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Ovine pulmonary adenocarcinoma (OPA) in sheep: an update on epidemiology, pathogenesis and diagnosis

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KEYWORDS

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Lung tumor

ABSTRACT

Ovine pulmonary adenocarcinoma (OPA) is a spontaneous lung tumor in sheep caused by Jaagsiekte sheep retrovirus (JSRV) belonging to the *Retroviridae*. The primary aim of this review work is to give brief insights into the epidemiological aspects of OPA based on a meta-analysis of available research work. This review article also discussed pathogenesis, diagnostic tests and control strategies available for OPA in Sheep. This will help in developing future strategies for disease-free status in India. This disease is endemic in Europe, Africa, Asia, and American continents, causing significant economic losses due to chronic respiratory illness and persistent infections in flocks. The virus is unique among retroviruses with selective affinity to lungs and is the only virus known to cause spontaneous lung tumors in sheep. The incubation time ranges for sheep with naturally occurring OPA ranged from one to four years. There are two pathological forms of the disease: classical and atypical. At an early stage, OPA is difficult to detect in sheep due to a lack of preclinical diagnostic methods, as JSRV is poorly immunogenic and doesn't induce an immune response. PCR, histopathology, and immunohistochemistry are recommended methods for OIE diagnosis. To become a JSRV-free country, mandatory surveillance, detection, and removal of positive animals are required, as OPA is difficult to control due to a lack of vaccines and preclinical diagnostic tests. Due to its similar histological and molecular pathogenesis to that of human lung cancer, OPA is considered an ideal large animal model of human lung adenocarcinoma.

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1 Introduction

An infectious and spontaneous lung tumor illness known as ovine pulmonary adenocarcinoma (OPA), which affects sheep and infrequently goats, is caused by Jaagsiekte sheep retrovirus (JSRV), which is a member of the *Retroviridae* family (Griffiths et al. 2010; Toma et al. 2020). The disease has similar histomorphological features and tumorigenic pathways to human lung adenocarcinomas, making it a vital prototype for understanding and studying human pulmonary cancers (Youssef et al. 2015; Gray et al. 2019a*)*. JSRV is the only known virus to cause spontaneous lung tumors by inducing the expression of the JSRV envelope protein (Env) through the binding of major receptor Hyl2 and the activation of the phosphatidylinositol 3-kinase/Akt-signalling pathway. This makes JSRV unique among other retroviruses (Palmarini and Fan 2001). Due to the identical envelope glycoproteins, JSRV and Enzootic Nasal Tumor Virus (ENTV) of sheep and goat nasal adenocarcinomas can cause cell transformation and malignancy (Monot et al. 2015). The occurrence of co-morbidities has been suggested by the frequent reports of JSRV-induced OPA in sheep flocks together with MVV and CAEV in recent years (Rosato et al. 2023). In a similar study in India, MVV, JSRV, and mycoplasma co-infection were reported in sheep and goats (Valecha et al. 2023).

OPA, also known as driving sickness, pulmonary adenomatosis of sheep, or sheep pulmonary adenocarcinoma (SPA), was initially identified as Jaagsiekte in South Africa during the 1800s. The name is taken from the Afrikaans word for "chasing sickness." (Jaagsiekte) (York and Querat 2003). In 1930, an outbreak of OPA occurred in sheep flocks in Iceland, and the disease was successfully eradicated by mass slaughter and culling of affected animals during the 1950s. OPA causes roughly 70% of lung tumors in sheep and is endemic in a few countries (Peru, Scotland, the UK, South Africa, and Spain). Although this disease posed substantial economic and animal welfare complications, particularly in sheep-farming countries, countries like Australia, New Zealand, the Frankland Islands, and Iceland are free from OPA (Sharp and DeMartini 2003). Iceland successfully eradicated OPA through a rigorous slaughter policy during the 1950s (Sanna et al. 2001). JSRV is a highly host-specific virus infecting sheep, occasionally goats and mouflon (ancestor of sheep) but not to other livestock species or humans (Sanna et al. 2001; De Las Heras et al. 2003; Wootton et al. 2006).

The first genetically modified livestock, Dolly, the cloned sheep, was also diagnosed with tumorous lung growth caused by JSRV and euthanized in 2003 at Roslin Institute. JSRV-infected sheep show symptoms like anorexia, progressive dyspnea, and debilitating condition. In severe cases, sheep may die due to respiratory failure or due to the secretion of massive amounts of fluid from proliferating Type II pneumocytes in lung tumors. The JSRV incubation period varies from six months to four years. However, it is shorter when newborn lambs are infected. Although OPA infects sheep of any age group, it is most frequently reported in adult sheep of 2 and 4 years of age (Fenner et al. 2015). The lack of T-cell responses and circulating JSRV-specific antibodies in sheep with naturally occurring or experimentally produced infection makes preclinical detection of JSRV challenging. However, JSRV capsid protein-specific antibodies were detected after the administration of recombinant JSRV proteins (Griffiths et al. 2010). For the epidemiological investigation and diagnosis of OPA, OIE has recommended PCR and RT-PCR methods targeting the LTR region of JSRV, particularly for the samples from lungs, mediastinal lymph nodes, bronchoalveolar lavage fluid, and PBMCs.

This review briefly discussed OPA and its causative agent, pathology and pathogenesis, epidemiology, and control aspects. We also gave brief insights into different diagnostic methods employed for the early detection of JSRV in Sheep. This will help in understanding JSRV and future strategies to be implemented for disease-free status in India.

