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Pulmonary adenocarcinoma in two alpacas (*Vicugna pacos*)

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SUMMARY

We describe here two cases of pulmonary neoplasia in alpacas: case 1 was a 19-year-old female huacaya alpaca that was submitted to investigate sudden death with possible previous weight loss. Gross and histological examination revealed changes consistent with primary lepidic-type pulmonary adenocarcinoma (previously bronchioalveolar carcinoma) with metastases to the thorax and abdomen. Case 2 was a six-year-old male castrated Suri alpaca with a 3-week history of weight loss and inappetance that was euthanased following a short period of recumbency and respiratory signs. Gross and histological features in this case were consistent with a diagnosis of metastatic adenocarcinoma affecting the lungs and lymph nodes. Masses from both alpacas were further characterised using immunohistochemistry for thyroid transcription factor 1 (TTF-1), pan cytokeratin AE1/AE3 and vimentin. We show that histological subtype and TTF-1 and AE1/AE3 immunoreactivity can be used to differentiate primary and metastatic pulmonary adenocarcinomas in the lungs of alpacas.

BACKGROUND

Primary diseases of the lower respiratory tract are uncommon in New World camelids (NWCs). Clinical signs of lower respiratory tract disease (dyspnoea or tachypnoea) usually occur secondary to an extrapulmonary condition, including cardiac disease, anaemia, hypoproteinaemia and oedema, metabolic acidosis, stress and pain.

Sudden death in any species, including NWCs, may be due to a range of infectious, non-infectious or toxicometabolic causes. Unfortunately, once an animal is dead, diagnostic tests, which are commonly used to investigate disease in live animals, for example, haematology and biochemistry, are of limited value. However, prompt postmortem examination of animals that die suddenly can provide valuable information that may help to establish a cause of death.

To the best of our knowledge, case 1 is the first report in an alpaca of a pulmonary lepidic adenocarcinoma with intrapulmonary metastases, as well as metastases to the thoracic lymph nodes, mediastinum, pleural cavity, kidney and liver. In contrast, findings in case 2 support a diagnosis of a non-primary pulmonary neoplasm affecting the lung and multiple lymph nodes, including bronchial, mediastinal and gastric nodes.

CASE PRESENTATION

Case 1 was a 19-year-old female huacaya alpaca (*Vicugna pacos*) that presented for postmortem examination at the Animal and Plant Health Agency (APHA) Veterinary Investigation Centre (VIC) in Thirsk. The animal had first presented to the private veterinarian 6 months previously (September 2018) with subcutaneous emphysema and muscle fasciculations in the neck. These resolved after treatment with antibiotics (the dose and route of administration are unknown). In March 2019, the alpaca was found dead with no clinical signs seen, although when the keepers examined the alpaca after death they thought it may have lost body condition. The alpaca was part of a private zoo collection, shared indoor and outdoor enclosures with exotic mammals and birds, and had been vaccinated with a combined vaccine against pasteurellosis and clostridial disease (Heptavac-P; MSD Animal Health, Milton Keynes, UK) within the previous 12 months.

Case 2 was a six-year-old male castrated Suri alpaca that presented with recumbency, increased respiratory effort and pain on abdominal palpation. The alpaca had a 3-week history of loss of body condition with inappetence progressing to anorexia. The alpaca was humanely euthanased by intracardiac injection with 200 mg/kg pentobarbitone sodium (Pentoject 20 per cent, Animalcare) and the carcase was frozen for 3 days (over the weekend) before submission for postmortem examination at the APHA VIC in Starcross.

INVESTIGATIONS

Case 1

On postmortem examination, case 1 was found to be in fair body condition. Compartment 1 of the stomach was full of semi-digested grass. The thoracic cavity contained a moderate amount of serosanguinous fluid with fibrin strands. Within the right caudal lung lobe, there were multifocal to coalescing nodular masses (figure 1) that were firm or oozed creamy liquid on cut surface. Similar lesions were present in the left caudal lung lobe. Within one mass, there was a focal area of black discolouration (figure 2). There were multiple, firm, round, 3 mm to 20 mm diameter, cream to red-brown nodules throughout the thoracic cavity, including the pulmonary and parietal pleura (figure 3), mediastinum and pericardium. Cranial and mediastinal lymph nodes were enlarged approximately two to three times the normal size. A single nodule was also present in the kidney (7 mm diameter) and in the liver (4 mm diameter).

