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REVIEW ARTICLE

Current Status of Ovine Pulmonary Adenocarcinoma (Jaagsiekte)

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Ovine pulmonary adenocarcinoma (OPA) / Jaagsiekte (Afrikaans: Jaag: Chasing, Driving; Siekte: Sickness - because affected sheep have the appearance of having being chased and the disease is most noticeable when sheep are being herded), earlier known as ovine pulmonary adenomatosis/ovine pulmonary carcinoma. OPA, a progressive respiratory disease, principally affecting adult animals, is a transmissible contagious tumour of lung, brocnchoalveolar carcinoma, caused by exogenous beta *retrovirus* which has an oncogenic protein that develops lung tumor in sheep and rarely, of goats and moufflons OPA was first described early 19th century in South Africa. OPA was first reported from India in Tamil Nadu State in 1960[1].

With India holding 74.26 million of sheep and 148.89 million of goat (Livestock Census, 2019), contributes nearly 5% and 10% of total meat (chevon and mutton) required in the world respectively implying economic importance. Occurrence of diseases leads to heavy economic losses to meat industry, wool and milk production. In sheep and goats, the most commonly affected system the respiratory system. Pneumonia inflicts heavy mortality and therefore adversely affects the profit in sheep and goat production.

The lungs are exposed to the external environment. The feed dust, pollutant, microorganism and noxious chemicals present in the air will cause respiratory disorder and distress to the animals. Pneumonia in sheep is one of the complex syndromes with multifactorial etiology, usually precipitated by physiological factors and stress in combination with infectious agents. OPA causes respiratory distress to the animal resulting in huge economic loss worldwide. Unlike antemortem diagnosis post mortem diagnosis has significant lesion in the lung like tumor growth and accumulation of fluid in the air ways. OPA has wide range of distribution in the world. Sheep and goat importing country from infested areas are largely at risk. Since the virus has a wide range of transmission it takes little time to infect the whole flock[2]. Hence, OPA is an important economic and animal welfare problem[3].

Aetiology

OPA is caused by a beta-retrovirus that cannot yet be cultured *in vitro,* but the virus has been cloned[4] and sequenced. The term Jaagsiekte sheep retrovirus (JSRV) is used in referring to this virus[5]. OPA caused by a betaretrovirus, JSRV, is distinct from the non-oncogenic ovine lentiviruses.

Exogenous JSRV (ExJSRV), responsible for lung cancer in sheep[6], is a single-stranded, positive sense RNA virus in the genus Beta-retrovirus, family Retroviridae, having approximately 7,460 nucleotides in length and as with other retroviruses four genes encode for essential viral proteins. These are i. gag encoding the structural internal virion proteins comprising matrix (MA), capsid (CA) and nucleocapsid (NC); ii. pro for an aspartic protease (PR); iii. Pol for the RT and integrase (IN) enzymes; and env the surface (SU) and transmembrane (TM) envelope glycoproteins. The viral proteins synthesized initially as large precursors and are later processed into the mature proteins by proteolytic cleavage. The genome also contains several cis-acting elements required for expression of the viral proteins[7-10].

JSRV also has a further open reading frame (orf-x) which overlaps the pol gene with undefined function. orf-x has a codon usage markedly different from that of other genes within JSRV and the predicted amino-acid sequence is extremely hydrophobic. Expression of the putative orf-x protein has not been demonstrated, although there appears to be a specific orf-x mRNA transcript in JSRV-transfected cells and in lung tumours[11]. The sequence is highly conserved in JSRV isolates collected from the UK, Italy, Spain, South Africa and the USA, which is consistent with the notion that orf-x encodes an authentic viral protein[12,13]. Sequence similarity with the adenosine 3A receptor that may give a clue to its function[12], but as most of the 'homologous' regions are in hydrophobic domains, the significance of this similarity is uncertain. orf-x is not essential for cellular transformation *in vitro*[14] or for oncogenesis *in vivo*[3,15-17].

ERVs (Endogenous retroviruses) are important in the OPA as the sheep genome contains many proviruses that are closely related to JSRV[10,11,18-20]. Twenty-seven endogenous JSRV-related proviruses (denoted enJSRV) have been identified. enJSRV was expressed in the ovine uterus[11].

Completely sequenced three strains of JSRV, including a South African isolate, denoted as JSRV-S[10,21]. The etiological agent of OPA is exogenous JSRV (exJSRV). A specific U3 long terminal repeat (LTR) sequence of exJSRV was detected in lungs from affected animals. U3 sequence and restriction profiles of the virus suggest that there are two types of exJSRV sequences: type I (Kenyan and South African) and type II (Wyoming, USA and UK isolates). exJSRV having a specific tropism for the differentiated epithelial cells, type II pneumocytes and non-ciliated bronchiolar Clara cells of the lung, is the only virus known to cause pulmonary adenocarcinoma in naturally infected animals[22].

The env protein induces oncogenic transformation due its interaction with cell growth pathways such as Hya12-RON, PI3K/Akt and Ras-MEK-ERK[6]. Though enJSRV genes were similar to exJSRV, but not associated with the development of tumors[3,8,9]. Despite this high homology, genetic differences are evident in the viral promoter region (U3) that allowed the development of the exJSRV-specific U3PCR. The genetic similarity is also smaller in the intracytoplasmic tail of the transmembrane protein encoded by the env gene[23] the latter being related to the mechanism of oncogenesis induced by env[11,23].

Epidemiology

Incidence: OPA has been reported in many sheep rearing countries of the world (Fig. 1), Europe, Africa, Asia and America, except in Australia, New Zealand and Falkland Islands. OPA was eradicated in Iceland in the 1950s, following a rigorous slaughter policy[3]. OPA was described in UK in 1888 by Dykes and McFadyean in the first volume of the Journal of Comparative Pathology and Therapeutics (1: 139-146), without knowing true nature of the disease, as lung disease in sheep, caused by the *Strongylus rufescens*. OPA reported in sheep or goats since 2015: UK[24], India[25-29], Ireland[30], Iraq[31,32], Algeria[33], Romania[22] and Mexico[34] using pathological and molecular studies.