2 Causative agent and genome organization

The mature virions are enveloped with glycoprotein spikes on the surface. The size of the virus ranges from 80-100 nm in diameter and has a three-layered structure. The genome has helical symmetry enclosed in an icosahedral capsid with a nucleoprotein complex (Figure 1). These glycoproteins (peplomers) have surface and transmembrane domains (SU and TM) vital in virus attachment to the host cell.

The retroviral genome is diploid with two RNA copies, linear positive-sense, single-stranded RNA. The four main genes that comprise the roughly 7.5 kb long JSRV genome are gag, pro, pol, and env. These genes encode several proteins with varied roles in viral replication (Figure 2; Table 1). The U3 nucleotide sequence of the LTR region varies between exogenous JSRV, endogenous retrovirus of sheep (enJSRV), and ovine enzootic nasal tumor virus (ENTV). Additionally, there are two types of exogenous retroviruses based on the U3 sequence and the presence of ScaI restriction sites in the viral genome: type I exJSRVs (isolates from South Africa and Kenya) and type II exJSRVs (isolates from Wyoming, USA and UK) (Cousens et al. 2009; Griffiths et al. 2010).

3 Epidemiology of OPA

3.1 Disease Transmission and Host Susceptibility

OPA is mainly spread via aerosols or droplets, according to epidemiological research. Feeding the colostrum and milk of OPA-infected sheep is another way the infection spreads spontaneously.

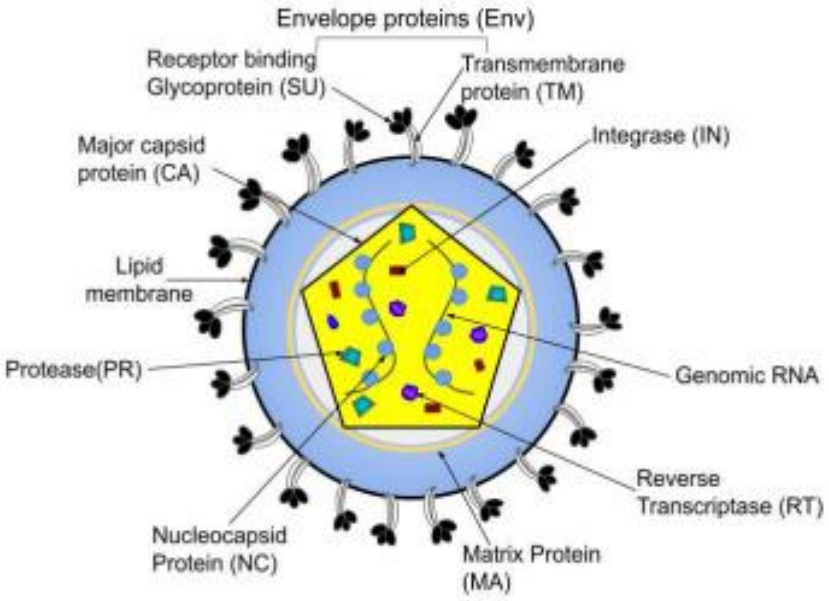

Figure 1 Structure of a mature JSRV.

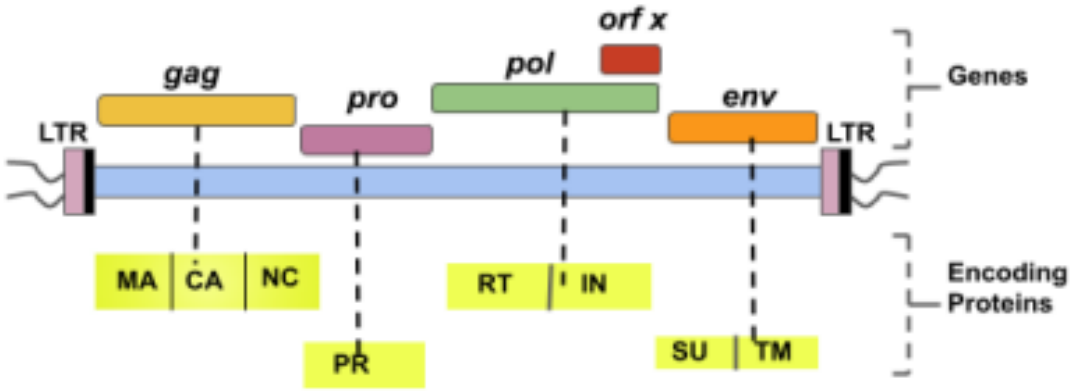

Figure 2 JSRV genome structure.

When a mother's milk is contaminated, JSRV proviral DNA can infect newborn lambs by crossing their gut epithelial barrier and infecting macrophages and somatic cells that have already experienced OPA (Voigt et al. 2007). The JSRV provirus was also found in the blood of lambs fed milk and colostrum from ewes infected with the virus (Grego et al. 2008). In spontaneous infections, the incubation time of OPA can take months or even years. However, under experimentally induced infection, particularly in lambs, it is as short as 2-6 weeks (De Las Heras et al. 2003). Sheep 2-3 years of age are more susceptible than older animals 4– 7 years. However, OPA-induced lung tumors were diagnosed in sheep up to 11 years of age at slaughter with no apparent clinical disease. Breeding flocks are more prone to disease persistence at the farm level, and few breeds are highly susceptible to OPA; for example, OPA afflicted 10% of Adalbol sheep, while the Gottorp breed in Iceland demonstrated greater susceptibility, with some farms losing nearly 90% of their Sheep (Palmarini et al.1996). Based on earlier reports, during the winter season, there are more occurrences of disease events (Griffiths et al. 2010).