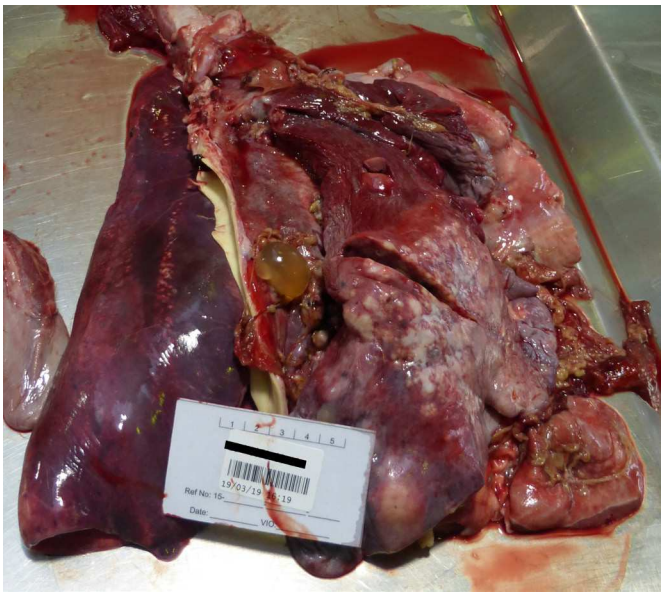

Figure 1 Case 1. Multifocal to coalescing neoplastic nodules affecting primarily the right middle and caudal lung lobes. Scale in centimetres.

Samples of tissues, including lung, lymph nodes, mediastinum, kidney and liver, were fixed in buffered formalin and submitted for histological examination.

Microscopically, the pulmonary parenchyma was multifocally effaced by infiltrative, non-encapsulated, poorly demarcated, nodular neoplasms composed of two different morphological types: the first type was characterised by neoplastic epithelial cells exhibiting lepidic growth along existing alveolar septa that were moderately to markedly expanded by a poorly cellular collagenous stroma (figure 4). Neoplastic cells were cuboidal to low columnar with variably distinct cell borders, a moderate amount of eosinophilic cytoplasm, and a round usually basally located nucleus with coarsely stippled chromatin and a single basophilic nucleolus. There was moderate anisocytosis, and anisokaryosis and mitotic figures were rare. There was multifocal squamous differentiation and a single, poorly defined focus where the cytoplasm of neoplastic epithelial cells contained marked amounts of granular brown material (melanin) (figure 5). This morphological type is consistent with primary pulmonary lepidic adenocarcinoma and was only observed in masses in the lung.

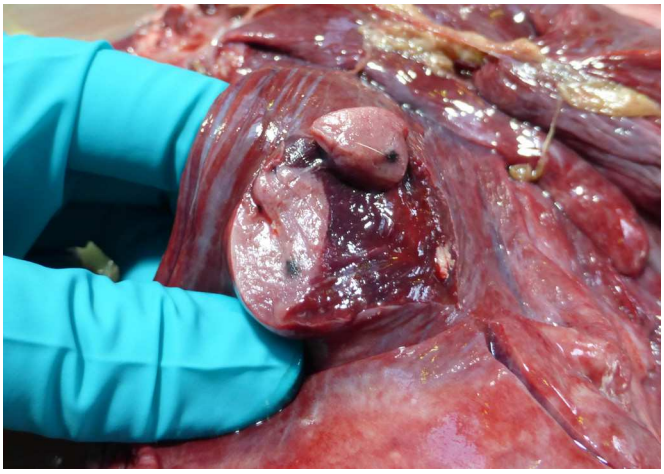

Figure 2 Case 1. Cut surface of a neoplastic lung nodule showing a focal area of black discolouration.

