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**Letter to Editor**

# CONSTRAINTS OF BLACKLEG CONTROL IN NIGERIA

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Sir, blackleg (also known as symptomatic anthrax, blackquarter or emphysematous gangrene) is a disease of cattle, sheep and other ruminants (1). In Nigeria, the disease was first reported in 1929 and has remained a major problem of cattle in the country (2). Infective spores of *Clostridium chauvoei* ingested during grazing are lodged in the gastrointestinal tract (GIT), livers and spleens of healthy cattle (3) and remain latent until their germination is triggered by punctured wounds (4). During growth, the organism is known to produce neuraminidase (sialidase), an enzyme that plays a key role in the pathogenesis of blackleg (5). Although Agba and Princewill (1986) (6) put the economic losses of cattle due to blackleg in Nigeria at less than a million United States Dollars (USD) annually, the current losses to the disease may approximate about 4.3 million USD annually (7). This is because of increased annual outbreaks over the years and the deregulation of Nigeria's economy. This letter to the Editor highlights the constraints associated with the control of blackleg of cattle in Nigeria and the possible ways of ameliorating the constraints.

The nomadic Fulani pastoralists of rural Nigeria, who own about 70-80% of livestock in the country, migrate from one place to another in search of grazing pasture for their livestock (8). It therefore follows that as they migrate, they encounter soils with high proportion of clostridial spores that constitute a health hazard. It is common knowledge that the best control strategy against blackleg is vaccination (9). Most times, potent vaccines are difficult to come by in Nigeria, because of the inability to maintain the cold chain. The nomads a times purchase the vaccines on their own from veterinary shops, without any machinery in place to maintain the cold chain. This practice has made it difficult to effectively control the disease in Nigeria. In line with this, therefore, some state governments in Nigeria do not vaccinate animals routinely against blackleg, because the nomads do not request for it, except in times of disease outbreaks. Even in the face of outbreaks, the attitude of the nomads in the control of disease spread to neighbouring herds leaves much to be desired. They do not report the outbreaks and may chose to move away from the area.

The drug of choice for treating blackleg is penicillin (7) but the nomads prefer the use of herbal remedies to treat the disease. They may report outbreaks to veterinarians and government officials only if the herbal remedies do not achieve the desired therapeutic results, accompanied by an upsurge of mortality which they can not control. Two herbal remedies (*Tamarindus indicus* and *Combretum fragrans*) are preferred to penicillin by the nomads for treating blackleg (10). The side effects of herbal preparations have been identified and they include: inappropriate dosing (11), intoxication leading to death of treated animals (12) or the problem of partial efficacy associated with some herbal remedies (13).

The control of blackleg has remained a major problem in Nigeria, since the nomads of rural Nigeria who are key players in the livestock industry are not settled and continue to move from one *Clostridium* infected soil to the other in search of grazing pasture. It is recommended that animal ranches (settled farms) should be established in areas free of clostridial spores. This is because of the danger posed by this on the health of animals and the role of the spores in the pathogenesis of blackleg. To effectively control the disease in cattle, vaccination of the animals using potent vaccines has been advocated. It is concluded that, while government should be prevailed upon to revive the available grazing reserves to settle the Fulanis, there is the need to encourage the use of the herbal remedies, as they are cheaper, effective and available in Nigeria. Research should be conducted to establish the dosage regiments, therapeutic index and side effects of these herbs.

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**Case Report**

# ADVANCED PERIODONTAL DISEASE IN A YORKSHIRE TERRIER WITH CONCURRENT NASAL CAVITY MALIGNANCY

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**Summary:** An eleven-year-old male Yorkshire terrier weighing 2.6 kg with a primarily indoor lifestyle was presented for cardiopulmonary examination due to a 6-month history of difficulty breathing. No cardiopulmonary abnormalities were detected but the history included bilateral serous nasal discharge and oral abnormalities were evident. Examination under general anaesthesia confirmed advanced periodontal disease with oronasal fistulae detected at the maxillary canine teeth. Dental treatment, including repair of the oronasal fistulas, appeared to resolve the respiratory signs but the discharge re-appeared at the left nostril 1 month later. There was no evidence of persistent or recurrent oronasal fistula, so rhinoscopy was performed identifying a mass in the left nasal cavity. Histopathological examination identified the biopsy specimen as a low-grade predominantly papillary-cystic adenocarcinoma combined with transitional carcinoma.

**Key words:** periodontal diseases; rhinitis; nose neoplasms – pathology; adenocarcinoma; dogs

## Introduction

Nasal discharge is a common problem in dogs (1). It may be the result of nasal or paranasal disorders or be related to systemic disease (1). In older animals chronic nasal discharge is commonly due to extension of dental disease to involve the nasal cavities (oronasal fistula or tooth root abscessation) or neoplasia (1, 2). Other differentials include fungal infection, chronic foreign bodies, allergic and non-specific rhinitis as well as systemic disease (1, 3). Both fungal rhinitis and nasal foreign bodies tend to be seen in dogs that spend a lot of time outdoors (1). Except for viral infections and systemic diseases, the nasal discharge in animals with nasal conditions is usually unilateral or may become bilateral with disease progression (1).

The case presented here illustrates some of the difficulties in diagnosis and treatment of nasal conditions in dogs.

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## Clinical case

*Presentation*

An eleven-year-old male Yorkshire terrier weighing 2.6 kg with a primarily indoor lifestyle was referred to the Clinic for Surgery and Small Animal Medicine of the Veterinary Faculty in Ljubljana for cardiopulmonary examination in January 2007. On presentation at the clinic the dog had a six month history of difficulty breathing, especially during the night and the owner mentioned a serous nasal discharge, more pronounced from the left nostril. The dog had been treated with furosemide (Edemid; Lek Ljubljana, Slovenia; 1mg/kg/day p.o.) and ramipril (Tritace; Aventis Pharma, Austria; 0.5 mg/kg/day p.o.) for one month prior to referral. No other problems were reported in the history. Complete blood count (CBC) values were within normal limits. On auscultation no cardiac abnormalities were detected, lung sounds and pulse were normal, but there was a pronounced expiratory stertor due to partial nasal airflow obstruction. Oral examination revealed the presence of advanced periodontal disease. As the dog's problem appeared to be related to the upper airway, cardiopulmonary examinations were post-

poned and the previously prescribed cardiologic treatment was discontinued pending the results of upper aerodigestive tract examination. The dog was scheduled for general anaesthesia the next day to permit a more thorough oral examination and treatment of the dental disease.

*Anaesthesia*

The dog was premedicated with methadone (Heptanon; Pliva, Croatia; 0.4 mg/kg i.m.) and carprofen (Rimadyl; Pfizer Animal Health S.A., UK; 4 mg/kg i.v.) prior to induction of anaesthesia using propofol (Propofol 1% Fresenius; Fresenius Kabi, Austria; 7.5 mg/kg i.v.). Following endotracheal intubation, anaesthesia was maintained with isoflurane (Forane; Abbott Laboratories Ltd., GB) given to effect (approximately 1.5%) in oxygen (2 l/min) using a Mapleson F anaesthetic circuit. Amoxicillin and clavulanic acid (Synulox; Pfizer Italia S.r.l., Italia; 20 mg/kg s.c.) was administered preoperatively as the start of a 10 day course of treatment (20 mg/kg/12h p.o.), carprofen treatment also being continued for 5 days (4 mg/kg/day p.o.) to maintain analgesia during the postoperative period. During anaesthesia body temperature, respiratory rate, inspired and expired isoflurane, heart rate, ECG, pulse oximetry, end tidal $CO_2$ and blood pressure with Doppler manometer were monitored. During the procedure and recovery from anaesthesia fluid homeostasis was maintained by administration of Ringer's lactate solution 26 ml/h (Hartman's solution; B.Braun Melsungen AG; Germany) i.v.

*Oral findings*

The dog's oral cavity was assessed by means of periodontal examination and recording, plus radiography of disease affected areas. Tooth presence, probing depth, periodontal attachment loss, furcation involvement and tooth mobility were graded. Supra- and subgingival scaling were then performed, followed by polishing and gingival lavage with water, prior to extraction of compromised teeth.