In European countries, the classical form is common, and more than 50% of animals in affected flocks die due to respiratory failure, causing considerable economic losses (Ortega et al. 2023). The atypical type of OPA is less common than the classical version and less contagious. It has been mostly described in Spain, Peru, Iran, and India (Garcia-Goti et al. 2000; Azizi et al. 2014; Mishra et al. 2021). In endemic countries, the mortality rate ranges between 1-5%. However, it may reach up to 50% in case of outbreaks in newly infected flocks (Griffiths et al. 2010; Toma et al. 2020). As OPA is not included in a list of notifiable diseases that require reporting, there are few epidemiological investigations of the disease. Hence, only a few countries have data on disease

outbreaks, prevalence, diagnostic and control aspects based on clinical, pathological, and molecular diagnosis. Recently, more attempts have been made to develop and employ sensitive and rapid preclinical tests based on genomic detection to diagnose OPA (Ortin et al. 2019).

A meta-analysis using a meta package in R-Software was carried out based on a systematic review of research conducted on OPA for the past 30 years (1988-2022). The prevalence of OPA was estimated from 1% (1-2%) with the common effect model and 14% (5-27%) with the random effects model (CI- 95%, p<0.01) (Table 2).

Table 1 JSRV genes, along with encoded proteins and their functions

Gene	Size	Proteins encoded	Functions
gag	1838 bp (263..2101)	Nucleocapsid (NC), matrix (MA), and capsid (CA)	Encapsulation of viral RNA and creation of viral core
pol	2333 bp (3108..5441)	Reverse transcriptase (RT) and integrase (IN)	viral life cycle
pro	869 bp (1993..2862)	protease	Assembly of virions and budding
Env	1847 bp (5350..7197)	surface (SU) and transmembrane (TM)	Virus-host receptor attachment to HlyA on the host cell membrane
orf-x	500 bp (4606..5106)	nonfunctional ORF X protein	Unknown function

Information is generated based on the Griffiths et al. (2010), Armezzani et al. (2014), Fenner et al. (2015)

Table 2 The epidemiological studies of JSRV/OPA in sheep across the world

STUDY	% Positivity	Total samples	Country
Sarkar et al. (1988)	3	1872	India
Garcia-Goti et al. (2000)	16	16	Spain
Sanna et al. (2001)	4	4	Italy
Gonzalez et al. (2001)	10	10	Spain
Morozov et al. (2004)	19	64	Africa
Uzal et al. (2004)	8	40	Argentina
De las Heras et al. (2005)	31	104	Scotland
Maeda et al. (2011)	0	40	Japan
Sayyari and Mohamadian (2012)	4	3985	Iran
Amini and Mostafa (2013)	132	5200	Iran
Azizi et al. (2014)	9	1000	Iran
Kumar et al. (2014a)	44	903	India
Cousens et al. (2015)	31	3385	UK
Sonawane et al. (2016)	6	75	India
Oda and Youssef (2011)	7	550	Egypt
Bahari et al. (2016)	26	99	Iran
Jassim et al. (2017)	10	180	Iraq
Mishra et al. (2018)	23	800	India
Singh et al. (2018)	31	1350	India
Lee et al. (2019)	30	1911	Ireland
Mansour et al. (2019)	25	250	Iraq
Samatha et al. (2019)	22	150	India
Al-Husseiny et al. (2020)	22	50	Iraq
Toma et al. (2020)	34	2693	Romania
Shi et al. (2021)	0	1372	China
Abd-Abass and Khudhair (2022)	21	195	Iraq

3.2 JSRV Prevalence in Indian Sheep

In India, for the first time, Damodaran (1960) reported OPA in Karnataka in a flock of sheep that had chronic pneumonia, and the diagnosis was made as Onderstepoort; the illness resembled Jaagsiekte in South Africa. From 1972 to 1977, the prevalence of OPA was up to 2.46% in Sheep of Andhra Pradesh (Devi et al. 2014). Based on the histopathological evaluation, pneumonic lesions were found in 204 out of 1872 sick sheep lungs in West Bengal, and three cases among them were identified as pulmonary adenomatosis (Sharp et al.1983). Similarly, out of 203 ovine lungs with various diseases, 21.68% of cases were detected as pulmonary adenocarcinoma (Kumar et al. 2014b). Further, based on the histological diagnosis, a 4.87% prevalence of OPA (44/903) in sheep was documented in the Southern parts of India (Kumar et al. 2014b). Sonawane et al. (2016) reported OPA during the necropsy examination of 75 sheep that had died naturally, and six cases had typical adenocarcinoma-like lung lesions positive for JSRV by PCR. In another study, 1350 lungs were examined in the Indian states of Delhi, Andhra Pradesh, and Uttar Pradesh, with 31 (2.29%) animals testing positive for JSRV (sheep-3.49%, 31/888; goats -0.00%, 0/462). Capsid antigen of JSRV was demonstrated by employing immunohistochemistry in alveolar macrophages, type II pneumocytes, lymphocytes, plasma cells, and in a few normal bronchiolar epithelial cells in lung tumours in sheep. PCR tests targeting the U3 and gag region of JSRV revealed the presence of JSRV DNA in sheep lung tumors (Singh et al. 2018). In a similar study, 150 sheep lungs suspected of having OPA in Andhra Pradesh were screened by pathological lesions and U3-hnPCR, and 22 samples (14.7%) were positive for JSRV (Samatha et al. 2019).