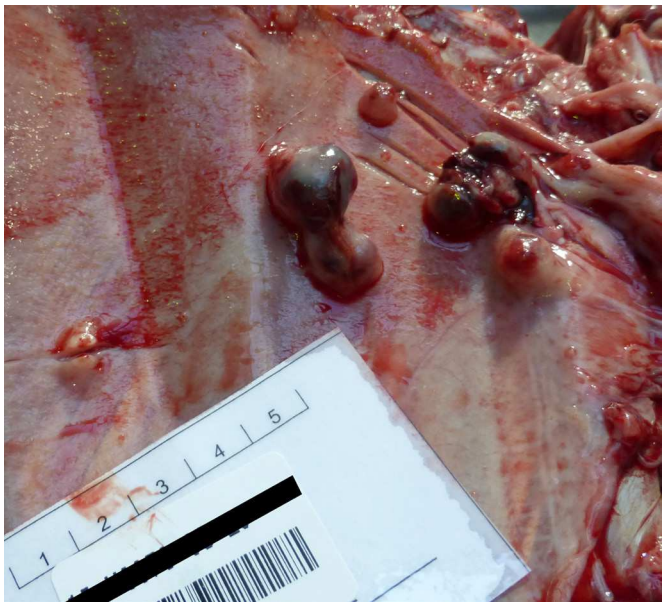

Figure 3 Case 1. Multiple, firm, round, cream to red-brown nodules attached to the parietal thoracic wall. Scale in centimetres.

The second morphological type was characterised by neoplastic cells arranged in irregularly shaped acini and papillary cords on a moderate amount of fibrovascular stroma (figure 6). Neoplastic cells were polygonal to fusiform with a moderate amount of granular eosinophilic cytoplasm, variably distinct cell borders, a single nucleus with finely stippled chromatin and one to three basophilic nucleoli. There was mild anisocytosis and anisokaryosis and the mitotic count was 1.5. Multifocally within and surrounding the neoplastic masses were areas of necrosis and haemorrhage. Neoplastic cells were present within mediastinal lymphatics and blood vessels. Squamous differentiation was observed within one mass. This second subtype is consistent with

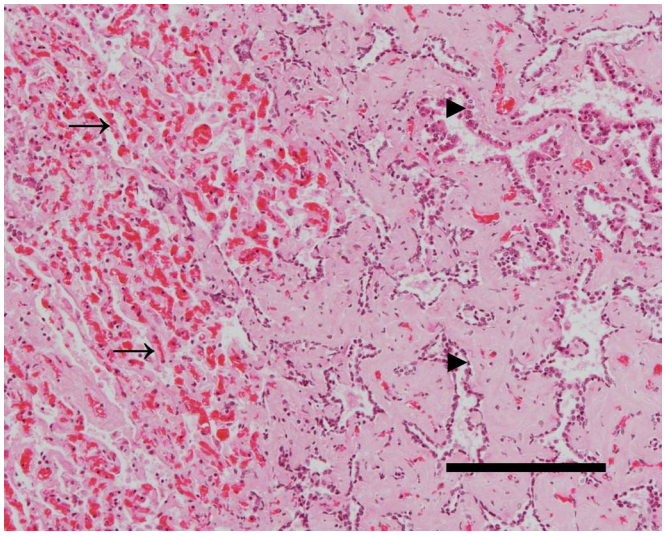

Figure 4 Case 1. Lung. Primary lepidic-type adenocarcinoma (right). Neoplastic epithelial cells exhibit lepidic growth along existing alveolar septa that are markedly expanded by a poorly cellular collagenous stroma (arrowheads). The left side of the image shows compressed but otherwise normal alveoli (arrows). H&E, original objective x 10. Scale bar 200 µm.

