

**Conclusions:** The presence of *A. vasorum* larvae in correlation with circulatory lesions and mononuclear infiltrates in the lungs confirmed that the cause of death was pulmonary collapse due to the parasitic infestation. This is the first case reported of a paediatric dog in Romania that died due to *A. vasorum* infestation.

#### INTRAOSSUEOUS LEISHMANIA INFECTION IN A DOG

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**Introduction:** Canine leishmaniosis is transmissible to vertebrate hosts by phlebotomine sand flies. It is a parasitic disease caused by protozoa of the genus *Leishmania*, endemic in several countries and with high prevalence in the Mediterranean basin. Manifestation of leishmaniosis in the oral cavity has been reported occasionally. We present a rare case of *Leishmania* infection in the mandibular bone associated with local bone and periodontal inflammation and oral discomfort.

**Materials and methods:** A 9-year-old neutered female Pitt Bull dog was attended at the Veterinary Teaching Hospital with hyporexia and progressive weight loss. At clinical evaluation, the patient revealed discomfort on oral manipulation, and swelling in the ventral surface of the left mandibular body was identified. Dental radiography was performed and signs of bone lysis and periosteal reaction were observed. A biopsy of bone and teeth was made and the material was sent to the Laboratory of Histology and Anatomical Pathology.

**Results:** Histological examination showed intense bone reabsorption and large macrophages with intracytoplasmic structures compatible with *Leishmania* spp amastigotes. Immunohistochemistry with an anti-*Leishmania* hyperimmune serum identified several *Leishmania* amastigotes inside macrophages and free in the surrounded tissue.

**Conclusions:** Oral leishmaniosis, affecting the tongue, has already been reported. In the present case, we unexpectedly identified the parasites in a diagnostic histopathological examination of mandibular periodontal bone, confirming that the use of biopsy and histopathology, including immunohistochemistry, are fundamental tools for the definitive diagnosis of leishmaniosis.

VARIA

LEFT FORELIMB MICROMELIA, ULNAR ABSENCE, PARTIAL ABSENCE OF CARPAL BONES AND PARTIAL ADACTYLIA IN A DOG

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**Introduction:** There are rare reports of congenital limb deformities in domestic animals.

**Materials and methods:** A 6-month-old stray intact mixed-breed dog presented for unwillingness to move and eat, failure to thrive, panting and with normal vital signs. On clinical examination, the dog appeared obtunded and could not stand or ambulate alone. Cranial nerves appeared intact, although the dog did not appear to be aware of its surroundings. The dog's conscious proprioception, although present, was sluggish and delayed in all four limbs. A left forelimb deformity was apparent. Euthanasia was elected for humane reasons. One of the two siblings had non-specific neurological signs that may be indicative of hydrocephalus but other congenital brain abnormalities could not be ruled out based on physical examination alone.

**Results:** Grossly and radiographically, the left forelimb was shorter (micromelia), with a malformed left humerus and missing ulna. The left radius was larger than the right. Many of the left carpal bones were missing, including the accessory carpal bone, and only one digit was present (partial adactylia). Autopsy revealed the following additional findings: a splenic fissure (1.5 cm) on the lateral surface, mandibular mesiocclusion, generalized lymphadenopathy and bilateral symmetrical hydrocephalus at the level of the lateral ventricles. Lymphoid follicular hyperplasia in the spleen and lymph nodes suggested an ongoing immune system stimulation. In the liver, there was multifocal, individualized hepatocyte necrosis and minimal periportal lymphocytic infiltration.

**Conclusions:** We describe a rare combination of congenital forelimb deformities in a dog, enriching the congenital pathology literature, often underdiagnosed or not reported.

#### CLINICOPATHOLOGICAL INVESTIGATION OF GALLBLADDER ULCER IN DOGS WITH COMPARISON TO GALLBLADDER MUCOCOELE AND CHRONIC CHOLECYSTITIS

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**Introduction:** Gallbladder ulcer (GU) manifests with acute to subacute onset of severe clinical signs usually in dogs that are on follow-up for asymptomatic gallbladder abnormalities such as gallbladder mucocele (GM) or chronic cholecystitis (CC). Understanding the aetiopathogenesis of GU is important for a better clinical approach towards prevention of the condition.

**Materials and methods:** We retrospectively investigated surgical specimens of 14 GU, 5 GM and 5 CC cases of client-owned dogs in Japan, using clinical data, histopathology, histochemistry (Giemsa, Warthin–Starry, high iron diamine/alcian blue (HID-AB) pH2.5, and PTAH stains), and immunohistochemistry (COX-1, COX-2).