4 JSRV viral pathogenesis

The JSRV virus replicates particularly in type II pneumocytes and bronchiolar Clara cells of the lungs (Toma et al. 2020). These cells express a specific receptor called glycosyl phosphatidyl inositol-anchored hyaluronidase-2 (Hyal2) and have been identified as the major receptor for JSRV. Major determinants of JSRV expression in hosts and cell tropism include the LTR and the JSRV envelope regions. The surface glycoprotein (SU) of JSRV attaches to the Hyal2 receptor and enters the cell via the endocytic pathway. The virion is penetrated by Env-Hyal2, which then causes the reverse transcriptase (RT) to transcribe the ssRNA and create a dsDNA. When the virus enters the nucleus during mitosis, viral integrase finds numerous places in the cell's nucleus to incorporate the viral DNA. A provirus enters the host genome and emerges from the cell as an immature virion. During this process, due to replication and infection of JSRV in lung epithelial cells, a large quantity of lung surfactant is produced from tumor cells and discharged from nostrils in sheep.

5 Clinical Pathology of OPA

An increased number of deaths in sheep flocks due to chronic pneumonia not responding to antibiotics can be a viable sign for suspecting OPA. When tumor progression and lesions are fully established, after a prolonged incubation period, affected sheep exhibit clinical symptoms such as debility, weight loss, and dyspnea. Later, as time passed, symptoms progressed to abdominal breathing, orthopneic posture, dilated nostrils, and open-mouth breathing (York and Querat 2003). The disease progression is usually acute in lambs, which frequently die within a few days,

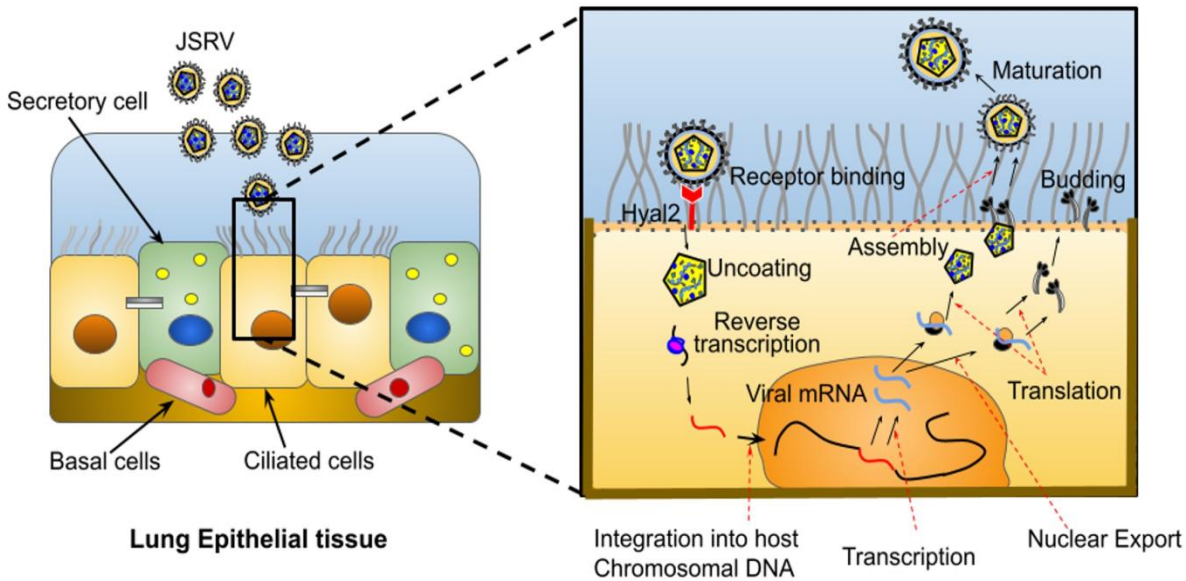

Figure 3 JSRV viral replication and pathogenesis in lungs

whereas in adult animals, it is slowly progressive, with clinical symptoms appearing over weeks or months before the animal dies (De las Heras et al. 2005). The clinical indications of the affected sheep are typical and include clear, foamy exudate coming from the nasal passages. The infected animal can produce frothy exudate up to 300-500 ml per day and can be collected by lifting the rear legs and lowering the head of the sheep. This is one of the practical diagnostic tests in OPA called the Wheelbarrow test (Cousens et al. 2009). When inoculated to healthy sheep, this fluid contains a large amount of JSRV viral particles, which induces disease conditions. Despite the characteristic clinical signs in affected animals, in some cases, the lung fluid is absent; therefore, diagnosis is based on gross and histopathological lesions and the detection of the JSRV genome by PCR in lung lesions (Griffiths et al. 2010).