Prevalence, morbidity and mortality: The prevalence of OPA appears to vary among the countries but, Peru, Scotland, South Africa, Spain are endemic[35]. In India, Jaagsiekte was first reported in Bikaneri breed of sheep. Two rams were aged one year four months each and two ewes, one aged two years eleven months and other ewe was seven years old[1]. Four Jaagsiekte cases were reported out of 1610 sheep, aged between 2-3 years, by examining the lung lesions obtained from slaughter house[36]. It was reported that the incidence in sheep was 0.060% (117/19342) and in goat was 0.13% (34/25467) from slaughter house samples[37]. Further, it was reported as the occurrence 0.57% (1/174) of Jaagsiekte in sheep and 0.54% (2/367) in goat[38]. Then, OPA has been reported in various parts of India[39-42].

The morbidity and mortality rates vary, but reported high in the first years after the emergence of the disease in a flock can reach up to 50% (Iceland in the 1930s), but as the disease becomes endemic the mortality rate falls to around 1-5%[3,43]. But, many cases of OPA can remain

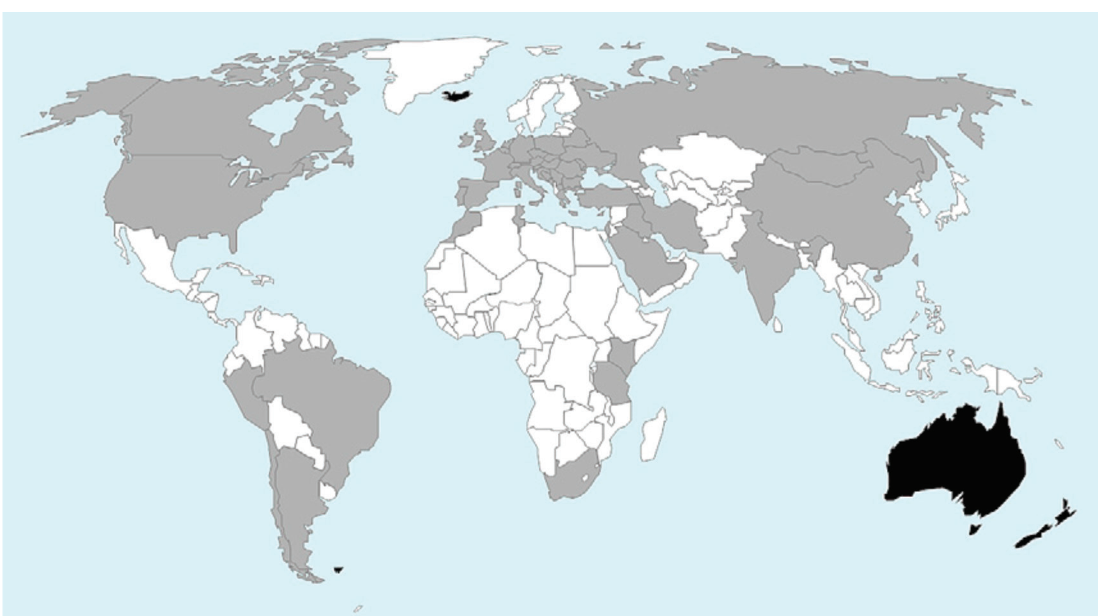


Fig. 1. Distribution of OPA. OPA present is shown in grey; Absent in black; Unknown in white.

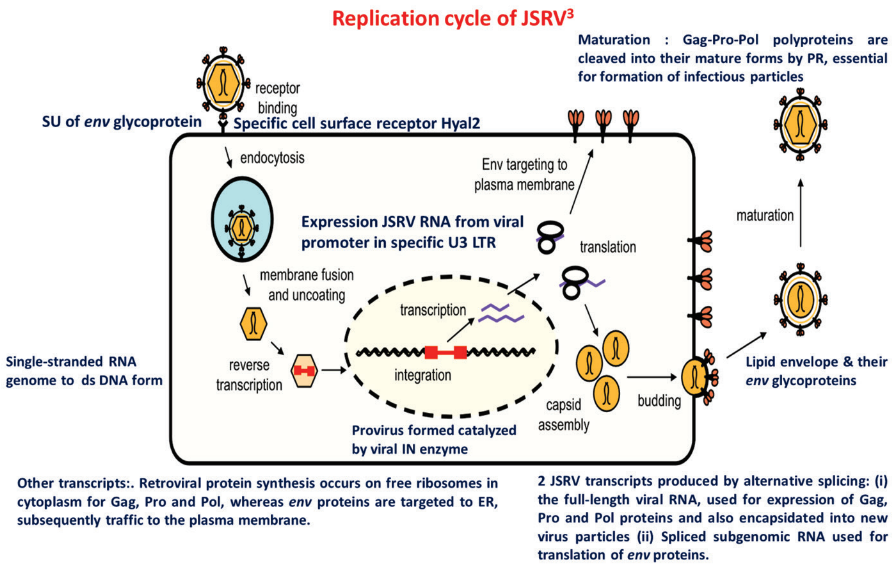


Fig. 2. Replication cycle of JSRV.

subclinical. Studies in flocks with endemic OPA in Scotland and Spain revealed that approximately 30% of the sheep had histologically confirmed lesions while the annual losses attributable to OPA were quite lower[35] and that 40% of animals with OPA lesions at necropsy were not clinically affected[3,9,23]. In our earlier slaughterhouse study conducted, because of limited sample size (n=95), we found 31.20% incidence of OPA[41]. In a later extensor study conducted with large sample size, out of 110000 sheep, 5000 goats studied, 247 affected lung tissues collected showed a prevalence of Jaagsiekte in 9.77% (21/215) in sheep and 6.25% (2/32) in goats[44].