Oral examination revealed advanced periodontal disease with generalised plaque and calculus accumulation. Many teeth were already missing. Oronasal fistulae were detected palatal to both maxillary canine teeth with deep periodontal pockets being present buccally; probing depths were 10 mm on the left and more than 12 mm on the right. Generalised gingival recession of about 2 mm was detected affecting those mandibular premolar and molar teeth that were still present. Of the remaining incisor teeth, only the left maxillary third incisor tooth was stable, the rest being highly mobile. There was generalized bleeding on periodontal probing, due to the extent of gingivitis and periodontitis.

*Dental treatment*

Left and right infraorbital and mental nerve blocks were performed using bupivacaine (Marcaine 0.5%; AstraZeneca, UK; 0.05 ml per site). Gross deposits were removed from the teeth and the oral cavity rinsed thoroughly prior to extraction of compromised teeth. Extraction was performed after sectioning of multirooted teeth (using cutting burs in a high-speed dental handpiece with copious water spray). A combination of closed elevation and luxation of mobile single-rooted teeth/tooth segments, and open extraction technique, raising muco-gingival access flaps with or without alveolar bone removal as required to facilitate elevation/luxation of the remaining teeth/roots. All the right maxillary incisor teeth, the canine, the right maxillary first, second and third premolar teeth, the left maxillary second incisor tooth, all the mandibular incisor teeth and the right mandibular canine tooth were extracted. There was extensive bleeding while raising mucogingival access flaps in the severely inflamed gingival tissues leading to significant blood loss (estimated from the number of swabs used and their degree of blood saturation to about 40 ml) resulting in a blood pressure drop which required use of a plasma expander (6% HES; Fresenius Kabi Deutschland GmbH, Germany; 10 ml bolus and Hartman's solution 50 ml/kg/h). In view of this, and a drop in the body temperature (to 35 °C), it was decided not to complete the treatment in a single session but to stage it with the aim of performing extractions of the remaining compromised teeth at a later date.

*Second presentation and dental treatment*

One and a half months after the initial treatment the dog was generally well, however, there was still a serous nasal discharge from the left nostril and an oronasal fistula was still present palatal to the left maxillary canine tooth. All CBC values were still within normal limits so, two months after the first treatment (March 2007), the dog was re-examined under general anaesthesia, using the same protocol as previously. Oral examination revealed severe plaque accumulation, however, gingival recession previously affecting the mandibular premolar and molar teeth has healed only after scaling and polishing, as had the mucogingival access flaps, al-

though the monofilament resorbable suture material (Biosyn 5-0; United States Surgical, USA) was still present. After thorough oral cleaning (scaling and polishing), the left maxillary canine tooth, left mandibular canine tooth and left mandibular first premolar tooth were extracted using the techniques described previously, the oronasal fistula being closed with a single layer mucogingival flap. A thorough examination of the larynx, pharynx, soft palate and caudal nasal cavity using a dental speculum, mirror and retractor was performed, but no additional abnormalities were found. Due to the presence of the oronasal fistula the dog was maintained on amoxicillin and clavulanic acid (20 mg/kg/12 hours p.o.) for 10 days following the dental treatment, with carprofen (4 mg/kg/day) also being given for the first 4 days.

### *Further presentations and diagnostic procedures*

One month after the second dental treatment (April 2007) the owner reported reappearance of the serous nasal discharge from the left nostril, it having stopped shortly after the previous treatment, and mild difficulties breathing. As no recurrence of the oronasal fistula was detected on clinical examination, the owner agreed to have another examination under general anaesthesia, but the owner scheduled this for 1 month later (May 2007). At this time all CBC values, urea, creatinine, alkaline phosphatase and alanine aminotransferase were still within normal limits. Examination under general anaesthesia (induced and maintained as previously, but without antibiotics and carprofen) revealed no abnormalities in the oral cavity. Radiographs of the head (lateral, open-mouth and intra-oral occlusal dorsoventral projections) were obtained but were not diagnostic. Rhinoscopy with a 2.7 mm rigid endoscope passed via the nostrils was performed revealing no abnormalities in the right nasal cavity, however, in the left nasal cavity at a depth of approximately 3 cm there was a mass estimated to be 1 $cm^3$ in size, appearing to be based caudally. The surrounding nasal tissues were visibly inflamed. Nasal flush was performed to clear any discharge before biopsy to obtain material for histopathology. As the drainage lymph nodes were small no attempt was made to perform fine needle aspiration at this stage, invasive biopsy remaining an option if the nasal biopsy confirmed neoplasia. The dog was discharged with a course of meloxicam for analgesia (Metacam; Boehringer Ingelheim Vetmedica GmbH, Germany; 0.1 mg/kg/day p.o.).

### *Histopathology results and further treatment*

Histopathology results revealed an inflamed low-grade malignant nasal tumour, composed of two distinct subtypes predominant papillary and cystic adenocarcinoma with mucus secretion and formation of small cysts (Figure 1) and a smaller part of the transitional carcinoma, which is also referred to as respiratory epithelial carcinoma or nonkeratinizing squamous cell carcinoma (Figure 2) (4). The adenocarcinomatous part was mostly composed of

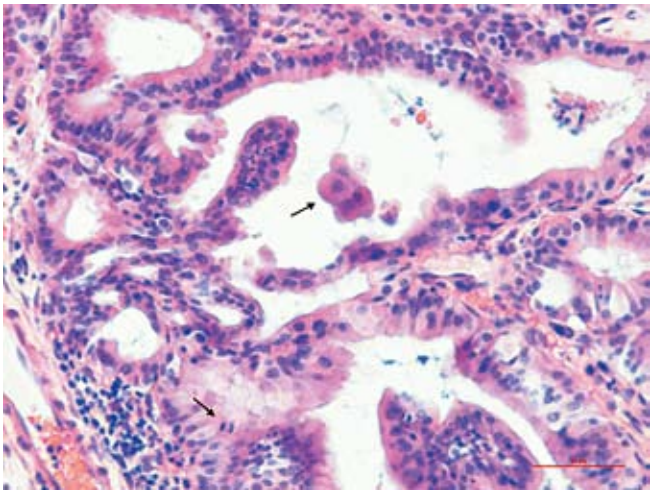

**Figure 1:** Papillary cystic adenocarcinoma. Part of the tumour shows a less well differentiated tall columnar epithelium with mild cellular and nuclear pleomorphism. A group of pleomorphic epithelial cells can be seen in the middle (arrow) and a mitotic figure in the left lower corner (arrow). There is abundant lymphocytic infiltrate and neutrophils in the stroma. HE staining, x200

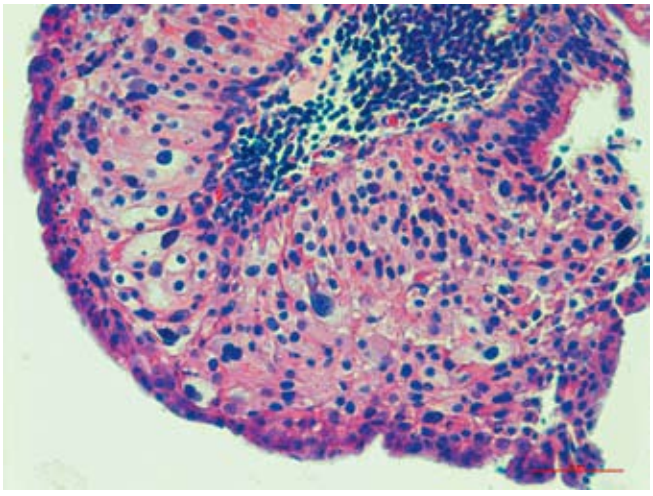

**Figure 2:** Transitional carcinoma. Cellular and nuclear pleomorphism is clearly evident. Abundant lymphocytic infiltration can be seen in the stroma. HE staining, x200

well differentiated cuboidal or tall columnar epithelial cells with mild pleomorphism, only limited areas showing greater pleomorphism. The cells of a transitional carcinoma subtype revealed greater variability of cell shape and size, from smaller basaloid cells to larger tall columnar and spindle-shaped or polygonal cells with moderate amount of pale eosinophilic cytoplasm and hyperchromatic nuclei containing one or two, and rarely several, small nucleoli. Mitotic figures were rare in both parts.