6 Gross and Histopathology of OPA

Post-mortem examination of the advanced stage of the naturally infected animal carcass appears to be a debilitating type with frothy exudate-filled trachea and nasal passages. When the thoracic cavity opens, there will be asymmetrically enlarged lung lobes, which fail to collapse and look dark, edematous, and bulky in weight. Upon palpation of the lungs, consolidated nodules/tumorous mass in cranioventral surfaces and other lobes will be distinctly visible. Incision of affected lobes shows a clear demarcation between the tumor and normal pink lung parenchyma. The consolidated nodule shows a grey, solid, granular surface of the tumor with frothy exudate oozing out of the lesions (Griffiths et al. 2010). The slashed surface of the tumor is moist, causing the bronchioles in the afflicted regions to discharge foamy exudates. This fluid also accumulates in the upper trachea and discharges from the nostrils. All these lesions represent a classical type of OPA (Garcia-Goti et al. 2000). A single or more white, spherical, tiny nodule of 3-5cm, buried in the diaphragmatic lobe may represent the appearance of a lung tumor in some OPA cases. These nodules seem hard, gritty, and stellate upon incision; the surface is dry cut, and there is no frothy discharge from the bronchi. This form is atypical OPA (De las Heras et al. 1992). Enlargement and edema of mediastinal lymph nodes with or without metastatic tumor lesions are consistent with both forms of OPA (Garcia-Goti et al. 2000).

Based on histomorphological lung lesions, OPA were classified into two distinct pathological forms: classical and atypical (Garcia-Goti et al. 2000; De Las Heras et al. 2003; Mishra et al. 2021). In classical form, the affected lung parenchyma shows mixed adenocarcinoma with papillary and acinar growth patterns with alveolar epithelial cells converted to cuboidal or columnar cells. There will be a clear demarcation between neoplastic foci and unaffected lung parenchyma in classical OPA rather than atypical form. A pathognomonic lesion of classical OPA is the localized infiltration of macrophages around a neoplastic foci that resembles acinar and papillary patterns and arises from the alveolar and bronchiolar epithelia (De Las Heras et al. 2003). The primary proliferating tumor cell type, representing over 80% of cell populations, is the Type II alveolar epithelial cells (Pneumocytes). This is followed by Clara cells (8–10%) and other undifferentiated cells (9–10%) (Platt et al. 2002). In malignant tumors, pleomorphic cells with high mitotic index form into solid masses of necrosis foci. The infiltration of macrophages and lymphocytes in the fibrovascular connective tissue scaffold around the tumor can also

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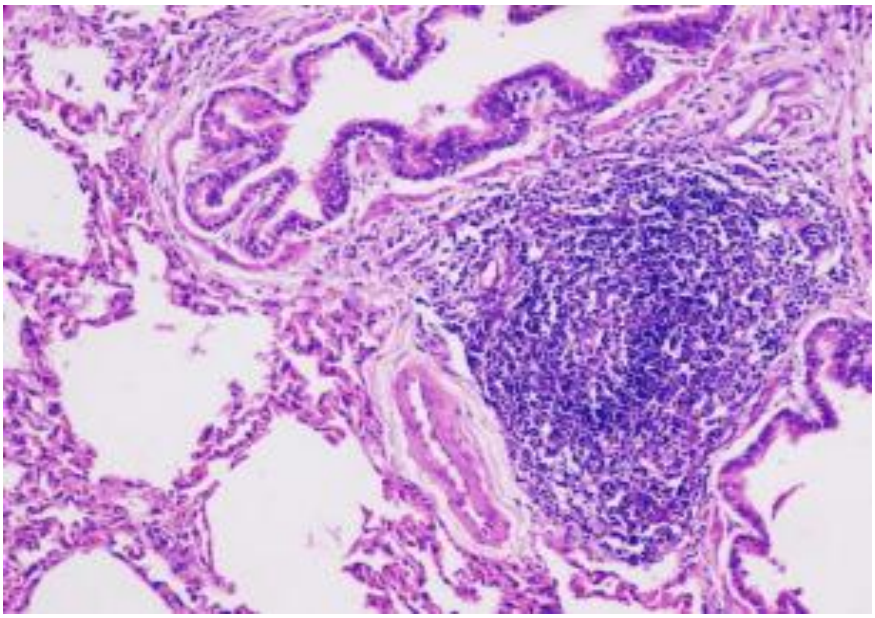

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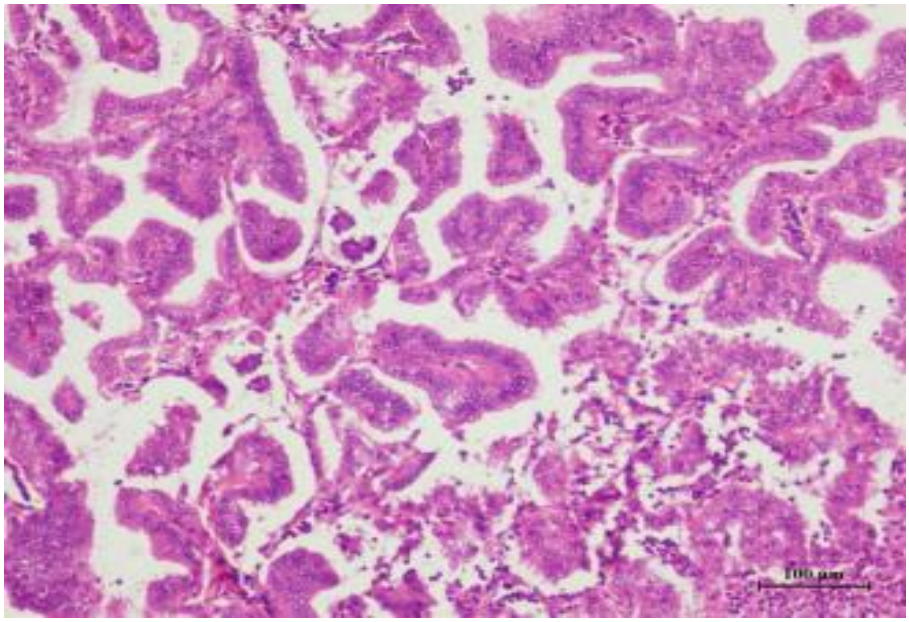