In Europe, the classical OPA has been reported in several countries, such as Ireland, UK, Scotland, Italy, Germany and Spain. The incidence of OPA is usually low, but in some locations it reaches up to 30% and mortality is >50%, causing important economic losses[45]. It can cause about 80% loss of flock for first exposure and 20% for endemic area[2]. Species: OPA mainly affects domesticated sheep *(Ovis aries)*. Sardinian moufflon (*Ovis musimon*, a species of wild sheep) can also become ill, and a few cases were reported in domesticated goat[5,28,44]. Breed and sex: Animals of either gender are affected. Although, there are no evidences that sex or breed can affect sensitivity, many sheep breeds around the world are susceptible to OPA. While some breeds or families seem more resistant to OPA[22,43], some breeds are more susceptible. In Iceland, Gottorp breed showed greater susceptibility, with some producers losing about 90% of the sheep, whereas only up to 10% of Adalbol sheep present in those farms presented clinical signs[23]. Age: In natural conditions, clinical disease is more often detected in sheep aged 1-4 years but age ranged from 2 months to 11 years, or even more, indicating a long incubation period. However, it seems shorter, 6-8 months, in previously unaffected flock. However, experimental data showed that sheep of all ages are susceptible and newborn lambs can develop OPA in 3-4 weeks. Season: Winter seems registering more cases[3,43].

Transmission

Portal of entry and source of virus: Epidemiological study showed that the OPA retrovirus is transmitted by droplets from respiratory fluid via aerosols or droplets, milk, and colostrum tumors, lung fluids, peripheral blood leucocytes and lymphoid organs; as a result, the agent has high chance of contaminating the environment from excess respiratory discharge. Horizontal transmission is demonstrated among sheep of all ages, but neonates appear particularly susceptible to the infection. There was no evidence that *in utero* vertical transmission is significant in the epidemiology of this disease; however, JSRV might be spread in milk or colostrum[46]. JSRV reaches Peyer's patches and mesenteric lymph nodes of lambs nursed by infected mothers[47] and further studies showed the presence of JSRV in milk macrophages but not in milk lymphocytes or mammary gland epithelia of naturally infected sheep[48]. Before tumors develop, the virus is detected in lymphoreticular cells. JSRV found in macrophages of infected mother's milk cross to cross the gut barrier of newborn lambs and spread from mesenteric lymph nodes and Peyer's Patch. The transplacental pathway does not appear to have any epidemiological significance[23,46-48].

The virus has long incubation period that varies according to age of the host. The period between infection and the appearance of clinical signs may be several months or years and many JSRV infected sheep do not exhibit clinical signs at all during their lifespan. This allows the spread of OPA into new flocks through contact with infected but apparently normal animals. This is due to the absence of an immunological response to JSRV in infected animals, which has hindered the development of serological diagnostic tests and vaccines. JSRV does not survive for long periods in the environment[49]. However, JSRV was reportedly present at high concentration in lung fluid produced by OPA affected sheep and can survive for several weeks at ambient temperatures[50]. In goats, few reports of natural infection, mostly unconvincing, possibly because of misdiagnosis[51]. However, in this species, the OPA acquires significant differences and virus replication does not take place within sensitive lung cells[23,52,53].

Incubation period

The incubation period of natural infections is prolonged, likely for several months to several years, 6 months to 3 years. The incubation period can be shorter in non-endemic herds (6-8 months) and in experimentally infected young lambs[11]. It appears to be age-dependent, and is longer in older sheep. The presence of tumours was detected in animals from 2 months to 11 years of age, although cases of natural disease are rarely observed in sheep less than 9 months old[43,50,54]. In herds where the disease is endemic, the mortality rate is generally low (1-5%), but can reach higher values (>30%) in newly infected herds[3,50]. In an experimental infection 1-week-old lambs developed clinical signs in 70 to 74 days, 1-month-old lambs in 92 to 209 days and 1 to 6-month-old lambs in approximately 160 days or longer. Experimentally infected adult sheep become ill in several months to years[55].

Pathogenesis

JSRV is transmitted primarily by the respiratory route and once inhaled the virus may infect a variety of cells including lymphocytes and myeloid cells[56], in addition to the epithelial cells of the lung. The functional consequences of infection of non-epithelial cells are unclear as very little virus gene expression appears to occur there. Within type II pneumocytes and Clara cells (or their precursors), expression of the JSRV env protein activates signaling cascades that promote cellular proliferation and drive malignant transformation of the cells. Initially, the tumour cells grow along the alveolar walls in a pattern reminiscent of human BAC, but subsequently become more invasive and metastasize to the local lymph nodes. In rare cases, extrathoracic metastases may occur. Larger tumours may be necrotic and fibromatous at their centre. For the weeks to months while the tumour grows, there may be little outward indication and most infected animals show no clinical signs. As the tumour grows, fluid production in the lung increases and this is likely to promote virus spread to other sheep. Only when the tumour reaches a size large enough to compromise lung function, or when fluid production reaches a noticeable level, do clinical signs appear. Critically, the majority of infected animals in endemic areas never show outward signs of infection, but they may be shedding virus, thus promoting inadvertent introduction of the disease into previously unaffected flocks and new geographical areas. Virus life cycle[3] is given in Fig. 2.

Clinical signs

The first indicator of OPA in a flock is often an increased number of deaths in adult sheep from pneumonia that does not respond to antibiotic treatment. Affected animals are afebrile, febrile in secondary bacterial infections, struggle to breathe, especially when exercised[57] and may become very thin despite having a normal/good appetite. Initially, affected animals are less active and have the appearance to have been chased and later dyspnoea and moist respiratory sounds, caused by the accumulation of fluid in the respiratory airways. A pathognomonic sign of OPA is the production of copious amounts of fluid in the lung that is frothy, clear, milky or at times pinkish and drains from the sheep's nostrils when it lowers its head. In the final stages of the disease variable amounts of frothy seromucous pulmonary fluid, from 10 to 40 mL up to 400 mL, are discharged from the nostrils when the sheep head is lowered or the rear limbs are raised (the 'wheelbarrow' test-raising/lifting the hind legs to lower the head of the animal-can be used to check for excess fluid in the lungs), although 40 mL per day is more common[50]. Once the clinical signs are seen the sheep usually lives for only a few more days and may die abruptly following exercise or exposure to cold. The severity of the signs reflects the extent of tumour development in the lung. Despite the unique clinical signs in some affected animals, in many cases no lung fluid is seen[2,3,9,58].