The dog was much better while on meloxicam and the owner was advised to proceed with staging. In view of the final diagnosis the owner was offered referral for a CT scan prior (3, 5) to possible radiation therapy.

## Discussion

Periodontal disease is the most common chronic infectious disease in dogs affecting a majority of the mature population (6, 7), with small breeds being predisposed to it (8). Tumours of the nasal and paranasal sinuses are rare in most domestic species but are recognised most frequently in dogs. The prevalence, however, is only 0.3 to 2.4%, with medium to large dolichocephalic breeds being more often affected. The higher risk associated with a long nose may be related to the larger surface area of nasal epithelium and with the filtering capability, although a genetic basis in some breeds is also suspected (9). Incidence of nasal tumours also increases with age of the dog, the mean age being reported as 9 to 10 years (10, 11). Despite the low prevalence, however, Tasker (12) reports that neoplasia is the most common diagnosis in dogs with persistent nasal disease (one third of cases), where as periodontal disease is only recognized as the cause in 10% of cases. Adenocarcinoma is the most frequent malignant nasal tumour recognized in dogs (9, 10) with transitional carcinoma being the second most common, both being rare or not reported in other animal species (4). Acinic cell carcinoma or even neuroendocrine carcinoma can not been ruled out completely in this case as differentiation requires immunohistochemistry, which was not performed. As neuroendocrine carcinoma is an uncommon sinonasal tract neoplasm with aggressive clinical behaviour (13, 14) this differential diagnosis was not consistent with clinical and histomorphological findings in the presented case.

The diagnostic approach to a patient with nasal discharge includes obtaining a complete history supported by thorough physical examination and routine blood tests to rule out systemic disease (1). If the results are normal as in the presented case, complete oral examination is the next step as well as nasal swabs for cytology and culture if fungal disease is suspected, before proceeding to imaging and rhinoscopy with biopsy sampling (1). However, blood test results may be normal in dogs with nasal neoplasia as paraneoplastic disorders associated with nasal tumours are rare in dogs (10).

As advanced periodontal disease with oronasal fistulae at the maxillary canine teeth was detected in this case, the chronic serous bilateral nasal discharge was suspected to be of dental origin, especially when the nasal discharge completely disappeared on the right side after the first dental treatment (extraction of the right maxillary canine tooth and closure of the fistula at that site), but remained on the untreated site. As the discharge from the left nostril also temporarily disappeared after extraction of the left maxillary canine tooth and antibiotic and anti-inflammatory treatment, dental disease must have had an influence on the nasal discharge, though the improvement may have been due to suppression of secondary bacterial infection in the nasal cavity with the use of antibiotics (11). After unilateral recurrence of the signs, inadequate healing of the oronasal fistula was considered the most likely differential diagnosis (15). Once this was ruled out further investigation was required to identify the cause. Nasal neoplasia most often presents initially with unilateral nasal discharge, epistaxis, epiphora and facial deformity occurring in more advanced cases (11). When there is only serous discharge and expiratory stertor, as our case, chronic rhinitis and nasopharyngeal dysfunction have also to be considered.

It is impossible to say, what the primary disease was or if there is any link between the two diseases in the present case as periodontal disease is extremely common in older small breed dogs and adenocarcinoma, although not a common condition, is seen most often in older dogs. Both conditions have chronic courses, clinical signs persisting for months (1, 6, 10, 11). It is well established that chronic inflammation and/or infection with certain organisms (particularly toxin producing spirochetes) prediposes to carcinoma in certain sites but there have not been any reports suggesting a link between periodontitis and nasal carcinoma (16, 17). Oral lichen planus, a chronic inflammatory disease seen in man is reported to be clinically associated with

development to oral cancer, most likely due to oxidative and nitrative DNA damage caused by chronic inflammation (17-19). Therefore it is possible that the two diseases in the reported case could be related as it has been established that cancer can be promoted and/or exacerbated by inflammation and infections and vice versa (17, 20, 21). The cytokines produced by activated innate immune cells are reported to be important components in this linkage (20, 22-24), many of them are also elevated in periodontal disease (25). TNF-α can contribute to tumour initiation by stimulating production of genotoxic molecules, such as nitric oxide (NO) and reactive oxygen species (ROS), that can induce DNA damage and mutations (20). Stimulated polymorphonuclear leucocytes (PMN), increased in periodontal disease, can also produce reactive oxygen and nitrogen species (ROS, RNS) (17, 25). In periodontal disease there is also a marked production of NO in gingival tissues (26-28). Additionally, activation of toll-like receptors (TLR) on host macrophages or directly on tumour cells by bacterial lipopolysaccharides (LPS), which leads to the activation of NF-κB signaling pathway can enhance tumour development and to a lesser extent tumour regression as well (17, 20, 29). Bacteria can add to cancer development also by production of carcinogenic metabolites (16).

After diagnosing the nasal mass, the dog was placed on meloxicam as the drug not only relieves pain and inflammation but also has the potential to slow the progression of some tumours this effect being suggested to be through the selective inhibition of cyclooxygenase-2 (COX-2), which is upregulated in epithelial nasal tumours (30, 31). Whilst side effects may occur with long term use of meloxicam (32), the risk is so low that it does not outweigh the potential benefits of its use. The owners of the dog have been recommended to consider more definitive treatment for the tumour, i.e. radiation therapy, as this currently provides the best results for nasal carcinomas (10).

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## NAPREDOVALA PARODONTALNA BOLEZEN IN MALIGNI TUMOR NOSNE VOTLINE PRI JORKŠIRSKEM TERIERJU

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**Povzetek:** Enajst let star, 2,6 kilograma težek jorkširski terier, ki živi pretežno v stanovanju, je bil napoten na kardiološki pregled zaradi 6 mesecev trajajočih težav z dihanjem. Pri pregledu kardiopulmonarne težave niso bile ugotovljene, pes pa je imel težave zaradi kroničnega seroznega nosnega izcedka ter sprememb v ustni votlini. Pregled ustne votline v splošni anesteziji je potrdil sum napredovale parodontalne bolezni z oronazalnima fistulama ob zgornjem desnem in levem grabilcu. Serozni nosni izcedek se je ponovil 1 mesec po sanaciji ustne votline, tokrat le iz leve nosnice. Ker oronazalne fistule ni bilo, je bila izvedena rinoskopija z odvzemom tkivnih bioptov mase v levi nosnici. Patohistološko je bil v levi nosni votlini dokazan maligni tumor, papilarno-cističen adenokarcinom, kombiniran s prehodnim karcinomom.

**Ključne besede:** parodontalne bolezni; rinitis; nos, novotvorbe – patologija; adenokarcinom; psi

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**Case Report**

# GENERALIZED SYMMETRIC ALOPECIA AND HYPEROESTROGENISM ASSOCIATED WITH CONCURRENT LYMPHOMA, SERTOLI CELL TUMOUR AND SEMINOMA IN A SAMOYED

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**Summary:** A 10-year-old Samoyed with unilateral right cryptorchidism was referred to the Clinic for Small Animal Medicine and Surgery, Veterinary Faculty of Ljubljana University, due to a symmetrical, noninflammatory, mildly pruritic alopecia of 6-month duration. It had a history of diarrhoea, which responded to cyclosporine treatment. Lymphoma and testicular neoplasia were suspected based on ultrasonography and fine needle aspiration cytology. The dog was castrated and splenic and gastric lymph node biopsies were obtained. Histopathology revealed three different tumours: Sertoli cell tumour and seminoma were present in the right inguinal testicle, and B cell lymphoma was present in the spleen and lymph node. After two months when the peripheral lymph nodes were considerably enlarged and the owners declined chemotherapy, they were advised to start corticosteroid treatment. Three months after the castration, the hair coat looked normal. Four months after the castration, the dog was euthanized at the owner's request by the referring veterinarian due to a lymphoma related disease.

**Key words:** unilateral cryptorchidism; testicular neoplasms – surgery – chemotherapy; ultrasonography; cytodiagnosis; biopsy, fine-needle; dogs

## Introduction

Noninflammatory alopecias are quite common in dogs and can be congenital, hereditary or acquired. The latter can result from endocrinopathies (e.g. hyperadrenocorticism, hypothyroidism, sexual imbalance), telogen or anagen effluvium, metabolic imbalance (disruptions in protein or fatty acid metabolism) and idiopathic disturbances of the hair growth cycle (1).