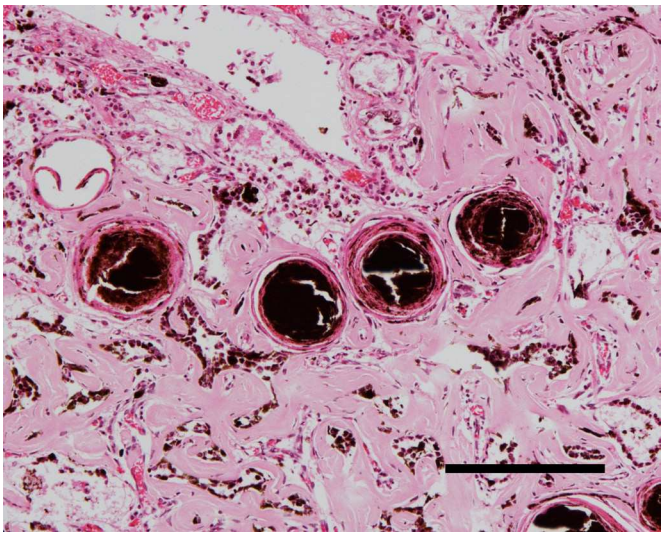

Figure 5 Case 1. Lung. Histological section taken from the focal area of black discolouration in figure 2. Neoplastic epithelial cells that exhibit lepidic growth along existing alveolar septa that are markedly expanded by a poorly cellular collagenous stroma. There is abundant dark brown granular material within neoplastic epithelial cells that frequently form round structures. H&E, original objective x 10. Scale bar 200 µm.

solid adenocarcinoma and was observed in the lung, cranial and thoracic lymph nodes, mediastinum and kidney.

Case 2

On postmortem examination, case 2 was found to be in poor body condition and no subcutaneous fat was visible. Multiple, firm, round, 2 mm diameter, raised, grey nodules were present over the pleural surface of both lungs with mild infiltration of the underlying parenchyma. Lymph nodes throughout the body, including the bronchial, mediastinal and gastric nodes, were enlarged two to three times the normal size by firm, white medullary expansion. A presumptive diagnosis of disseminated neoplasia was made.

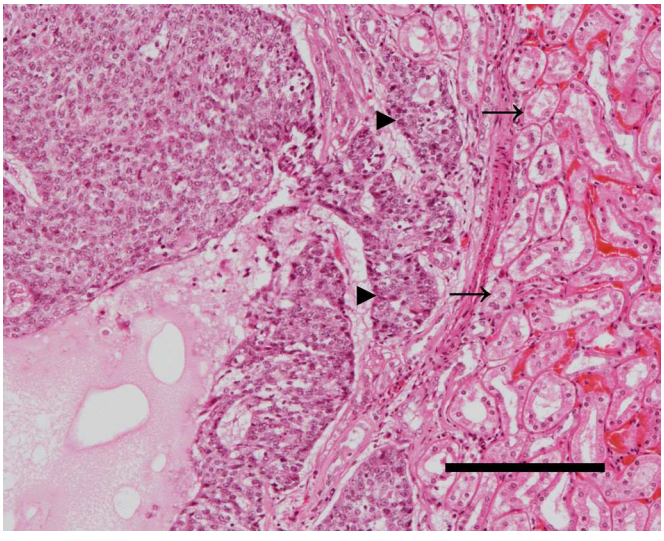

Figure 6 Case 1. Kidney. A metastatic solid-type adenocarcinoma (left, arrowheads) compresses adjacent renal tubules (right, arrows). H&E, original objective x 10. scale bar 200 µm.