OPA occurs in domestic and wild sheep species and affects no other livestock except goats, in which natural cases have been described only subclinically in affected animals[3]. As the OPA has a long incubation period, clinical disease is encountered most commonly in sheep over 2 years of age, with a peak occurrence at the age of 3-4 years. In exceptional cases, the disease occurs in animals as young as 2-3 months of age. The cardinal signs are those of a progressive respiratory embarrassment, particularly after exercise; the severity of the signs reflects the extent of tumour development in the lungs. Death is often precipitated by a super imposed bacterial pneumonia, particularly that due to *Mannheimia* (formerly *Pasteurella multocida*) *haemolytica*. In clinically affected animals, a peripheral lymphopenia characterized by a reduction in CD4+ T lymphocytes and a corresponding neutrophilia may assist clinical diagnosis, but the changes are not pathognomonic and are not detected during early experimental infection. Moist rales may be heard on auscultation, but coughing is not usually prominent. The clinical signs are slowly progressive, ending in severe dyspnea. Death usually occurs in days to a few months, often from secondary bacterial pneumonia[2]. Not with standing, this period is shortened and fever appears in the case of secondary bacterial infections[9,43,50,58]. As the pulmonary tumor burden increases, the production of surfactant-containing fluid by the transformed epithelial cells increases, resulting in copious pulmonary effusion, nasal discharge, and progressive dyspnea, which can be accentuated by exercise[58].

Cytology

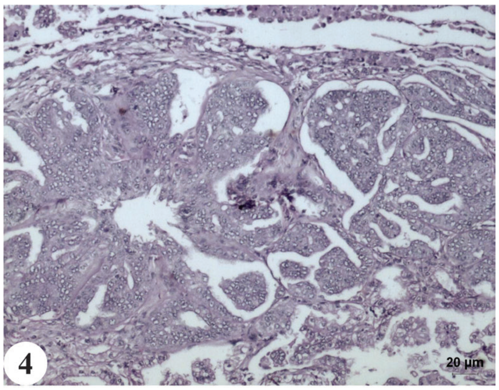
Cytopathology show high epithelial cellularity seen as individual, acinar or papillary arrangements. Leishman-Giemsa stained smears revealed high cellularity as individual to acinar or papillary arrangements. Neoplastic cells were round. Acinar pattern of neoplastic cells showed pleomorphism with variable sized nuclei and nucleoli. Nuclei showed coarse chromatin. Nucleoli were basophilic. Cytoplasm was scanty to moderate in amount and pale basophilic. Columnar type of cell, showed basally placed spherical to oval nucleus[44]. Both classical and atypical forms of OPA reveal numerous well differentiated cuboidal or polygonal neoplastic epithelial cells arranged in small acini, clusters or individually. The neoplastic cells had a moderate amount of pale blue, finely granular cytoplasm, moderate nuclear: cytoplasmic (N/C) ratio and round to oval, centrally located, nuclei with finely stippled chromatin and 1-2 distinct blue nucleoli. Anisocytosis and anisokaryosis were mild to moderate with rare mitotic figures. Large numbers of macrophages and mature lymphocytes were admixed with the neoplastic cells. OPA containing MGs were poorly cellular and composed of spindle, stellate and elongated cells with round to oval nuclei and inserted in a pale blue slightly vacuolar extracellular myxoid material[22].

Types of OPA

There are two recognized forms of OPA which show gross, histological and immunohistochemical (IHC) differences. Although mixed or intermediate forms of OPA have also been reported. i. Classical OPA: Clinically, the affected sheep develop chronic and progressive respiratory distress, especially when exercised. A common sign of classical OPA is mucous nasal discharge because of production of large fluids amounts in the lung. The lesions in classical OPA predominantly affect all pulmonary lobes and are located in the cranioventral area. They can be either nodular or exhibit a diffuse growth type, showing a grey moist appearance on the cross section. The classical form is more common than the atypical type. ii. Atypical OPA: The atypical OPA is less contagious than classical form. The atypical OPA has been reported in Spain, Peru, Iran and India, but there is no evidence of its occurrence in other countries where OPA is commonly found. Due to the restricted tumour development in atypical OPA and the lack of overproduction of lung fluid, the atypical form of OPA occurs only as a subclinical finding in abattoir studies or when the animal is necropsied for unrelated reasons. Lesions consist of hard nodules, pearly-white that have a dry cut surface; the tumors are well-delimited by the surrounding pulmonary tissue[3,22,45]. However, nodules resembling atypical lesions have been noted in the same lungs in which the classical form of the tumour is present. Therefore, these two forms of OPA appear to represent two extremes of a spectrum of histological presentations in this disease[3]. No molecular differences have been found between JSRV associated with these two forms which may represent two extremes of the OPA disease spectrum rather than two separate forms, and atypical OPA might constitute the initial lesions of this spectrum[43,45].