Testicular tumours comprise 4-7% of all tumours in male dogs (2). They arise either from Sertoli cells (i.e. Sertoli cell tumours), germinal epithelium (i.e. seminoma) or interstitial cells (i.e. Leydig cell tumours) (3), all of which occur with equal prevalence (2, 4). Other primary tumours may also arise, but are extremely rare. Dogs with non-descended testicles have an increased risk of up to 13.6 fold to develop a testicular tumour compared to dogs with descended testicles (5). Since right-sided cryptorchidism is more common, the right testis is more often affected (4). Multiple tumours within one testis occur in up to 46% of the cases and are also described in macroscopically normal testeses (6). Hyperestrogenism occurs in 25-50% of dogs with Sertoli cell tumour and less frequently with seminomas, and may lead to signs of feminization (7). It can lead to bone marrow hypoplasia with consecutive thrombocytopenia, non-regenerative anaemia and granulocytopenia. Serum testosterone and progesterone concentrations may be increased, but usually testosterone concentration is low to undetectable (5). As these changes may be subtle and are not specific and sensitive changes, castration with a histopathological examination is still the diagnostic and therapeutic approach of choice (8). An alternative and perhaps more sensitive marker for canine Sertoli cell tumour is increased serum inhibin concentration (9).

Malignant lymphoma is the most common canine haemato-lymphatic tumour, most commonly classified based on anatomic location, clinical stage or immunophenotype. The multicentric form with

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peripheral lymphadenopathy is the most common form, followed by the alimentary, mediastinal and extranodal (kidneys, skin, and brain) forms (10-12). The aetiology of canine lymphoma is unknown, but hypotheses include retroviral infection, exposure to herbicides and magnetic field, chromosomal abnormalities and immune dysfunction (13).

## Case report

A 10-year-old, intact male Samoyed with unilateral right cryptorchidism was presented at the Clinic for Small Animal Medicine and Surgery, Veterinary Faculty of Ljubljana University for an investigation of a symmetric alopecia of 6-month duration. It was currently vaccinated and dewormed and has lately gained weight although water and food intake seemed unchanged. The dog had a history of large bowel diarrhoea eight months before the presentation at our clinic, which was treated with metronidazole and spiramycin for 1 month without obvious improvement. This was followed by cyclosporine treatment for 40 days, which led to complete recovery, however, dermatologic abnormalities were observed. Initially, alopecia was present only in the neck region, and later symmetrically spread to the axillae, abdomen as well as the inguinal and tail areas. Mild pruritus was observed during the disease course. An abdominal ultrasound performed by the referring veterinarian did not reveal abnormalities. Serum hormone concentrations were determined and included free T4 (1.1 ng/dl, reference range 0.6 – 3.7), cTSH (0.13 ng/ml reference range <0.40). testosterone (0.0667 ng/ml, reference range 0.3 – 5.8)[1] and 17-β-oestradiol (87.9 pg/ml, reference range <15 (9)).

Physical examination revealed, in addition to the alopecia (Figure 1 and 2) and cryptorchidism, a bilateral purulent ocular discharge. Skin scrapings for *Sarcoptes* and *Demodex* were negative. Cytology of the skin was positive for *Malassezia*. Faecal examination was positive for *Giardia*. CBC (Bayer Technicon H*1, Bayer-Technicon, Germany) and serum biochemistry analysis (biochemical analyzer Technicon RA-XT, Bayer Technicon, Germany) including glucose, urea, creatinine, calcium, phosphorous, alkaline phosphatase, alanine-aminotransferase and cholesterol) were unremarkable, with exclusion of a mild normocytic normochromic anaemia (haematocrit 0.35 l/l, reference range 0.37-0.55 (14)).

[1] References values of the Vet Med Lab laboratory in Trieste, Italy.

**Figure 1:** Skin at the beginning of the therapy

**Figure 2:** Skin at the beginning of the therapy

**Figure 3:** Hair re-growth after 3 weeks of the hypoallergenic diet and essential fatty acid supplementation

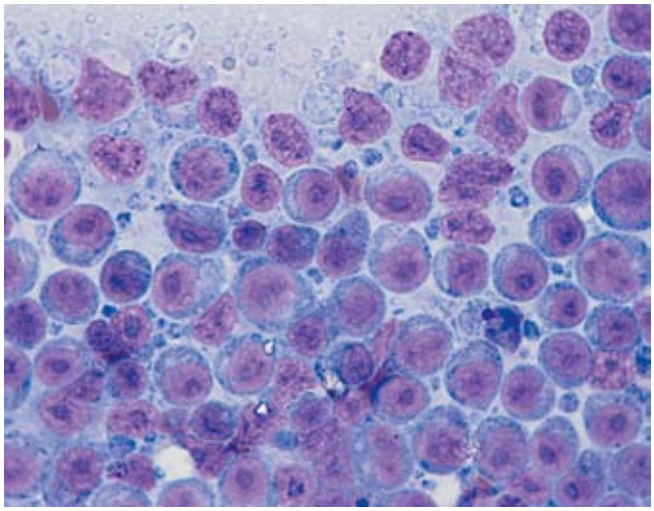

**Figure 4**: Lymphoblasts in the spleen (×1000)

**Figure 5:** Dog three months after surgery

**Figure 6:** Dog three months after surgery

Treatment was initiated using anthelmintics praziquantel 5 mg/kg and fenbendazole 50 mg/kg (Zantel, CAM) PO q24h for 5 days, ketoconazole 14 mg/kg PO q24h (Oronazol, Krka) and topical ophthalmic gentamicin (Garamycin, Krka) ointment bilaterally q12h. After one month, there was no improvement and total serum T4 and TSH analyses were repeated (30.6 nmol/l and 0.47 ng/ml, respectively). A hypoallergenic diet (Eucanuba FP dermatosis and homemade diet (horse meat, rabbit meat, fish) and an oral omega 3 and omega-6 fatty acids preparation (Dermanorm, Vetoquinol, 2 capsules/dog PO q24h) were prescribed for three weeks, when an improvement in general health and growth of hair was seen (Figure 3). The same therapy was continued for 2 additional months. At that time, the dog was rechecked due to deterioration in the condition of the skin. It had pruritus, alopecia, erythema, ventral hyper- and hypopigmentation and dorsal hypotrichia, seborrhoea and crusts. An abdominal ultrasound revealed nonhomogeneous splenomegally and nonhomogenous masses: two cranio-dorsal to right and two cranio-dorsal to the left kidney. These masses were interpreted as lymph nodes by the radiologist. The testicle present within the scrotum had a hypoechoic discrete nodule of 8 mm diameter. A nonhomogeneous 3×4 cm mass in the right inguinal area was observed. Other abdominal organs were ultrasonografically normal. A fine needle aspiration of the inguinal mass (probably testicle) and the spleen was performed. The first was non-diagnostic while the latter revealed a population of large lymphoid cells with large nuclei, scant deeply blue cytoplasm, some of which had a pronounced perinuclear reactive zone and 1-3 prominent nucleoli of different size and shape (Figure 4).

According to these findings we planned to castrate the dog and take samples of the spleen, enlarged lymph nodes and skin.

A preoperative CBC showed a mild normochromic normocytic nonregenerative anaemia (RBC 5.02 × $10^{12}$/l, reference range 5.5-8.5 × $10^{12}$/l; haemoglobin 115 g/l, reference range 120-180 g/l; haematocrit 0.32 l/l, reference range 0.37-0.55 l/l) and mild thrombocytopenia (platelets 141 × $10^{9}$/l, reference range 200-500 × $10^{9}$/l)(14). Serum biochemistry was unremarkable. Under general anaesthesia, the dog was castrated and the right inguinal mass was excised. This mass was 5 cm in diameter and had an appearance of a testicle. Then, a midline exploratory laparotomy was performed. The spleen as well as the gastric lymph node were enlarged and were removed. The mesenteric lymph nodes were also

mildly enlarged (5 cm diameter). No other abnormalities were observed. The biopsy of the skin was also performed.