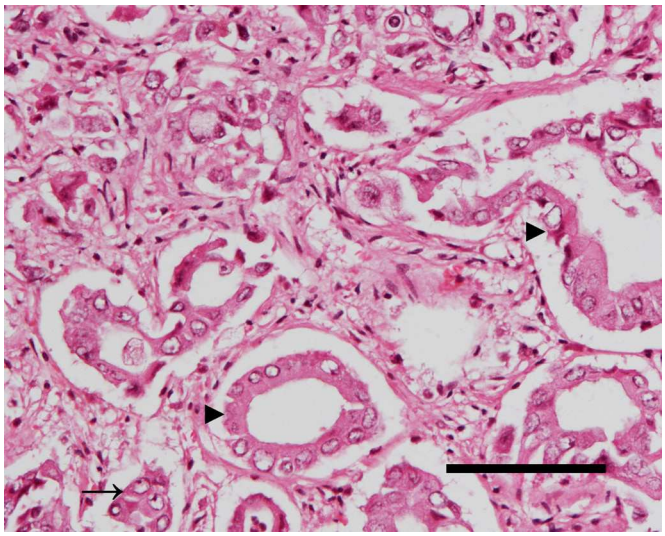

Figure 7 Case 2. Lung. Metastatic adenocarcinoma. Neoplastic cells are arranged in tubules (arrowheads) and acini (arrow) on a moderate fibrovascular connective tissue stroma. H&E, original objective x 20. Scale bar 100 µm.

Samples of tissues, including lung, mediastinal lymph node and liver, were fixed in buffered formalin and submitted for histological examination.

On histological examination of the lung, there were multifocal, expansile, poorly demarcated, non-encapsulated, nodular neoplasms composed of neoplastic cells arranged in tubules and acini on a scant to moderate fibrovascular connective tissue stroma (figure 7). Neoplastic cells were cuboidal to pleomorphic, with well-defined cell borders, a moderate amount of eosinophilic granular cytoplasm and a round to oval nucleus with marginated chromatin and single basophilic nucleolus. There was moderate anisocytosis and anisokaryosis, and the mitotic count was 5. In the mediastinal lymph node, the normal lymph node architecture was almost completely effaced by neoplastic cells with a morphology similar to that in the lung, and there was marked diffuse fibrosis. Neoplastic cells in the lymph node showed a higher degree of cellular pleomorphism compared with the lung, including occasional signet cells. Based on the morphology of neoplastic masses in the lung and lymph node, a diagnosis of multicentric adenocarcinoma with marked desmoplastic response was made.

To further characterise neoplastic masses in the lungs and other tissues immunohistochemistry for thyroid transcription factor 1 (TTF-1, SPT24 clone, dilution 1:200; Novocastra, Newcastle upon Tyne, UK), pan cytokeratin AE1/AE3 (MFN116 clone, dilution 1:100; Dako/Agilent, Stockport, UK) and vimentin (V9 clone, dilution 1:5000; Dako/Agilent) was performed on representative sections of masses from the lung, kidney and mediastinum/pleura from case 1, and lung and mediastinal lymph nodes from case 2. Positive control tissues were alpaca thyroid for TTF-1 and alpaca small intestine for AE1/AE3 and vimentin.

In lung samples from case 1, there was widespread nuclear TTF-1 immunoreactivity in bronchiolar epithelial cells and alveolar pneumocytes in areas with a lepidic pattern (figure 8) and no immunoreactivity in masses with a solid or squamous morphology. There was no TTF-1 immunoreactivity in the masses in the kidney or mediastinum/pleura. There was strong cytoplasmic immunoreactivity for pan cytokeratin AE1/AE3 in solid masses and areas of squamous differentiation in the lung, kidney (figure 9) and mediastinum/pleura, and moderate,

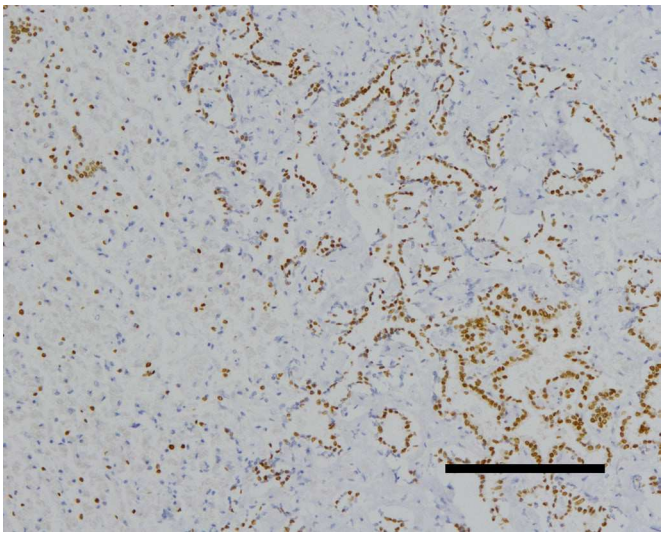

Figure 8 Case 1. Lung. Primary lepidic-type adenocarcinoma. Neoplastic epithelial cells exhibit strong intranuclear immunoreactivity for thyroid transcription factor 1. Original objective x 10. Scale bar 200 µm.

