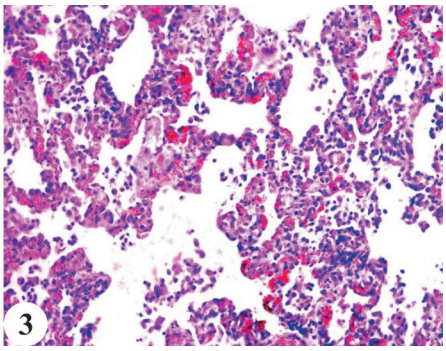


Fig. 1. Goat lungs showing greyish to purple coloured, enlargement, heavy, diffusely oedematous and consolidation; **Fig. 2.** Microscopic sections of lungs showing diffuse marked intra-alveolar septa thickened with infiltration of chronic inflammatory cells and erythrocytes along with mild oedema (H&E X200); **Fig. 3.** Microscopic sections of lungs showing diffuse thickening of intraalveolar septa with infiltration of macrophages and erythrocytes (H&E X400).

for post mortem examination in the Department of Veterinary Pathology, CVS & AH, Himmatnagar, Gujarat from Teaching Veterinary Clinical Complex, CVS & AH, Himmatnagar, Gujarat. Necropsy was performed and the lung samples from different lobes were collected in 10% neutral buffered formalin for histopathological investigation and lung tissue was also collected and stored at -20^{O}C for PCR. For histopathological processing, the tissue samples were rehydrated in tap water, dehydrated in increasing grades of alcohol, cleared in xylene and embedded in paraffin. From paraffin embedded tissue blocks, 4-5µm thick tissue sections were cut on clean, grease free glass slides and haematoxylin and eosin staining was done by routine standard protocol[8]. Then sections were examined under the light microscope for histopathological evaluation of tissue.

To confirm the presence of OPA, PCR was performed. The genomic proviral DNA was extracted from the frozen lung samples by using phenol chloroform extraction method and the extracted genomic DNA was used as a template and the primer of forward 5' TGGGAGCTCTTIGGCAAAAGCC 3', reverse 5' CACCGGATTITTACACAATCACCGG 3' flanking a region of 176 bp region of U3-LTR gene as described earlier[9] and primer of forward 5'GCTGCTTTGAGACCITATCGAAA3', reverse 5'ATACTGCAGCTCGATGGCCAG 3' for a product size 229 bp region of gag gene published by researchers[10] (1µM of each primer/PCR reaction, for both reactions) with the thermal cycle conditions as follows: initial denaturation at 94^{O}C for 3 min; 35 cycles of denaturation at 94^{O}C for 30 s for U3 or 60 s for gag, annealing at 54^{O}C for U3 or at 52^{O}C for gag, for 90 sec and extension at 72^{O}C for 1 min and final extension at 72^{O}C for 4 min. PCR products were electrophoresed in 1.5% agarose gel and examined in Gel Documentation system.

Grossly, goat lung was enlarged, heavy, diffusely edematous and consolidated during necropsy (Fig. 1). Mediastinal lymph nodes were also slightly enlarged and edematous. Histopathological examination of lung tissue showing diffuse marked intra alveolar septa thickened (Fig. 2) with infiltration of chronic inflammatory cells and erythrocytes along with mild oedema (Fig. 3). In PCR, an expected size of amplified DNA product of 176 bp for U3-LTR (Fig. 4A) and 229 bp for gag primer (Fig. 4B) was found. There was no amplification in negative control. Hence, based on history, clinical signs, necropsy findings, histopathology and PCR it can be concluded

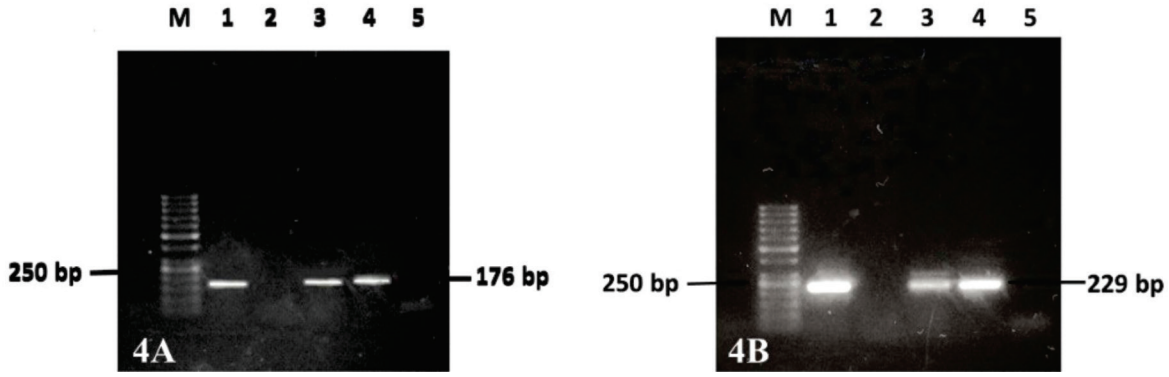


Fig. 4. Detection of JSRV proviral DNA in pneumonic lung tissue: **A.** PCR for U3-LTR gene: Lanes 3 and 4 are positive samples (176 bp); Lanes 2 are negative; Lane 1 as positive control and Lane 5 as negative control and M-50 bp ladder. **B.** PCR for gag gene: Lanes 3 and 4 are positive samples (229 bp); Lanes 2 are negative; Lane 1 as positive control and Lane 5 as negative control and M-50 bp ladder.

that goat is susceptible to JSRV infection, although reports of OPA in goat are very rare. The susceptibility of goats to JSRV infection may be due to rearing of goats mostly along with sheep[4].

Based on gross and histopathological analysis, the lungs examined in this study exhibited a greyish to purple coloration, a swollen and moist surface, and signs of edema with consolidation, which are in concurrence with the classical presentation of OPA (Garcia-Goti *et al*. 2000)[10]. In typical cases, tumours are solid, grey or light purple and have a glossy translucent sheen in the diaphragmatic lobe, accompanied by several smaller nodules in the main lobes. These nodules usually present a hard, pearly white appearance with a dry cut surface, as reported by previous studies[9,12]. However, such features were not observed in the current investigation.

In the present study, U3 LTR and gag-specific PCR products confirmed the presence of JSRV proviral DNA in the lung samples of infected goats. The use of PCR to detect OPA-causing proviruses in lung samples from abattoir cases has proven valuable technique for identifying infected sheep and goats. This method has been effectively applied in multiple studies for OPA diagnosis and it can greatly support control and eradication activities[11].

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