Histopathology of the right inguinal mass revealed the presence of seminoma and Sertoli cell tumour in the right testis. The left testis was not examined. Lymphoma was present in the spleen and gastric lymph node. Subchronic dermatitis with a mixed cell (predominantly eosinophils) population was present in the skin biopsies. Immunohistochemistry examination of splenic and gastric lymph node biopsies with CD3 and CD79 antibodies (DAKO REALTM, EnVision Detection System) confirmed a B cell lymphoma.

Postoperatively a bloody-purulent urine was noticed. Dipstick and urine sediment analyses confirmed a diagnosis of purulent cystitis. Culture and sensitivity were not performed. Amoxicillin with clavulanic acid (Synulox, Pfizer) 20 mg/kg PO q12h for 14 days was prescribed.

The dog was rechecked one month later, seemed healthy, gained two kilograms of body weight and its hair-coat showed signs of improvement. The palpable peripheral lymph nodes were unchanged and CBC was unremarkable (mild anaemia, haematocrit 0.36 l/l). A month later, the dog had mild mandibular and praescapular lymphadenopathy. CBC was normal, with the exception of a mild anaemia (haematocrit 0.34 l/l). The owners were offered a multidrug chemotherapy, but declined and were thus advised to start corticosteroid treatment - methylprednisolone (Medrol, Pfizer; 2 mg/kg/day PO until relaps). An additional month later, three months after the operation, Lord's hair looked normal again (Figure 5, 6). A month later, after two months of methylprednisolone treatment, the dog deteriorated, was depressed and anorexic and was euthanized at the owner's request by the referring veterinarian.

## Discussion

This is an unusual case with 3 different neoplasias occurring concurrently in a single dog. Multiple tumours occurring in the brains (15, 16) or in the mammary tissue (17) were reported, but authors are not aware of any report about concurrent multiple tumour occurrence in different tissues.

Preoperatively seen mild nonregenerative anaemia with the haematocrit of 0.32 l/l and thrombocytopaenia could have developed secondarily to lymphoma (18,19), but haematocrit of 0.35 l/l was also seen 3 months earlier, when lymphoma was not diagnosed yet. Such a long course of the disease is unusual for lymphoma (18).

In addition to symmetrical truncal alopecia other male patients, but not the present case, may also present other signs of feminization (8). The subsequent improvement of the skin after surgery in contrast to the partial response to previous therapy supports the assumption that these changes were secondary to hyperoestrogenism due to Sertoli cell tumour. Plasma oestradiol concentrations in male dogs should be less than 15 pg/ml. In dogs with Sertoli cell tumours, eostradiol concentrations are usually 10 to 150 pg/ml, as was in the present case (87,9 pg/ml) (9).

Sertoli cell tumours metastasize in 2-14% of cases; seminomas do so even less frequently. Most commonly they metastasize to the sublumbar (3), inguinal and iliac lymph nodes, lungs, liver, spleen, kidneys, pancreas (9), subcutis, brain and eyes (20). The above mentioned lymph nodes were not enlarged in our case, as observed upon the exploratory laparotomy. However, gastric and mesenteric lymphadenopathy was observed. While the gastric lymph node presented lymphoma and had no evidence of a testicular cancer metastasis, the mesenteric lymph nodes were not examined histologically. Although we cannot rule out that they were enlarged due to metastasis of the testicular tumours, it is more likely that lymphoma was the cause. A postoperative serum oestrogen could have aided in determination of Sertoli cell tumour metastasis. Retrospectively, considering the chronic diarrhoea, unresponsive to antibiotics, but responsive to cyclosporine, the mesenteric and gastric lymphadenopathy, and diagnosis of lymphoma in the latter as well as in the spleen, we believe this was a case of alimentary lymphoma. It is presumed that they are most likely B-cell in origin (18), but newer studies are conflicting (21). However, it could be also a stage IVa of the multicentric form (18), which is perhaps more likely regarding subsequent development of generalised lymphadenopathy.

The chronic diarrhoea, mentioned in the history, could develop due to other causes such as lymphocytic-plasmacitic enteritis (LPE) or giardiasis, as the dog was positive for the latter. Giardiasis seems to be a less likely cause for the diarrhoea as the dog failed to respond to a prolonged metronidazole and spiramycin treatment, and did respond to cyclosporine (22, 23). It has been suggested, that LPE may be a prelymphomatous change in the gastrointestinal tract (18). Cyclosporine treatment has been reported to induce lymphoma or other tumours oc-

casionally (18, 24, 25). The drug hinders lymphocyte T activity and recognition of neoplastically changed cells (26), which, presumably, can consequently induce additional concurrent neoplastic disease.

Dogs with lymphoma are known to be less immunocompetent because of the incompetence of neoplastic lymphocytes/lymphoblasts (11, 18) or fewer total lymphocytes, especially T cells (27). This could have led to the observed postoperative cystitis.

Lymphoma is usually treated with combination chemotherapy protocol (28-30), rather than corticosteroids a single agent, however, the latter was attempted due to the owner's decline of the former. Corticosteroid effects are usually short-lived in canine lymphoma, and relapses are extremely common (18), and thus it is not surprising that the present dog was represented shortly later with signs of systemic disease, most probably a relapse of the lymphoma.

## Conclusion

We presented a case of multiple neoplasia in a dog including B cell lymphoma, Sertoli cell tumour and seminoma. The dog had a chronic dermatopathy, probably due to hyperoestrogensim secondary to the Sertoli cell tumour. The latter is supported by recovery of the skin following castration. The lymphoma was probably alimentary, although a multicentric lymphoma cannot be ruled out. The cause of lymphoma is unknown, but it may have been associated with a previous cyclosporine therapy.

## Acknowledgement

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## GENERALIZIRANA SIMETRIČNA ALOPECIJA IN HIPERESTROGENIZEM PRI SAMOJEDU Z LIMFOMOM, SEMINOMOM IN TUMORJEM SERTOLIJEVIH CELIC

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**Povzetek:** 10 let star samojed, desnostranski kriptorhid, je bil napoten na Kliniko za kirurgijo in male živali Veterinarske fakultete zaradi šest mesecev trajajoče nevnetne alopecije. V anamnezi živali so lastniki navajali drisko, ki je bila uspešno zdravljena s ciklosporinom. Na podlagi ultrazvočne preiskave in citološke preiskave tankoigelnega punktata smo postavili sum na limfom in neoplazijo mod. Psa smo kastrirali ter odvzeli biopsat vranice in bezgavke. Patohistološka preiskava je potrdila tumor sertolijevih celic in seminom desnega moda ter B celični limfom vranice in bezgavk. Dva meseca po operativnem posegu smo opazili povečane periferne bezgavke, vendar se lastniki niso odločili za citostatično zdravljenje. Svetovali smo jim začetek zdravljenja s kortikosteroidi. Tri mesece po kirurškem posegu je bila odlakanost psa spet normalna, evtanaziran je bil štiri mesece po kastraciji zaradi obolenja, povezanega z limfomom.

**Ključne besede:** enostranski kriptorhidizem; testis, novotvorbe – kirurgija – kemoterapija; ultrazvok; citodiagnostika; biopsija, tankoigelna; psi

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**Case Report**

# NOSOCOMIAL *KLEBSIELLA OXYTOCA* INFECTION IN TWO DOGS

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**Summary:** Despite a large amount of published data on nosocomially-acquired intravenous catheter-related *Klebsiella oxytoca* infection in human medicine there is a lack of information in veterinary medicine.
Intravenous catheter-related *Klebsiella oxytoca* infection was strongly suspected in two dogs that underwent dental procedures under general anesthesia. Dog 1 was presented with severe osteomyelitis of the shoulder joint from the same leg that was used for intravenous catheter placement during implantation of bilateral direct acrylic inclined plane approximately 3 weeks ago. According to the bacteriology identification that revealed *Klebsiella oxytoca* infection and susceptibility testing of the synovial fluid from the shoulder joint the dog was treated with amoxicillin+clavulanic acid for one month. In spite of clinical improvement the radiographic examination revealed severe osteoarthritis of the affected joint at the end of the course. Dog 2 died of septic shock associated with disseminated intravascular coagulation 7 hours after the extraction of left mandibular fourth premolar tooth. *Klebsiella oxytoca* was isolated from several abdominal organs post-mortem.
Bacteriology examination of hospital environment and equipment was carried out because of possible common-source epidemic and among other microorganisms, Klebsiella oxytoca was isolated. Additional infection control measures were instituted and so far, eigteen-months after that no nosocomial infection was confirmed.