multifocal cytoplasmic staining of respiratory epithelial cells in areas with a lepidic pattern. In case 2, there was weak to moderate cytoplasmic immunoreactivity for AE1/AE3 in neoplastic epithelial cells (figure 10) and no TTF-1 immunoreactivity. In both alpacas, neoplastic cells were vimentin negative.

DIFFERENTIAL DIAGNOSIS

An important differential diagnosis for multifocal nodular lesions in the lung and thoracic lymph nodes is infection with *Mycobacterium* species, most commonly *M bovis*. Alpacas and llamas appear to be very susceptible to infection with *M bovis*, and tuberculosis (TB) is becoming an increasingly important issue for the camelid industry in a number of countries, including the UK.[1] Transmission occurs through inhalation or ingestion of infectious material from ruminant or wildlife hosts. Transmission between camelids and from camelids to humans is also

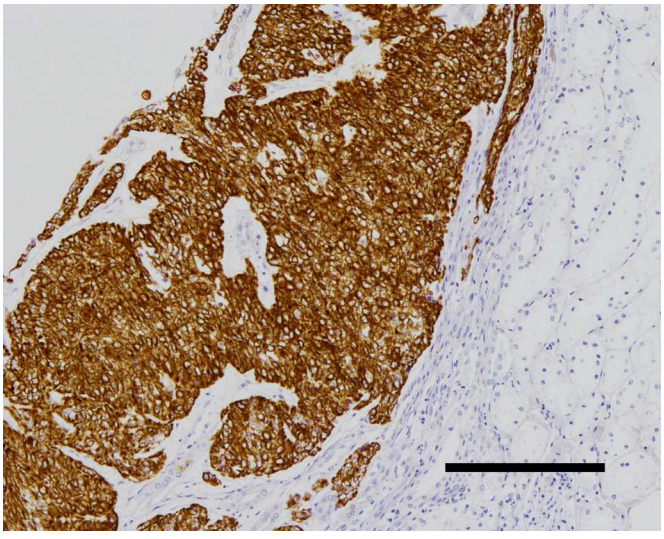

Figure 9 Case 1. Kidney. Metastatic solid-type adenocarcinoma. Neoplastic epithelial cells exhibit strong cytoplasmic immunoreactivity for pan cytokeratin AE1/AE3. Original objective x 10. Scale bar 200 µm.

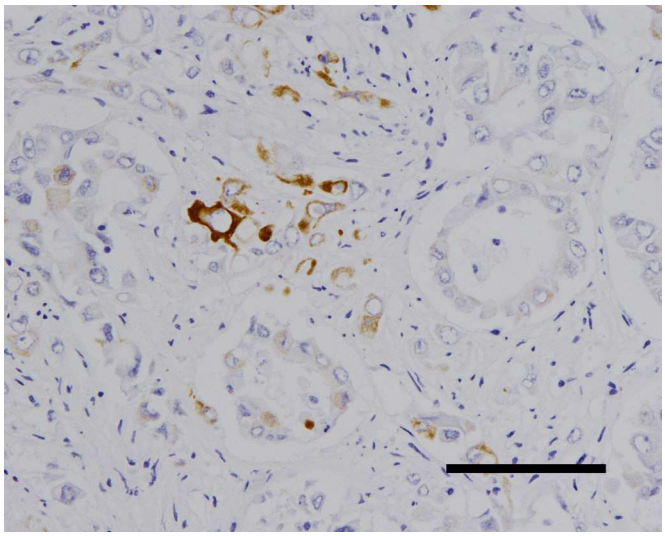

Figure 10 Case 2. Lung. Metastatic adenocarcinoma. Neoplastic cells exhibit weak to moderate cytoplasmic immunoreactivity for pan cytokeratin AE1/AE3. Original objective x 20. Scale bar 100 µm.