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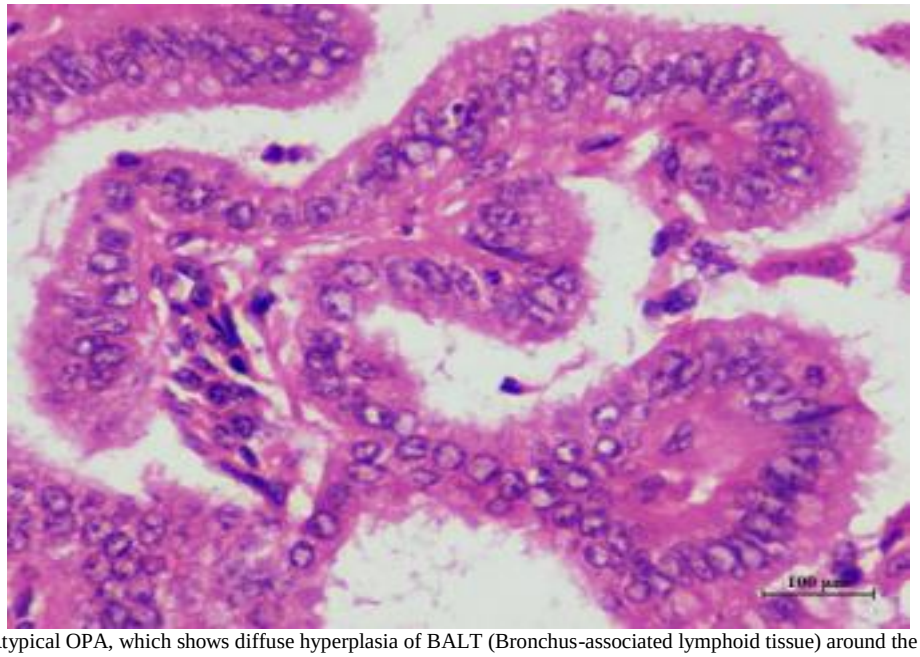

Figure 4A - Atypical OPA, which shows diffuse hyperplasia of BALT (Bronchus-associated lymphoid tissue) around the bronchiole in OPA-affected sheep (H&E, X 100); 4B - Classical OPA in which Lung parenchyma showing extensive hyperplasia and acinar type projections of adenocarcinomatous lesions (H&E, x100); 4C. Acinar and papillary growth patterns of alveolar epithelial cells converted to cuboidal or columnar cells (H&E. x400)

be observed. Mediastinal lymph nodes show metastatic neoplastic cells in acinar or papillary forms similar to lung lesions. However, there is a lower frequency of metastasis in the atypical form of OPA (De Las Heras et al. 2003). As with classical OPA, the histological alterations in atypical OPA are strikingly similar. In the abnormal variant, the lungs get invaded with connective tissue fibers, lymphocytes, and plasma cells. There is no clear-cut demarcation of tumor foci with surrounding lung parenchyma in an atypical form. The excessive proliferation of Bronchus-associated lymphoid tissue (BALT) can be observed around the neoplastic areas (Figure 4). Due to the limited tumor progression and the absence of lung fluid, the atypical form of OPA appears only as a subclinical form and is usually diagnosed in slaughterhouse specimens. Few studies also documented the peribronchial, peribronchiolar, and perivascular infiltration of mononuclear cells in atypical OPA (Sonawane et al. 2016).

7 Diagnosis of OPA/JSRV

7.1 Molecular Diagnosis of OPA by PCR and Sequencing

The U3 region in the long terminal region of JSRV is the target for single-step and hemi-nested PCRs, widely employed for epidemiological research in sheep flocks (Palmarini et al.1999). LTR-PCR is an instrumental test to detect JSRV proviral DNA specifically in peripheral blood mononuclear cells (PBMCs) in both clinical and experimentally induced infection in Sheep (De las Heras et al. 2005; Lewis et al. 2011), bronchoalveolar lavage fluid in sheep (Voigt et al. 2007), lung tumor lesions, lymphoid tissues (Singh et al. 2018; De las Heras et al. 2021), milk, and colostrum (Quintas et al. 2021). PCR testing in bronchoalveolar lavage proved more sensitive than the blood PCR (Cousens et al. 2009).

In sheep and goats, the endogenous Jaagsiekte Sheep Retrovirus (enJSRV) is present in at least 15–20 copies of the genome. Exogenous (exJSRV) and endogenous (enJSRV) retroviruses can be distinguished from one another by the presence of the ScaI restriction site in the Gag gene and the "LHMKYXXM" motif in the envelope protein. However, the ScaI restriction site is found in oncogenic JSRVs but absent in endogenous retroviruses in sheep, and the "LHMKYXXM" pattern is found in all exJSRVs but not in enJSRVs (Shi et al. 2021). The whole genome sequencing analysis of JSRV from India revealed a close relationship with China and USA isolates (Mishra et al. 2018). The hemi-nested PCR and partial gene sequencing of the U3 region of JSRV have shown 96–100% homology to the UK strain and 88–93% homology to the South African strain in South India.