**Key words:** cross infection – microbiology; infection control; *Klebsiella* infections – etiology – microbiology; postoperative complications; dogs

## Introduction

Nosocomial infections are infections that may be derived from endogenous flora of the patients or from exogenous microorganisms acquired by patients during their stay in the hospital (1, 2). In the latter case, the infectious agent is often resistant to multiple antibiotics and may be transmitted to or between several patients, thus leading to clusters or outbreaks of nosocomial infections due to the same strain (3). A limited number of small outbreaks is documented in companion animals due to *Klebsiella sp.* (4), *Serratia marcescens* (5), *Enterobacter cloace* (6), *Escherichia coli* (7, 8), *Enterococcus faecium* (2), *Clostridium difficile* (9, 10), *Clostridium perfringens* (11), *Acinetobacter baumanni* (2, 12) and *Pseudomonas aeruginosa* (13). In human medicine, there are several documented outbreaks of catheter or contaminated disinfectant-associated infections with *Klebsiella oxytoca* (14, 15, 16, 17, 18, 19). The data on catheter associated *Klebsiella oxytoca* infection in veterinary medicine are sparse (4, 20).

Bacteria from genus *Klebsiella* are non-motile, rod-shaped, Gram-negative aerobic bacteria that possess a prominent polysaccharide capsule. Some of these bacteria produce an extracellular toxic complex that has been shown to be lethal for and produce extensive lung pathology in mice. It is composed of capsular polysaccharide, lipopolysaccharide, and protein; when introduced to experimental animals in sublethal doses, the animals built up immunization due to antibody production (21). *Klebsiella oxytoca* and *Klebsiella pneumoniae* are both opportunistic pathogens found in the environment and in mammalian mucosal surfaces; they are usually passed by the hands of hospital personnel. *Kleb-*

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*siella oxytoca* and *Klebsiella pneumoniae* are common isolates in clinical microbiology and important producers of extended spectre beta-lactamases (ESBL). Enterobacteriaceae with beta-lactam resistance due to the production of ESBL were discovered in the eighties and since that time became epidemic and endemic in hospitals worldwide (22).

The purpose of this report is to describe nosocomial *Klebsiella oxytoca* infection in two dogs that underwent dental procedures at Clinic for Small Animal Medicine and Surgery, Veterinary Faculty, University of Ljubljana (CSAMS-VFLJ).

## Case 1

Dog 1, a 7-months-old intact male Airedale terrier, considered healthy after physical examination, weighing 19.5 kg was presented to CSAMS-VFLJ for orthodontic movement of right mandibular canine tooth. Bilateral direct acrylic inclined plane was implanted under general anaesthesia. The dog was premedicated with medetomidine (Domitor, Pfizer, Karlsruhe, Germany) 0.018 mg/kg i/m. Intravenous catheter was placed into cephalic vein 15 minutes later after standard aseptic procedure including clipping the hair with the clipper and disinfection of the skin with a mixture of 2-propanol and benzalkonium chloride (Cutasept, Beiersdorf GsmbH, Wien, Austria). The dog was induced to general anaesthesia with propofol (Propofol 1%, Fresenius Kabi, Graz, Austria) 1.5 mg/kg i/v, endotracheally intubated and maintained with isoflurane (Forane, Abbott, Queenborough, UK) in 100% oxygen for 20 minutes. Atipamezole (Antisedan, Pfizer, Karlsruhe, Germany) 0.038 mg/kg i/m was administered at the end of the procedure to antagonize the effects of medetomidine. During general anaesthesia, Lactated Ringer's solution (B Braun, Melsungen, Germany) was administered at 10 ml/kg/h. One hour and half after atipamezole administration, metoclopramide (Reglan, Alkaloid, Skopje, Macedonia) 0.4 mg/kg s/c was given to treat postoperative nausea. The dog was discharged to a home care few hours later.

The dog was brought to local veterinary practitioner because of lameness of right front leg that was used for intravenous catheter placement the next day. Intense pain was observed at palpation of right shoulder joint and the dog was given carprofen (Rimadyl, Pfizer, Animal Health S.A., Dundee, UK) 4 mg/kg s/c. The lameness resolved the day after that, but the mild pain was still present at palpation of affected joint. Radiographic examination showed no abnormalities and the dog was prescribed carprofen (2 mg/kg p/o q12h).

Five days later, the dog returned to veterinary practitioner with extensive swelling of right front leg. The dog was prescribed amoxicillin+clavulanic acid (Synulox, Pfizer Italiana, Latina, Italy) 20 mg/kg p/o q12h and carprofen as before. The dog's condition improved and therapy was terminated 10 days later.

The day after, the dog was presented again with intense pain of right shoulder joint, painful hind part of the body and stiffed gait. The dog was reluctant to walk. According to veterinary practitioner, radiographic examinations of right shoulder joint and hip joints were normal as well as neurological examination. Routine haematology profile was within reference range. The dog was tested for dirofilariosis, boreliosis and ehrlichiosis with commercially available ELISA kit (SNAP 3Dx, Canine Heartworm Antigen/Borrelia Burgdorferi/Ehrlichia Canis Anibody Test Kit, IDEXX Laboratories, Westbrook, Maine, USA), and the results were negative. Despite negative results, the dog was prescribed doxycycline (Clinofug D, Dr. August WOLFF GmbH&Co, Bielfeld, Germany) 10 mg/kg p/o q24h and carprofen 2 mg/kg p/o q12h. The dogs' condition worsened in the course of therapy, and a week later the dog was referred to CSAMS-VFLJ.

The dog was presented to CSAMS-VFLJ with intense pain of the whole right front leg. Radiographic examination revealed osteomyelitis of the right humeral head. Definitive pattern of lysis and solid pattern of periosteal new bone were seen on the humeral head (Figure 1). Synovial fluid from right shoulder joint was taken and sent to bacteriology identification and susceptibility testing to commonly used antimicrobial agents.

Bacteriology examinations were performed in Columbia agar supplemented with 5% of ovine blood. *Klebsiella* microorganisms were identified on the basis of colony morphology, microscopic Gram stain characteristics, indole and oxidase activity. The final confirmation was done with API-20E® enteric identification system (bioMerieux, Marcy l'Etoile, France). The antibiotic susceptibility of isolated strains was determined by disc-diffusion method according to the NCCLS (National Committee on Clinical Laboratory Standards) guidelines (Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Second Edition, M31-A2, Vol. 22 No.6).

The following antimicrobials were assayed: azithromycin, amikacin, amoxicillin, amoxicillin/clavulanic acid, cephalexin, cephalotin, ciprofloxacin, enrofloxacin, ceftriaxone, gentamicin, neomycin, piperacilin, metronidazole, trimethoprim/sulfamethoxazole and oxytetracycline. Initial beta-lactamase activity was detected by the hydrolysis of nitrocefin (cefinase). Screening tests for extended-spectrum-β-lactamase activity were evaluated regarding their susceptibility to cefpodoxime, ceftazidime, cefotaxime and cefuroxime (BBL-Difco). Confirmation tests for ESBL were done by E-test (E-test, AB-Biodisk, Dalvägen, Solna, Sweden) for cefotaxime/ cefotaxime+clavulanic acid (CT/CTL) and ceftazidime/ ceftazidime+clavulanic acid (TZ/TZL).

Bacteriology identification revealed infection by *Klebsiella oxytoca*, β-lactamase positive by nitrocefin testing, but ESBL negative (Table 1). According to the susceptibility testing, the dog was prescribed amoxicillin+clavulanic acid 20 mg/kg p/o q12h for one month and carprofen 2 mg/kg p/o q12h, the dose of latter to be gradually decreased by the owner according to the intensity of pain.