suspected. Clinical signs are non-specific, so TB may not be suspected and therefore the disease is likely under-reported.[1 2]

In case 1, there were areas of necrosis in the lung and lymph nodes but no caseating granulomas typical of *M bovis* infection, and no acid–fast bacteria were seen on Ziel-Neelsen stained impression smears of the cut surface of thoracic nodules or formalin-fixed paraffin-embedded sections of masses in the lung and other tissues. In addition, no *Mycobacterium* species were cultured from tissue sent for statutory diagnosis. Therefore, infection with *M bovis* was ruled out in this case.

Other possible bacterial causes of chronic pneumonia with abscessation in include *Arcanobacterium pyogenes*, *Actinomyces lamae*, *Streptococcus equi* subspecies *zooepidemicus* (usually associated with acute disease), *Fusobacterium necrophorum*, *Corynebacterium pseudotuberculosis*, *Rhodococcus equi* and *Burkholderia pseudomallei*.[1 2]

In case 2, the gross distribution and histological pattern of lung lesions were reminiscent of that observed in ovine pulmonary adenocarcinoma associated with infection with Jaagsiekte sheep retrovirus (JSRV). To the authors' knowledge, JSRV infection has not been reported in camelids despite PCR testing (Cousens, personal communication).

DISCUSSION

Primary pulmonary adenocarcinomas have previously been reported in llamas (*Lama glama*) with confirmed metastases to the pleura, liver and possibly the femur in one case[3] and pleura, liver, kidney and heart in another case.[4] Recently, Moser *et al*[5] reported a case of bronchioalveolar carcinoma in a six-year-old huacaya alpaca presenting with respiratory signs (dyspnoea and abdominal breathing) and weight loss despite having a good appetite. Multiple pulmonary nodules were detected on ultrasonographic, radiographic and CT examination and were diagnosed as bronchioalveolar carcinomas on histological examination. No extrapulmonary nodules were identified. Other tumours previously reported in the lungs of NWCs include multicentric lymphosarcoma in llamas and alpacas,[6–9] metastatic malignant melanoma in alpacas,[10 11] metastatic cholangiocarcinoma in a llama[12] and metastatic nephroblastoma in a guanaco (*L guanicoe*).[13]

In 2014, the WHO guidelines for the classification of lung tumours in humans were updated such that classification is now based on histological pattern rather than possible cell of origin. This resulted in a change in the nomenclature of pulmonary adenocarcinoma so that tumours that were previously classified as bronchioalveolar carcinomas were renamed 'lepidic adenocarcinomas'.[14] In dogs, most pulmonary adenocarcinomas are of bronchioalveolar origin, while in cats and cattle, adenocarcinomas are more common.[15]

In the lung, TTF-1 is a marker for pulmonary tumours derived from club cells and type II pneumocytes and is considered to be relatively specific for tumours of bronchioalveolar origin.[15] Occasional weak TTF-1 immunoreactivity has been reported in a llama with pulmonary adenosquamous carcinoma without metastases.[4]

A combination of histopathology and immunohistochemistry can be used to characterise pulmonary adenocarcinomas as primary pulmonary adenocarcinomas, that is, originating in the lung, or metastatic extrapulmonary adenocarcinomas, that is, originating outside of the lung. In human pathology, primary pulmonary adenocarcinomas are more commonly of the lepidic subtype and TTF-1 positive, while metastatic pulmonary adenocarcinomas are more commonly of the solid adenocarcinoma subtype and lack TTF-1 positivity.[14 16] However, this distinction can be complicated by a number of factors, including variable TTF-1 positivity in primary lung tumours,[17] loss of TTF-1 positivity associated with transdifferentiation of primary lung tumours[15] and the presence of intrapulmonary metastasis arising from primary lung tumours.[18]