Gross pathology

In over 100 years of OPA reports two pathological forms of OPA are recognized, classical and atypical[43]. In the classical form the lungs appear considerably enlarged, heavy, oedematous and they do not collapse when the chest is opened. On necropsy, naturally infected animal in the advanced stages of disease will show a thin carcass with frothy fluid filling the trachea and exuding from the nares. Careful palpation will reveal consolidated foci or diffuse areas within the bulk of some or all of the lung lobes. This represents tumour, which, when extensive, can grossly distort the normal architecture of the organ. Externally, affected areas appear darker (purple/greyish) than adjacent normal tissue, but the smooth contour of the overlying pleura generally remains uninterrupted. Sectioning the lesion displays the solid, grey, granular surface of the tumour, which frequently exudes frothy fluid. There is a clear boundary between this and adjacent normal aerated, pink, functional lung tissue. Affected lungs have variably sized, coalescing neoplastic nodules (Fig. 3)[44], associated with regions of pulmonary atelectasis and the tumors fail to protrude from the cut surface of the lung. Affected lungs may exude clear fluid on sectioning; additional frothy fluid may be present in the bronchi and trachea and concurrent pneumonia is often present. In larger masses, the centre is more resistant to the knife and may feel gritty. In addition, firm foci of fibrosis or soft pockets of necrosis and abscessation are also frequently found. Mediastinal and tracheobronchial lymph nodes may be enlarged and occasionally contain metastasis. Extrathoracic spread of the tumour has been occasionally reported (Up to 10% in the liver, kidney, heart and skeletal muscle), but are generally rar[3,45]. Animals that have died naturally often have a secondary bacterial pneumonia, detailed dissection is important as



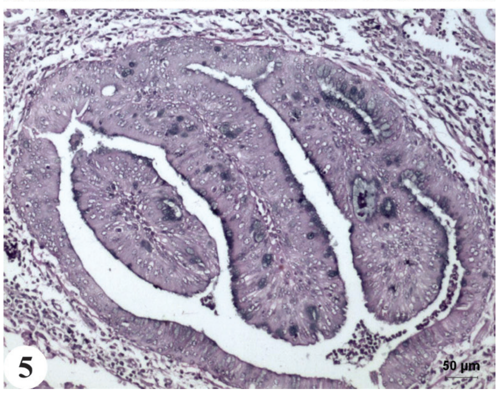


Fig. 3. Jaagsiekte-Diffuse multiple variable sized grayish-white nodules; **Fig. 4.** Jaagsiekte-Alveoli-Pulmonary papillary adenocarcinoma-Cuboidal cells (H&E Bar = 20 µm); **Fig. 5.** Jaagsiekte-Bronchogenic adenocarcinoma-Columnar cells (H&E Bar = 50 µm).

extensive pleurisy and adhesions can mask the underlying pathology of OPA[3].

The neoplastic lesions occur particularly in the cranioventral parts of the lungs, although any part may be involved. They are diffuse or nodular, grey or purple in colour and have an increased consistency. The neoplastic areas vary from small discrete nodules, in the early stages, to diffuse extensive lesions, which often are bordered by small satellite nodules. The cut surface of the tumour lesion has a granular appearance, is moist and frequently exudes frothy fluid. Concurrent lesions of other lung diseases are frequent and may hide the tumour lesion pattern[42,58,59].

Atypical form tends to be more nodular, well-demarcated from surrounding parenchyma, solitary, or multifocal nodules, dry sectioned surface, distributed primarily in the caudal pulmonary/diaphragmatic lobes. Such nodules may be present either underneath the pleura or deep in the lung tissue, and fluid is absent in the bronchi. The neoplastic foci are more solid than in the classical form, and tend to exude less fluid. Atypical and classical OPA may coexist in a flock and in individual sheep. Little is known about possible differences in the pathogenesis of classical and atypical OPA[3,42,45].

Histopathology

OPA reveals several non-encapsulated neoplastic foci that emanate from the alveolar and bronchiolar epithelia, forming acinar and papillary proliferations predominantly that expand into adjacent structures and compressing the adjacent structures. These are supported by a fibrovascular connective tissue framework that can dominate the centre of large tumour nodules. OPA were composed of a single layer of neoplastic cuboidal, columnar or polyhedral epithelial cells lining the alveolar lumina or bronchioles, surrounded by a fine to moderate fibrovascular stroma (Figs. 4-5)[44]. Tumour cells vary in shape and malignancy both within and between tumour nodules. Classically, they are cuboidal or columnar, with or without cytoplasmic vacuolation, and have a low mitotic index (0.002% in any tumour). In areas of increased malignancy, solid masses of pleomorphic cells with a high mitotic rate and scattered foci of necrosis are found. Occasionally, nodules of loose mesenchymal tissue appear admixed with tumour, as part of the fibrovascular core or on their own, which are also assumed to be neoplastic tissue. The fibrovascular connective tissue acts as a scaffold for infiltrating inflammatory cells, which vary according to the size and age of tumour and the presence of secondary infectious agents[3,45].

Natural stroma of the tumour may show variable amounts of lymphocytes, plasma cells and connective tissue fibres. Another common feature of OPA is the infiltration of large numbers of macrophages in and around the neoplastic alveoli and bronchioles. In secondary bacterial infection, neutrophils can also be often found. Mesenchymal tissue foci (myxoid nodules or growths) in a variable proportion of tumours interming led with the neoplastic epithelial component may be seen[3,43].

The diameter of individual neoplastic epithelial cells ranged from 20 to 30 µm, showed moderate amount of pale acidophilic, finely granular cytoplasm and intermediate N/C ratio. Anisokaryosis and anisocytosis were low to moderate; the nuclei were round to oval, centrally located, with a fine granular to lacy chromatin with and 1-2 distinct, basophilic nucleoli. The average of mitotic rate was 2 per 10 HPF without atypical features. The adjacent parenchyma of the neoplastic mass show atelectasis, and at the periphery of the tumors, the neoplastic cells were occasionally arranged in a lepidic growth pattern. The myxoid growths consisting of sparsely cellular structures of spindles cells embedded in an abundant extracellular matrix were observed. The MGs were admixed with the neoplastic epithelial component, or in some areas were disposed as multiple poorly demarcated masses. In some cases, the neoplastic epithelial component was absent. The neoplasm is composed of short bundles and streams of spindle, stellate or individual cells. These mesenchymal cells were embedded into an abundant myxoid matrix that contains moderate amounts of foamy, amphophilic, AB-PAS positive material (mucin). The individual cells had variable distinct borders, intermediate N/C ratio and moderate amounts of pale acidophilic, homogenous cytoplasm. The nuclei were oval to elongated, 20-30 μm in diameter, in transverse section, centrally located with clumped chromatin and indistinct nucleoli. No mitotic figures were present; anisocytosis and anisokaryosis were low. Presumptive diagnosis is pulmonary myxomas. Overall, mesenchymal proliferations and myxomatous changes, named as myxoid growths (MGs), were identified (myxoma-like nodules)[22].