**Table 1:** Antimicrobial susceptibility profile from microorganisms isolated from dog 1 and dog 2

| | Dog 1 | Dog 2 | |
|---|---|---|---|
| | osteomyelitis | deceased | |
| | *Klebsiella oxytoca* (synovial fluid) | *Klebsiella oxytoca* (liver, kidney, spleen, peritoneal fluid, small intestine) *Escherichia coli* (small intestine) | |
| Susceptibility testing | *K. oxytoca* | *K. oxytoca* | *E. coli* |
| azithromycin | R | R | R |
| amikacin | S | S | S |
| amoxicillin | R | R | I |
| amoxicillin+clav.acid | S | S | S |
| cephalexin | S | I | I |
| cephalotin | S | S | S |
| ciprofloxacin | S | S | S |
| enrofloxacin | S | S | S |
| ceftriaxone | | S | S |
| gentamycin | S | S | S |
| neomycin | S | S | S |
| piperacillin | | S | S |
| metronidazole | | R | R |
| trimethoprim/sulpha | S | S | S |
| oxytetracycline | | S | S |
| cefpodoxime | S | S | S |
| ceftazidime | S | S | S |
| cefotaxime | S | S | S |
| cefuroxime | S | S | S |
| β-lactamase activity by nitrocefin testing | + | + | + |
| E-test, ESBL CT/CTL | - | - | - |
| E-test, ESBL TZ/TZL | - | - | - |

S, sensitive; I, intermediate sensitive; R, resistant; E-test ESBL CT/CTL, extended specter β-lactamase, cefotaxime/ cefotaxime+clavulanic acid; E-test ESBL TZ/TZL, extended specter β-lactamase, ceftazidime/ ceftazidime+clavulanic acid; +, positive; -, negative

Two weeks after the commencement of the therapy, the dog was much less in pain and able to use right front leg, although it was still lame. The dog was brought for a control check 6 weeks after the end of amoxicillin+clavulanic acid therapy. No lameness or pain was observed at the clinical examination and according to the owners it was doing well.

**Figure 1:** Lateral radiograph of the right humeral head 3 weeks after the intravenous catheter-related infection with *Klebsiella oxytoca*. Osteomyelitis with definitive pattern of lysis and solid pattern of periostal new bone is seen.

**Figure 2:** Lateral radiograph of the right humeral head 6 weeks after the end of amoxicillin+clavulanic acid therapy (20 m/kg p/o q 12h for one month) shows resolution of the lytic process. The shape of the humeral head is irregular and severe osteoarthritis of the shoulder joint is visible.

Radiographic examination of the right shoulder joint showed resolution of the lytic process. The shape of the humeral head was irregular and severe osteoarthritis of the shoulder joint was visible (Figure 2).

## Case 2

Dog 2, a 2 years-old intact working male German shepherd weighing 30.6 kg, considered healthy after physical examination including pulse and respiratory rate and heart and lung auscultation, was brought to CSAMS-VFLJ for extraction of left mandibular fourth premolar tooth three weeks after the dog 1. Body temperature was not measured because of the dog's uncooperativeness. The extraction was performed under general anaesthesia with isoflurane in 100 % oxygen after premedication with medetomidine 0.025 mg/kg i/m and butorphanol (Torbugesic, Fort Dodge Animal Health, Iowa) 0.1 mg/kg i/m and induction to anesthesia with propofol at a dose of 2 mg/kg i/v. Intravenous catheter placement procedure was the same as in the dog 1. The dog was given perioperatively carprofen 4 mg/kg i/v and Lactated Ringer's solution at 10 ml/kg/h. At the end of the procedure, medetomidine was reversed with atipamezole 0.05 mg/kg i/m. Single dose of methadone (Heptanon, Pliva, Zagreb, Croatia) 0.4 mg/kg s/c was administered for postoperative analgesia. Panting that was observed soon thereafter was thought to be a consequence of methadone administration or relatively warm recovery room (23 to 24°C). The dog's body temperature was not measured because it's aggressive temper. Two hours later, the dog was discharged to a home care with carprofen 2 mg/kg p/o q12h to be given following three days. At that time, it was alert but still mildly ataxic and weak.

Later in the afternoon, the dog's caretaker noticed that the dog is apathetic and sleepy, and it refused water and food. Its pulse was thready. The dog was given additional dose of atipamezole (dose not known) by staff veterinarian and sent back to CSAMS-VFLJ. On the admission (7 hours after the end of procedure) the dog was in cardiopulmonary arrest. Cardiopulmonary resuscitation (CPR) was performed with no success. During CPR, considerable amount of smelly dark reddish intestinal contents were released.

Post-mortem examination carried out at Institute for Pathology, Forensic and Administrative Veterinary Medicine, Veterinary Faculty, University of Ljubljana revealed severely congested kidneys, liver and small intestinal wall. The small intestine was filled with hemorrhagic contents, while large intestine was empty. The spleen and lungs were congested as well. The subcutaneous blood vessels were congested with non-coagulated blood and the muscles were pale. Non-coagulated blood was found in right ventricle.

Histopathology showed disintegrated epithelium of small intestine. Small intestinal mucosal venous dilatation was accompanied with lymphocyte and macrophage infiltration in the lamina propria. Inflammatory cells, predominantly lymphocytes, were present in liver vessels. Liver sinusoids were congested and hepatocellular atrophia was observed in centrilobular zone. In both kidneys, hemorrhagic renal cortical necrosis with tubular casts was found. Reactive hyperplasia of spleen involved lymphoid tissue of the white pulp and macrophages of the red pulp. Several small colonies of bacteria were found in blood vessels and in parenchymatous tissue of the spleen. Severe congestion with multiple focal intraalveolar haemorrhages was found in bilateral lungs, and a small number of alveoli contained air. In the heart, prominent multiple epicardial haemorrhages and myocardial hyperaemia were observed. Abdominal organs were sent to bacteriological examinations and *Klebsiella oxytoca* was isolated abundantly from liver, kidneys and spleen. No bacteria were isolated from lungs. *Klebsiella oxytoca* and haemolytic *Escherichia coli* were isolated from small intestine while *Klebsiella oxytoca* and *Clostridium sporogenes* were isolated from the small amount of peritoneal fluid, the latter probably contaminating abdominal cavity post mortem. Initial tests of isolated bacteria regarding beta-lactamase activity by nitrocefin were strongly positive (Table 1). Screening tests for extended-specter-β-lactamase (ESBL) were negative and confirmation tests with E-test were negative as well. The antibiotic susceptibility of isolated strains of *Klebsiella oxytoca* and *Escherichia coli* was determined as in dog 1 (Table 1).

The anaesthesia staff was concerned about a possible common-source epidemic of *Klebsiella oxytoca* infection after the second case occurred, and environmental surfaces and equipment in pre-/post-operative rooms (PPR), operating theatres and wards (tables, cages, incubator, clippers, muzzles, Doppler pulse detector and pulse oximetry probes, breathing circuits, endotracheal tubes, laryngoscope blades, dentistry equipment) as well as opened vials of propofol, acepromazine and carprofen and intravenous fluids were bacteriologicaly examined. β-lactamase negative Klebsiella oxytoca (by nitrocefin testing) was isolated from PPR cages and *Staphylococcus intermedius*, *Enterococcus sp.*, and *Bacillus sp.* from muzzles. β-lactamase positive *Klebsiella oxytoca* and β-lactamase positive *Escherichia coli* (by nitrocefin testing) were isolated from tables, cages and clippers in wards.

Additional infection control measures for PPR, operating theatres and wards were instituted, including restrictions on the personnel entrance into PPR and operating theatres, strict disinfection of clippers, tables, cages and other equipment between the patients and regular disinfection of hands before handling another animal or before intravenous, arterial and urinary catheter placement. Particular care was taken at manipulation of catheters and disconnection of infusion lines. The use of single-use gloves was strongly encouraged when manipulating the animals. So far, 24-months after the additional infection control measures were instituted; no nosocomial infection was confirmed at CSAMS-VFLJ.

## Discussion

90% of nosocomial bloodstream infections in human hospitals are related to intravenous catheter use (23). These infections can range in severity from localized phlebitis to fatal bacteraemia and sepsis, and are caused by contamination of the catheter either at the time of insertion or during use (24). Microorganisms may be introduced on the hands of the person placing or handling the catheter. They may be a part of the health care worker's own skin flora, may be part of the patient's skin flora acquired from a site that has not been disinfected, or may be faecal or other types of bacteria that the health care worker has handled (1).