In the lung of case 1, masses with a predominantly lepidic pattern were TTF-1 positive and often also included areas of squamous transdifferentiation that were TTF-1 negative. These masses likely represent multicentric primary tumours, although intrapulmonary dissemination following intra-airway seeding and reaspiration cannot be ruled out.[14 18] Other masses in the lung, as well as masses in the lymph node and kidney, were a mixture of papillary and solid patterns with variable amounts of fibrosis and necrosis and were TTF-1 negative and cytokeratin AE1/AE3 positive. Unfortunately, no fixed liver tissue was available, but since the gross morphology of the liver nodule was similar to that in the kidney, it is reasonable to assume that the liver nodule was also a metastatic pulmonary adenocarcinoma. The absence of a primary tumour in a distant organ detected at postmortem, combined with the gross distribution of the lesions in the lung, supports a diagnosis of (multiple) primary pulmonary adenocarcinomas with intrapulmonary metastases rather than extrapulmonary metastases from another location.

Granular brown pigment within a focal area of squamous differentiation (figure 5) was confirmed to be melanin. Although melanocytes are not commonly found within the lung, they can migrate to the lower respiratory tract during embryonic development, resulting in, for example, congenital melanosis of calves, lambs and pigs.[17]

In case 2, the gross distribution—particularly the involvement of the gastric lymph node—and histological and immunohistochemical features are most consistent with extrapulmonary metastatic adenocarcinoma. However, no primary tumour was identified at postmortem and, given the variation in TTF-1 expression by primary pulmonary adenocarcinomas discussed previously, an intrapulmonary origin cannot be entirely ruled out.

Cytokeratins are structural proteins that form part of the cytoskeleton of epithelial cells.[19] In case 2, tumour cell morphology and immunoreactivity for cytokeratin marker AE1/AE3 support a diagnosis of a carcinoma arising from glandular epithelium (ie, adenocarcinoma) and possible locations of origin include the colon, biliary duct, prostate, mammary gland or bronchial glands. Vimentin is a structural protein found in mesenchymal cells, including muscle, blood vessels and white blood cells.[19] In both case 1 and case 2, tumour cell morphology is not typical of a tumour arising from a mesenchymal tissue (ie, sarcoma), and this is supported by a lack of vimentin immunoreactivity.

In previous reports of primary pulmonary adenocarcinomas in llamas,[3 4] both animals presented with weight loss and anorexia. This clinical presentation is similar to that of case 2 but different from that of case 1, which initially presented with subcutaneous emphysema followed 6 months later by sudden death. Given the presence of multiple neoplastic metastases on the pulmonary and parietal pleura, the subcutaneous emphysema may have been caused by disruption of the pleural lining by neoplastic growths and subsequent escape of air into the mediastinum and subcutis.

To the author's knowledge, this is the first report in alpacas of non-lymphomatous, multicentric neoplasia affecting the lungs, thorax and abdomen. In addition, this is the first application of TTF-1 to characterisation of pulmonary adenocarcinoma in alpacas.

Learning points

- Pulmonary neoplasia is relatively rare in New World camelids but loss of body condition is a common clinical finding.
- Clinical signs of lower respiratory tract disease in NWCs usually occur secondary to an extrapulmonary condition rather than a primary lung disease.
- Tuberculosis should be considered a differential diagnosis in NWCs presenting with weight loss and/or multiple, variably sized, white nodules in the thoracic and/or abdominal cavities and organs.
- Tuberculosis affecting NWCs is suspected to be zoonotic, so field veterinarians should take appropriate precautions when interacting with suspect cases.
- Thyroid transcription factor 1 can be used to characterise pulmonary adenocarcinomas in camelids.

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Contributors ZVT and HMcF had primary responsibility for the cases, performed the postmortems and coordinated ancillary testing. SJM and MW performed histopathological and immunohistopathological examinations. FB validated immunohistochemistry markers and performed immunohistochemical investigations. SJM led the drafting of the manuscript. All coauthors critically reviewed and approved the final version of the manuscript.

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