Histologically, OPA lesions are consistent with a well-differentiated, multicentric, bronchioloalveolar carcinoma (adenocarcinoma). Based on predominant architectural patterns OPA is classified as lepidic, papillary, acinar, or mixed. The lepidic subtype was characterized by a layer of cuboidal epithelium following an alveolar pattern. The papillary subtype presented exophytic growths of cuboidal-to-columnar cells arranged around a fibrovascular core. The acinar subtype was characterized by palisades of taller columnar cells that formed tubular structures often surrounded by fibrovascular stroma. Some of the acinar structures showed mucous differentiation. Mixed patterns were the most frequently identified, and they were composed of a combination of the previously described subtypes or the intermediate forms between them. In sheep with subtle 1-2 mm diameter pulmonary lesions (the mixed form of OPA), the diagnosis of OPA was only confirmed after histological examination. In several cases, proliferative pulmonary nodules were composed of neoplastic cells associated with a moderate amount of extracellular matrix, with myxoid differentiation (fibromyxoid nodules)[58].

Atypical OPA

Atypical forms though essentially similar in histological appearance as that of classical OPA, the pattern of epithelial neoplasia is more often acinar and the stroma is more heavily infiltrated by mononuclear cells, mostly lymphocytes and plasma cells and connective tissue fibres. Lymphoid proliferations are also consistently seen around the bronchioles of neoplastic areas. The distinguish of atypical OPA features were represented by more prominent limits between normal tissue and neoplastic masses due to higher fibroblast proliferation and inflammatory infiltrates, predominated by mature lymphocytes and macrophages[43,45].

Electron microscopy

Electron microscopy document the early growth of the tumour, to identify ultrastructural characteristics of neoplastic cells and to support the histological findings. The tumours appear to originate from cells resembling fetal pneumocytes and type II pneumocytes. These cells proliferate to line alveoli with cuboidal or columnar cells, before forming papilliform or varicose clusters that can extend into bronchioles. Tumour cells have microvilli, basal or centrally located nuclei and are connected by desmosomes. They contain variable numbers of secretory granules appearing as electron-dense structures or are filled with myelinoid whorls, in comparison with the electron-lucent granules found in normal granular pneumocytes. The cells have rough endoplasmic reticulum, large numbers of free polysomes, a well-developed Golgi apparatus and hypertrophic mitochondria. Cytoplasmic glycogen granules have also been identified, sometimes in large quantities and confirmed using periodic acid-Schiff staining. The macrophages identified by histology are highly 'ruffled' and enlarged, confirming their activated state. They either attach to the surface of normal or neoplastic cells, or form separate clusters. In addition to lysosomes and phagolysosomes, they contain ingested JSRV particles, bacteria and mycoplasma-like organisms[3]. Ultrastructurally, proliferation of type II pneumocytes (Type B cells) having microvilli and well-developed junctional complexes. Non-ciliated bronchiolar epithelial cells (Clara cells) with electron dense cytoplasmic granules without any surrounding membrane and microvilli and no virus particles were traced[27].

Diagnosis

A definitive diagnosis of OPA is made based on the characteristic gross and histopathological lesions. Lack of antibody development against the virus in infected sheep limits the use of serological test and vaccine production; due to this the diagnostic option becomes minimum. Further, the virus can't grow in any laboratory animal except sheep and goat. JSRV cannot yet be propagated *in vitro*, therefore routine diagnostic methods, such as virus isolation, are not available for diagnosis. Diagnosis relies, at present, on clinical history and examination, as well as on the findings at necropsy and by histopathology and IHC. Viral DNA or RNA can be detected in tumour, draining lymph nodes, and peripheral blood mononuclear cells by polymerase chain reaction. Lambs become persistently infected by JSRV at an early age, and, in an OPA-affected flock, most sheep are infected. Transthoracic ultrasound diagnosis of ovine pulmonary adenocarcinoma in adult sheep can be helpful in natural cases and experimental sequential study[60].

Serological tests

Antibodies to the retrovirus have not been detected in infected sheep; therefore, serological tests are not available for diagnosis.

Serum biochemical markers

Among the serum markers tested, CEA levels remained similar, whereas CA 125, CA 19-9, CA 15-3, and AFP-3 levels were significantly higher in the OPA group than the control group. In all OPA animals, CA 125 levels were higher than 1 U/mL[61].

Oxidative stress, apoptosis and autophagy

To elucidate pathogenic mechanism, effects of OPA infections on lung tissue oxidative DNA damage, inflammation, apoptosis and autophagy with IL1-β, TNF-α, 8-OHdG, Bcl-2, BAX, Caspase3, LC3B and LC3A levels investigated in sheep showed that severe OPA cases showed more severe levels of IL1-β, Tnf-α, 8-OHdG, BAX, Caspase3, LC3A and LC3B expressions than mild cases did. Thus, where the severity of tumor foci due to OPA in lung tissues increased, oxidative stress, inflammation, apoptosis and autophagy were found to be significantly increased[62].