7.2 Immunohistochemistry of JSRV in OPA lungs

A crucial diagnostic tool for OPA is detecting sheep lung cells exhibiting JSRV antigens. JSRV has been widely demonstrated in alveolar Type II pneumocytes, bronchiolar epithelial cells, macrophages, lymphocytes, and cytoplasm using monoclonal and polyclonal antibodies produced in rabbits against JSRV capsid proteins and envelope proteins (Murgia et al. 2011; De las Heras et al. 2014; Mishra et al. 2021). In some studies, SP-A, SP-B and SP-C (Surfactant Protein), Proliferating cell nuclear antigen (PCNA) and clara cell secretory protein (CCSP) have also been shown to be present in Type II Pneumocytes in the lungs, mediastinal lymph nodes, heart, kidneys, liver, diaphragm, gut, skeletal muscle, spleen, skin, and adrenal glands (Leroux et al. 2007; Beytut et al. 2009). Mishra et al. (2018) demonstrated tumor biomarkers such as PCNA, MYC, MMP2, FOXO3a, MHC I and MHC II in alveolar pneumocytes, bronchiolar epithelium and mononuclear cells lungs in addition to JSRV-capsid antigen in natural infection in sheep. The details of characteristic histological features, target proteins for immunohistochemistry and target genes for genomic detection of JSRV in lungs and other organs are given in Table 3.

Table 3 Target organs, histopathological changes, target proteins for immunohistochemistry, and target genes for PCR detection of JSRV in Sheep

Study	Sample/organs	Histopathological changes	Target proteins (IHC)	Target Gene (PCR/hnPCR/ RT-PCR)
Palmarini et al. (1999)	Lungs, PBLs	Papillary adenocarcinoma with proliferations of cuboidal cells, interstitial myxoid or fibrotic nodules	JSRV- CA protein	U3 -LTR JSRV
Garcia-Goti et al. (2000)	lungs, MLN, mammary gland, mammary LN, spleen, brain and kidney	Infiltration of lymphocytes and macrophages in connective tissue fibres and hyperplasia of BALT	JSRV-CA protein in neoplastic alveolar cells	U3 LTR (U3-hn-PCR)
Gonzalez et al. (2001)	Lungs, PBLs, kidney, spleen, mammary gland, mammary lymph node, MLN	Classical and atypical OPA lesions in the lungs	-	U3 region of exogenous JSRV (U3-hn-PCR)
Salvatori et al. (2004)	PBLs, lungs, MLNs, Lung fluid	The papillary and acinar adenocarcinoma of the alveoli and hyperplasia of BALT	JSRV-CA protein and JSRV-SU in the cytoplasm of neoplastic alveolar cells	JSRV-U3 region
Uzal et al. (2004)	Lungs	Cuboidal cell proliferation and infiltration of macrophages in the lungs	JSRV-CA protein	U3 LTR-JSRV
Caporale et al. (2006)	Lungs	Papillary and acinar nodules	JSRV-Env	JSRV-Env gene
Leroux et al. (2007)	Lungs	Bronchioloalveolar, acinar and papillary form of adenocarcinoma in lungs	SP-A (Surfactant Protein A)	-

Study	Sample/organs	Histopathological changes	Target proteins (IHC)	Target Gene (PCR/hnPCR/ RT-PCR)
Beytut et al. (2009)	Lungs and MLNs and bronchial LNs	Adenocarcinoma-type lesions in lungs	CA, Surfactant protein-A (SP-A), SP-B and SP-C, Clara cell secretory protein, proliferating cell nuclear antigen	
Griffiths et al. (2010)	Lungs	Tumors of the alveolar and bronchiolar epithelium into the acinar and papillary type and nonencapsulated tumors	JSRV Env proteins in type II pneumocytes using an anti-Env (SU) monoclonal antibody.	U3 LTR
Minguijón et al. (2013)	Lungs, MLNs, heart, Kidneys, liver, diaphragm, intestine, skeletal muscle, spleen, skin and adrenal glands	Metastatic nonencapsulated acinar and papillary type of adenocarcinoma with infiltration of macrophages, neutrophils, lymphocytes	JSRV env and surfactant protein C (SPC) in all tissues	JSRV-LTR
Azizi et al. (2014)	Lungs	Atypical form OPA, lymphoid infiltration	-	LTR
Singh et al. (2018)	Lungs	Papillary and acinar patterns of neoplastic cells in alveoli with infiltration of lymphocytes and alveolar macrophages	JSRV capsid antigen	Gag, U3 LTR
Mishra et al. (2018)	Lungs	Classical form-Proliferation of alveolar pneumocytes into papillary type with infiltration of neutrophils, macrophages Atypical form- bronchiolar hyperplasia fibroplasia, infiltration of MNCs	JSRV-CA in pneumocytes, bronchiolar epithelium, and MNCs Tumor biomarkers- PCNA, MHC I, MHC II MYC, MMP2, FOXO3a in lungs	JSRV-LTR
Samatha et al. (2019)	Lungs	Nonencapsulated alveolar papillary and acinar projections and bronchoalveolar growth pattern in the lungs	-	U3 LTR by U3-hn PCR
Mansour et al. (2019)	Blood, lungs, LNs	Glandular neoplastic cells bronchiolar hyperplasia lymphocytic necrosis	-	Env of JSRV by RT-PCR
Lee et al. (2019)	Lungs	Clear-cut demarcated areas of acinar-type adenocarcinoma supported by a fibrous stroma and infiltration of macrophages	Envelope protein of JSRV	RT-PCR of JSRV
Toma et al. (2020)	Lungs	Tubular, papillary and myxoid-type lesions in classical form. In atypical form, fibroblast proliferation and infiltration of lymphocytes and macrophages in stroma	JSRV-MA, multi-cytokeratin vimentin (Vim), alpha smooth-muscle actin (alpha-SMA), desmin, S100 protein, Ki67 and Thyroid Transcription Factor 1 (TTF-1)	JSRV-LTR
Belalmi et al. (2020)	Lungs	Papillary, acinar or glandular patterns with infiltration of lymphocytes and macrophages	JSRV-Env protein	-
Abd Abass and Khudhair (2022)	Nasal secretions, lungs, MLN	Glandular transformation and hyperplasia of alveolar cells	-	RT PCR of JSRV-Env