**Results:** Lethargy, vomiting and abnormal liver-related blood chemistry were common among GU patients. Open cholecystectomy was performed 7.35 days on average (range: 0 to >30 days) after the onset of clinical signs among GU patients, while the gallbladders of GM and CC patients were often electively removed. Gallbladder rupture was reported in five dogs in the GU cohort only. The average gallbladder wall total thickness in GU patients was 1,685 µm (i.e. 3 times higher than in normal gallbladders). Bacteria were detected in 8/14 GU, 5/5 GM and 0/5 CC cases. Expression of COX-1 and COX-2 by gallbladder epithelial cells was infrequent in GU cases but common in the GM and CC groups. HID-AB stain yielded equivocal results.

**Conclusions:** Based on COX-1 and COX-2 expression patterns, GU may be associated with alterations in the arachidonic acid cascade in mucosal epithelial cells. Further studies to elucidate the gene and protein expression profile of inflammatory mediators in these cells would be of value.

#### SPONTANEOUS NEOPLASIA IN RHINOCEROTIDAE: COMBINED RETROSPECTIVE CASE SERIES AND LITERATURE REVIEW

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**Introduction:** The family Rhinocerotidae includes five near-threatened to critically-endangered species (IUCN Red List). Scientific literature around rhino neoplasia is sporadic and unreviewed.

**Materials and methods:** Fifty-nine neoplasms from 49 rhinos were reviewed from IZVG Pathology (n = 7 individuals) and current scientific literature (18 peer-reviewed publications, using Google Scholar/PubMed keywords “rhinoceros”, “tumor”, and “neoplasia”). Species included greater one-horned (n = 27), southern white (n = 15), southern black (n = 4), Sumatran rhinoceros (n = 2) and non-specified (n = 1).

**Results:** In 20.3% of individuals, neoplasia led to death or was life-threatening without treatment. 6.8% of all tumours metastasized (no organ predisposition). Benign/malignant tumours comprised 55.9/42.4% respectively. Primary female non-ovarian reproductive tract (NORT)

tumours were most frequent (57.6%; 12.1% malignant) followed by cutaneous squamous cell carcinomas (SCCs) (13.6%). Of nine SCCs, eight affected southern white rhinos. By species the commonest tumours were: greater one-horned - NORT leiomyoma (79.3%); southern white - SCC (35%); southern black - melanocytic tumours (2/4); and Sumatran - NORT leiomyoma (2/3). Southern black rhinos were younger (average 8.4 years) and mostly male with malignant tumours (acute lymphoblastic leukaemia, cutaneous malignant melanoma, malignant seminoma). Greater one-horned (average 22.9 years) and Sumatran rhinos were mostly females with NORT leiomyomas (87% and 2/3 tumours, respectively). White rhinos (average 39 years) exhibited a 50:50 sex ratio and 70% malignant tumours (eight SCCs, two malignant seminomas and single cases of ovarian adenocarcinoma, prostatic carcinosarcoma, adrenocortical carcinoma and cutaneous soft tissue sarcoma) and a 10% metastatic rate.

**Conclusions:** NORT leiomyomas and SCCs are most common in greater one-horned and southern white rhinos, respectively. Metastatic neoplasia appears rare.

#### CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF FOLLICULAR GASTRITIS (LYMPHOID FOLLICULAR HYPERPLASIA) IN DOGS

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**Introduction:** Follicular gastritis (FG) or lymphoid follicular hyperplasia is characterized by the presence of large lymphoid nodules (LNs) in the lamina propria of the stomach. Its cause, mechanisms and immunohistochemical features in dogs are still unknown.

**Materials and methods:** In this study, tissue samples from 34 dogs with FG were included. The clinical and endoscopic aspects were evaluated. Tissues were routinely processed and stained with H&E, Giemsa and primary antibodies recognizing CD3, PAX5, Ki67, Bcl-2, Bcl-6 and *Helicobacter pylori*.

**Results:** Young French Bulldogs were the most affected. *Helicobacter* organisms were identified in 74% of cases. Endoscopy revealed small nodules, ranging from 2–5 mm. Microscopically, the LNs were distributed as follows: 9% in the antrum, 9% in the body and 81% were disseminated. Lymphoplasmacytic (27/34) and eosinophilic (4/34) gastritis were the main associated changes. The large-sized active follicles were composed of PAX5+ B lymphocytes in the centre and CD3+ T lymphocytes at the periphery. Small LNs were composed of CD3+ T cells. Furthermore, germinal centres showed a variable Ki67 expression (8.67–51.05%) and strong Bcl-6 immunolabelling, but were negative for Bcl-2. Pearson's test showed a moderate positive correlation between the size of LNs and the grade of *Helicobacter* colonization ( $r(32) = 0.59$ ,  $P = 0.000177$ ) and a negative correlation between the size of LNs and associated gastritis ( $r(32) = -0.1022$ ,  $P = 0.5659$ ) and the grade of *Helicobacter* colonization and associated gastritis ( $r(32) = -0.0649$ ,  $P = 0.71$ ).