Immuno-histochemistry/fluorescence

Epithelial tumour cells in the lungs of sheep with OPA are the major sites of replication for JSRV[63]. As defined by IHC, type II alveolar epithelial cells are the principal neoplastic cell type (82%), with Clara cells (7%) and undifferentiated cells (11%) making up the remainder[3]. JSRV proteins were demonstrated in myxoid nodules and infiltrating interstitial cells of some OPA initial lesions suggesting that the participation of lymphoreticular cells may form part of the sequence of events leading to lung cell infection and tumour development[64]. JSRV nucleic acids can be also detected in the tumour tissue by specific PCR techniques[9,23]. Antibodies raised against JSRV proteins confirms the presence of JSRV in the transformed cells by IHC. LTR and ENV genes are targeted. The long terminal repeats of Jaagsiekte sheep retrovirus (JSRV) are preferentially active in type II pneumocytes[65]. IHC showed expression of *env* antigen in the cytoplasm of neoplastic epithelium by specific *env* oncogene monoclonal antibody. Cytokeratin identified epithelial component and vimentin fibroblasts of neoplasm by cytoplasmic expression[44]. IHC analysis reveals fewer JSRV-positive cells in atypical tumours than in the classical form of OPA[43,45].

JSRV protein is detected in all tumor epithelial cells, histologically normal alveolar type II cells and few bronchiolar epithelial cells, alveolar macrophages, lymphocytes and plasma cells[2]. A comparative study was conducted by IHC differential expression of JSRV capsid antigen and tumour biomarkers in classical and atypical OPA[66]. OPA cases were positive for MCK and TTF-1. MGs showed IHC reaction to vimentin, desmin and SMA; Ki67 expression of classical OPA was higher than atypical OPA and MGs. JSRV-MA was identified by IHC in both epithelial and mesenchymal cells of OPA. IHC and EM also confirmed the JSRV within the neoplastic cells[22].

Angiogenesis

The bFGF; VEGF-C and PDGF-C have roles in the pathogenesis of OPA. The bLF may activate macrophages and plasma cells in these lesions, but limited expression of bLF by neoplastic cells may be a consequence of defective or impaired function of this molecule[2].

Apoptosis marker studies

Immunofluorescence staining of lung tissues revealed no BAX, Caspase 3, LC3A and LC3B expression in negative controls, whereas moderate and severe BAX, Caspase 3, LC3A, and LC3B expression was observed in OPA positive cases[62]. Apoptosis, known as programmed cell death, can cause differentiation in cells and tissues by going beyond the body's control mechanisms in cases such as oxidative stress, inflammation and cancer[67]. Apoptosis and carcinogenesis mechanism work together. In the occurrence of apoptosis in cancer formation, apoptosis occurs faster in the cell as a result of decreased Bcl-2 molecule level and increased BAX molecule level[68,69]. Bcl-2 molecule level decreased significantly in the lung tissues with OPA, as in the tumoral mechanism and BAX increased severely and increased Caspase 3 expression in the cells. Thus, the tumoral foci developed and grew faster than normal due to the acceleration of the apoptotic process, especially in severe cases. Autophagy increases in cells under oxidative stress and starvation[70]. Although studies revealed that OPA causes oxidative stress[71], no study evaluated in terms of autophagy. OPA infections increased autophagy in cells depending on the severity of the disease in lung tissues.

In immunofluorescence staining revealed that LC3A and LC3B expression levels in anaplastic cells increased severely in advanced cases. Oxidative stress caused by the disease in the tissue is the cause of such an increase in autophagy in the cells. OPA infection triggered oxidative stress in sheep lung tissues, causing DNA damage and autophagy in cells and increased inflammation due to cancer. Apoptosis is also accelerated in cells according to the severity of the tumor and accordingly, the tumor spread to larger areas. These findings will be useful in further clarifying the pathogenesis of OPA[62].

PCR

PCR with primers specific for JSRV is always positive when applied to samples of OPA lesions in lung tissue. ExJSRV was identified by PCR in 97.05% of analyzed samples. Phylogenetic analysis revealed the presence of the exJSRV type 2 in Romanian sheep affected by lung cancer and showed a high similarity with the UK strain. PCR using *ltr* and *env* nucleotides were done[22,44]. NA (enzootic nasal adenocarcinoma) associated with JSRV infection was reported first time in a sheep of Ireland origin, which shows the necessity of using PCR in combination with IHC to reach an accurate etiologic diagnosis, which is of importance in countries currently free of ENTV-1[72]. ENA, a neoplasia of glands of the nasal mucosa associated with enzootic nasal tumour virus 1 (ENTV-1), is similar to JSRV. ENA enzootically occurs in many countries of the world with the exception of Australia and New Zealand[73].

Transcriptional response to JSRV

In a RNA sequencing study conducted for the transcriptional response of ovine lung tissue to infection by JSRV, 1,971 ovine genes were differentially expressed in JSRV-infected lung compared to non-infected lung, including many genes with roles in carcinogenesis and immunomodulation, confirmed using IHC and reverse transcription-quantitative PCR. There was activation of anterior gradient 2, yes-associated protein 1 and amphiregulin in OPA tumor cells, indicating a role for this oncogenic pathway in OPA. There was also differential expression of genes related to innate immunity, including genes encoding cytokines, chemokines and complement system proteins; also macrophage function reflecting the increased abundance of these cells in OPA-affected lung tissue. There was little evidence for the upregulation of genes involved in T-cell immunity. Comparison of the genes differentially regulated in OPA with the transcriptional changes occurring in human lung cancer revealed important similarities and differences between OPA and human lung adenocarcinoma. This provided valuable new information on the pathogenesis of OPA and strengthens the use of this naturally occurring animal model for human lung adenocarcinoma[74].

Treatment, control measures and vaccines

Since effective treatment and a vaccination procedure are not currently possible and control and eradication of the disease are difficult[9]. Till date there is no effective methods of controlling its spread and OPA remains an important problem sheep farming countries[61]. There are no vaccines available[75].