7.3 Imaging techniques in OPA diagnosis

In the preclinical form of OPA, imaging diagnostic techniques such as computed tomography, ultrasonography, and X-rays can be valuable techniques to confirm the disease. These techniques can identify any pulmonary lesions, including tumorous growths. Transthoracic ultrasonography may detect small nodular tumors of 1-2 cm in diameter even before the clinical form of OPA. Further, the computerized three-dimensional color CT scan is handy for studying the pathogenesis of OPA (Quintas et al. 2021).

8 JSRV-induced OPA as a model for human lung cancer

Sheep are good models for researching human diseases such as malignancies, respiratory syncytial virus infection, cystic fibrosis, chronic obstructive pulmonary disease, and asthma (Gray et al. 2019b). Retroviruses are an excellent model for investigating the causes of carcinogenesis because of their capacity to integrate into the host cell's genome and interfere with its regulatory mechanisms (Palmarini et al. 1997). Regarding histomorphological features, there are many similarities between OPA and the human lung adenocarcinoma; therefore, OPA is regarded as a crucial big animal model for comprehending the mechanisms behind retroviral oncogenesis (Mornex et al. 2003). Although JSRV was shown in human lung neoplastic epithelial cells, its significance in human carcinogenesis remains unclear (Miller et al. 2017). Type II pneumocytes are transformed by JSRV by insertional mutagenesis (Palmarini et al. 1997). The membrane receptor Hyal2 mediates the interaction between oncogenic JSRV envelope proteins and target cells. This causes cellular change, the activation of the PI3K/Akt and MAPK pathways, and telomerase activity (Leroux et al. 2007). Human lung alveolar cells were shown to contain the JSRV-receptor Hyal2. However, human adenocarcinoma did not have any JSRV DNA. There is no indication of JSRV transfer from sheep to humans. Additionally, there is proof that JSRV envelope protein may occasionally cause human cell neoplastic transformation. DNA sequences linked to JSRV were found in a small number of African American lung cancer cases. OPA is currently categorized as a mixed adenocarcinoma exhibiting acinar, papillary, and bronchioloalveolar development patterns by the World Health Organization's (WHO) revised classification system for human lung malignancies (Travis et al. 2012).

9 Prevention and control of OPA

OPA is an incurable disease, and death in clinical cases is inevitable due to the progression of lung lesions. Vaccines and therapies to cure OPA are not currently available. The stringent quarantine, cleaning, and disinfection of contaminated farms and equipment and removing diseased animals and their lambs from flocks continue to be the most effective methods for managing the disease. Control and prevention of JSRV are the same as that of the eradication programs in Maedi-visna. In breeding flocks, mainly JSRV transmission is considered through feeding of milk/colostrum and therefore, newborn lambs may be immediately separated from ewes and fed with heated colostrum or milk (Voigt et al. 2007). Eliminating JSRV-positive sheep from flocks as soon as possible, conducting routine epidemiological surveillance, and early diagnosis are all effective control methods. A combination of diagnostic techniques such as clinical, pathological, immunohistochemistry, PCR, and sequencing should be chosen so that the complete absence of the infection may be achieved in a sheep population.

Conclusion and Future Prospects

Due to intensive and migratory sheep farming practices in India, there is a considerable risk of OPA infections in sheep flocks. There is an urgent need to relook into the slow viral diseases, including OPA in sheep, for differential diagnosis of pneumonia, as OPA often goes unnoticed in farms. As no serodiagnostic tests are available due to poor immune reaction in JSRV-affected sheep, in recent years, more efforts have been focused on developing molecular tests for early detection of the disease to eradicate OPA in sheep effectively. Future control strategies should be directed to establish low-risk status for OPA in sheep herds by employing genomic methods such as RT-PCR and sequencing to detect JSRV at early stages. The only proven method to eradicate OPA from sheep was a rigorous slaughter policy involving massive culling of affected flocks. But, under Indian conditions, it is not a practically viable solution. Hence, early detection, quarantine, and good husbandry practices are to be followed to control OPA in sheep effectively.

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