**Conclusions:** French Bulldogs are predisposed to FG. In this species FG lesions are commonly associated with lymphoplasmacytic inflammation. *Helicobacter* spp infection may play a role in the development of FG.

#### A COMBINED APPROACH FOR DETECTION OF SMALL RUMINANT RETROVIRUS CO-INFECTIONS

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**Introduction:** Results of a project on gross changes in slaughtered animals alerted us of the likely underreporting of Jaagsiekte retrovirus (JSRV)-induced ovine pulmonary adenocarcinoma (OPA) in Switzerland.

Furthermore, ovine lungs with OPA frequently exhibited lesions suggestive of Maedi Visna virus (MVV) or caprine arthritis encephalitis virus (CAEV) infection, indicating that co-morbidities might occur.

**Materials and methods:** Lungs and pulmonary lymph nodes were sampled from suspected OPA cases, inflammatory lung lesions and control lungs (total of 110 cases). Tissues were (a) processed for histology and immunohistology (IH), and (b) underwent DNA extraction and real-time PCR for JSRV, MVV and CAEV. Peptide sequences were used to generate virus-specific customized polyclonal antibodies. PCR-positive OPA cases and formalin-fixed and paraffin-embedded MVV- and CAEV-infected synovial cell pellets served as positive controls.

**Results:** Fifty-two lungs were histologically diagnosed with OPA. Histological evidence of MVV/CAEV infection was seen in 25 lungs. JSRV was detected by qPCR in 85% of the suspected OPA cases; six were co-infected with MVV, one with CAEV. MVV was detected by qPCR in 14 cases; four lungs were positive for CAEV, three had MVV/CAEV co-infection. JSRV was detected by IH in 91% of the PCR-positive cases, whereas MVV and CAEV immunoreactivity was seen in 79% and 25% of PCR-positive lungs, respectively.

**Conclusions:** Although qPCR has a higher sensitivity than IH, the combined approach allows investigations on viral cell tropism and pathogenic processes in co-morbidities, including their potential interdependency.

#### PULMONARY BLEEDING IN RACEHORSES: A GROSS AND HISTOLOGICAL COMPARISON OF EXERCISE-INDUCED PULMONARY HAEMORRHAGE AND EXERCISE ASSOCIATED FATAL PULMONARY HAEMORRHAGE

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**Introduction:** Exercise-induced pulmonary haemorrhage (EIPH) is a common condition in Thoroughbred racehorses that is usually responsible for reduced performance, while exercise associated fatal pulmonary haemorrhage (EAFPH) is characterized by severe pulmonary bleeding resulting in sudden death during strenuous exercise.

**Materials and methods:** Anamnestic data together with pulmonary gross and histological changes (haemorrhage, haemosiderophages, iron encrustation, vascular remodelling, type II pneumocyte hyperplasia and bronchiolar inflammation) were compared semiquantitatively in racehorses with EIPH (n = 10), EAFPH (n = 10) and control horses (n = 5).

**Results:** No differences in anamnesis were identified between the three groups. Grossly, cranial lobe reddening and oedema were significantly more prevalent and severe in the EAFPH group compared with the EIPH and control groups. Histologically, haemorrhage scores were higher in the EAFPH group, while haemosiderophages, iron encrustation of collagen and elastin fibres and vascular remodelling scores were significantly higher in the EIPH group compared with the EAFPH and control groups (except the control group for the vascular remodelling). Haemosiderophages and iron encrustation in EIPH horses were more prevalent in the intralobular small veins and in the pulmonary arteries, compared to other micro-compartments.

**Conclusions:** This study demonstrates that lungs of horses that experienced EAFPH show significantly less vascular remodelling and other chronic pulmonary changes that instead characterize horses with EIPH.

#### DIETARY INCLUSION OF OREGANO ESSENTIAL OIL IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) DIET: EFFECT ON GROWTH PERFORMANCE, SKIN AND INTESTINAL MORPHOLOGY, ANTIOXIDANT STATUS, AND FILLET FATTY ACID PROFILE

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