In silico analysis for vaccine development: An attempt was made to construct an effective multi-epitopes vaccine against JSRV eliciting B and T lymphocytes using immunoinformatics tools. The designed vaccine was composed of 499 amino acids. Before the vaccine was computationally validated, antigenicity, allergenicity, toxicity and stability were considered. The physiochemical properties of the vaccine displayed an isoelectric point of 9.88. According to the Instability Index (II), the vaccine was stable at 28.28. The vaccine scored 56.51 on the aliphatic index and -0.731 on the GRAVY, indicating that the vaccine was hydrophilic. By applying the RaptorX server for vaccine's tertiary structure, Galaxy WEB server refined the structure, and the Ramachandran plot and the ProSA-web server validated the vaccine's tertiary structure, Protein-sol and the SOLPro servers showed the solubility of the vaccine. The high mobile regions in the vaccine's structure were reduced and the vaccine's stability was improved by disulfide engineering. The vaccine construct was docked with an ovine MHC-1 allele and showed efficient binding energy. Immune simulation remarkably showed high levels of immunoglobulins, T lymphocytes, and INF-γ secretions. The molecular dynamic simulation provided the stability of the constructed vaccine. Finally, the vaccine was back-transcribed into a DNA sequence and cloned into a pET-30a (+) vector to affirm the potency of translation and microbial expression. A novel multi-epitopes vaccine construct against JSRV, was formed from B and T lymphocytes epitopes, and was produced with potential protection. This study might help in controlling and eradicating OPA[76].

Ovine JSRV induced OPA model

Necessity of JSRV to induce a lung cancer in sheep is reported[4,54]. Experimental induction of OPA in sheep results in pathological changes similar to those observed in natural conditions and are compatible with the classical form, whereas atypical presentations have not been recorded in these cases[9,43]. Such studies will improve OPA research by providing novel insights into JSRV infectivity and OPA disease progression[77]. In experimentally induced OPA JSRV replicates actively in the transformed cells (type II pneumocytes and club cells) where viral antigen can be shown by IHC methods[3,43].

Human lung cancer model

Similarities in pulmonary anatomy and physiology potentially make sheep better models for studying human lung function and disease. OPA shares many histological similarities with the human pulmonary adenocarcinoma, representing an important animal model for understanding the mechanisms of viral oncogenesis. Although JSRV was found in human pulmonary neoplastic cells, its role in the development of lung cancer in humans is not fully elucidated[22]. The potential for OPA to be used as a pre-clinical animal model for assessing human lung cancer treatment strategies are yet to be fully exploited.

Antibodies against JSRV capsid were identified in human lung adenocarcinoma, but an association between JSRV and this neoplasm in humans could not be demonstrated[23,53]. Since, natural infection of Jaagsiekte is common in sheep caused by retrovirus globally, OPA is considered as model for human adenocarcinoma especially pneumonic type bronchioalveolar carcinoma[23,78,79]. JSRV can infect human cells but it plays little if any role in human lung cancer[80]. We can integrate techniques commonly used in the treatment of human lung cancer patients to further strengthen the effectiveness of OPA as a pre-clinical cancer research model[7,81]. Comparison of transcriptional changes in the genes differentially regulated in OPA and human lung cancer revealed important and OPA can be a model for studying pathogenesis of OPA for human lung adenocarcinoma[74].

Virus entry into cells is initiated by binding of the viral envelope (env) protein to a specific cell-surface receptor, Hyal2. Unlike almost all other retroviruses, the JSRV env protein is also a potent oncoprotein and is responsible for lung cancer in animals. Of concern, Hyal2 is a functional receptor for JSRV in humans. JSRV is fully capable of infecting human cells, as measured by its reverse transcription and persistence in the DNA of cultured human cells. Role for JSRV in human lung cancer both agreed and disputed. A highly-specific mouse monoclonal antibodies and a rabbit polyclonal antiserum against JSRV env to test for JSRV expression in human lung cancer was done. JSRV env expression was undetectable in lung cancers from 128 human subjects, including 73 cases of bronchioalveolar carcinoma (BAC; currently reclassified as lung invasive adenocarcinoma with a predominant lepidic component), a lung cancer with histology similar to that found in JSRV-infected sheep. Neutralizing antibodies in sera from 138 Peruvians living in an area where sheep farming is prevalent and JSRV is present, 24 of whom were directly exposed to sheep and found none. While JSRV can infect human cells, JSRV plays little if any role in human lung cancer[81].

Naturally occurring OPA cases are readily available from infected flocks due to the endemic nature of the disease in many countries and pre-clinical cases can be identified by the use of ultrasound scanning programmes. The use of naturally occurring cases could decrease the use of experimentally induced OPA tumors in lambs, reducing ethical concerns with this model. Future studies that can integrate techniques commonly used in the treatment of human lung cancer patients, such as ultrasound, general anesthesia, CT and surgery, would further strengthen the effectiveness of OPA as a pre-clinical cancer research model and in ovine natural cases model[7] and is comparable to TTNB in human patients in terms of procedure duration, radiation exposure and complication rate. This model can be developed further for other pre-clinical uses, such as the procurement of biopsy specimens, the development of medical devices for the local delivery of chemotherapeutic agents, monitoring the tumor microenvironment and in the assessment of the effectiveness of RT or systemic chemotherapeutic agents and has a great potential to not only advance the molecular understanding of human lung cancer[81].

Recommendations

1. Herd replacement from infected area should be banned to avoid spread of infection in naïve population.
2. Avoiding OPA introduction: Need for stringent bio-security measures and replacements only from flocks free from respiratory diseases including OPA.
3. Environmental hygiene to be maintained from discharges and any virus reserving matter.
4. To prevent OPA transmission through milk, weaning and feeding neonates with milk replacement and colustrum from cow rather than sucking their dam.
5. Stamp out the affected flocks to eradicate the diseases from the area.
6. Research fields for the future: Mechanism of tumor production, vaccine development, public health importance and nature of tumor.
7. Considering lack of diagnostics, development of better preclinical diagnostic tools is essential to control[2,24].
8. Cell culture studies suggested a new mechanism of retroviral transformation having direct relevance to design JSRV-based vectors targeting the differentiated epithelial cells of the lungs[11].
9. Novel methodologies to explore the possibility of generating transgenic sheep resistant to JSRV infection by redirecting enJSRVs expression in type II pneumocytes and Clara cells with caution[19].

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