There are few data in the veterinary literature documenting the incidence of catheter-related bloodstream infections in companion animals (2, 4, 5, 6, 7, 9, 10, 11, 12, 13). Most catheter-related infections involve organisms that are ubiquitous to the patient's skin flora, nasopharynx or intestinal tract and organisms carried on the hands of hospital personnel (25). Surveillance study in a veterinary hospital reported 26% positive jugular catheter bacterial cultures after an average of 2.7 days. More than 50% of the organisms were *Klebsiella spp.* and *Enterobacter spp* (1).

In the present report, the cages and clipper from wards were contaminated with β-lactamase positive Klebsiella oxytoca, which was also isolated from both affected dogs. None of dogs entered the wards; therefore the only possible way of transmission could be by the hands of personnel rotating between PPR and wards or the clipper from the wards although the rotation of clipper between the wards and PPR is not a common practice at CSAMS-VFLJ. However, since the culturing of microorganisms from environmen-

tal surfaces and equipment was carried out 4 days after the dog 2 died, regular cleaning and disinfection procedures in a meantime might have an influence on the microbiology results and other sources of intravenous catheter contamination can not be excluded. While there is the possibility that the infection entered the bloodstream at the oral cavity during dental procedure in dog 2, it is highly unlikely in dog 1 because during the implantation of bilateral direct acrylic plane the integrity of gingival mucosa was not interrupted. However, the lack of catheter cultures makes it hard to be certain of the source of the infection.

Clinical presentation of *Klebsiella spp.* bacteraemia is indistinguishable from that of bacteraemia caused by other microorganisms. If bacteraemia develops, symptoms include fever or hypothermia, leukocytosis with left shift or neutropenia, and shock. The incidence of septic shock is remarkable; in a five-year study in human hospital 22% of the patients with documented bacteraemia due to *Klebsiella spp*. developed septic shock (26). In the present report, shock was overlooked in a dog 2, because the dog was discharged soon after the end of procedure due to his aggressive temper and uncooperativeness, and the early signs of impending shock such as panting and change in mental status were unfortunately misinterpreted.

The results of post-mortem examination of dog 2 indicate that the dog died because of septic shock associated with disseminated intravascular coagulation, and β-lactamase positive *Klebsiella oxytoca* was isolated from several abdominal organs, including small intestine. The authors can not affirm, whether *Klebsiella oxytoca* was introduced into the dog through intravenous catheter or during dental procedure or the dog was already the reservoir for *Klebsiella oxytoca*, which spread to extraintestinal sites through mesenteric lymph node complex as a result of bacterial translocation. Bacterial translocation in hosts with an intact intestinal barrier can occur by intracellular route, however the primary mechanism promoting bacterial translocation in these cases is bacterial overgrowth due to recent antimicrobial therapy (27), which was not the case in dog 2. The other mechanism, which promotes bacterial translocation is increased permeability of intestinal mucosal barrier, for example due to hemorrhagic or endotoxic shock, where indigenous bacteria translocate intercellulary (28). The various species of indigenous bacteria do not all translocate at the same rate. Gram-negative, facultative anaerobic *Enterobacteriaceae*, such as *Escherichia coli*, *Klebsiella spp*., and *Proteus mirabilis*, translocate from gastrointestinal tract at a greater rate than the other bacteria (27). In the present report, the authors doubt that uneventful short-term anaesthesia could provoke bacterial translocation and sepsis by itself in previously healthy dog.

The susceptibility profile of *Klebsiella oxytoca* isolated from dog 1 and dog 2 showed that the organism was not resistant to a broad spectrum of antibiotics including those commonly used in the hospital (amoxicillin+clavulanic acid, gentamicin, enrofloxacin). Dog 2's antimicrobial sensitivity profile showed an intermediate resistance to cephalexin, where dog1's organism was sensitive. This might represent a developing and broadening resistance pattern as the organism has been presumably in a hospital setting for a longer period of time, but on the other hand cephalexin is not an antibiotic used on a regular basis in a hospital setting.

Amoxicillin+clavulanic acid to which *Klebsiella oxytoca* was sensitive, is used most often for prophylactic antibiotic treatment of surgical and dentistry patients at CSAMS-VFLJ. The lipopolysaccharide capsule of *Klebsiella oxytoca* that protects the organism from phagocytosis and aids in adherence (21) might be the cause for the severity and rapid development of the infection in dog 2.

In conclusion, the present report demonstrates the potential for nosocomial infection with *Klebsiella oxytoca* in small animal hospitals. Strict infection control measures, particularly regular disinfection of hands and use of disposable gloves should be strongly encouraged as the most important factors in preventing nosocomial infections.

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# BOLNIŠNIČNA OKUŽBA DVEH PSOV Z BAKTERIJO *KLEBSIELLA OXYTOCA*

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**Povzetek:** Kljub številnim literaturnim podatkom o bolnišničnih okužbah prek intravenskega katetra z bakterijo *Klebsiella oxytoca* v humani medicini so podatki s področja veterinarske medicine pomanjkljivi.
Predstavljena sta dva primera suma okuženosti intravenskega katetra z bakterijo *Klebsiella oxytoca* pri psih, pri katerih je bil opravljen stomatološki poseg v splošni anesteziji. Pri prvem psu se je 3 tedne po vstavitvi obojestranske direktne akrilatne opornice razvil osteomielitis ramenskega sklepa na nogi, ki smo jo ob posegu uporabili za intravensko kateterizacijo. Bakteriološka identifikacija sklepne tekočine je potrdila okužbo z bakterijo *Klebsiella oxytoca*, psa pa smo glede na rezultate antibiograma mesec dni zdravili z amoksicilinom s klavulansko kislino. Kljub kliničnemu izboljšanju, smo z rentgenskim slikanjem ob zaključku zdravljenja potrdili osteoartritis ramenskega sklepa. Drugi pes je poginil 7 ur po izdrtju levega mandibularnega četrtega ličnika zaradi septičnega šoka z diseminirano intravaskularno koagulacijo. Po smrti smo izolirali bakterijo *Klebsiella oxytoca* iz več organov trebušne votline.
Zaradi suma glede skupnega izvora okužbe smo opravili bakteriološko preiskavo bolnišničnih prostorov in opreme ter med drugim identificirali tudi bakterijo *Klebsiella oxytoca*. Poostrili smo postopke za preprečevanje prenosa morebitnih bolnišničnih okužb in 18 mesecev po opisanih primerih nismo potrdili novih bolnišničnih okužb.

**Ključne besede:** bolnišnična okužba – mikrobiologija; infekcija, nadzor; *Klebsiella* infekcije – etiologija - mikrobiologija; pooperacijske komplikacije; psi

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**Case Report**

# LARGE SCALE OUTBREAKS OF PESTE DES PETITS RUMINANTS IN SHEEP AND GOATS IN THAR DESERT OF INDIA

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**Summary:** Severe peste des petits ruminants (PPR) outbreaks were recorded in Thar desert of Rajasthan state (India) affecting large population of sheep and goats over a huge geographical area, taking a toll in tens of thousands of animals. These outbreaks were investigated for epidemiological, clinical, pathological, haematological and biochemical features. The outbreaks started in migratory sheep which returned back from adjacent PPR endemic areas and later spread to goats with estimated case fatality rates of 70% and 80%, respectively. Younger animals were more affected than the adult ones. The stress of migration coupled with low environmental temperatures bolstered by humidity and nutritional deficiency was the reason of precipitation of the disease. Typical clinical and pathological findings were recorded and significant ($P \leq 0.05$) changes were observed in most of the haemato-biochemical parameters. The free-range grazing system had been responsible for the involvements of larger number of flocks in distant areas with prevalence for longer duration. The disease waned with increase in the environmental temperatures.

The present situation warrants strict sero-surveillance and monitoring of PPR together with uninterrupted vaccination of migratory flocks at district or state borders for its effective control.

**Keywords:** sheep diseases – diagnosis – virology; goat diseases – diagnosis – virology; disease outbreaks; peste-des-petits-ruminants – diagnosis – virology; pathology, clinical; blood chemical analysis: antibodies, viral – blood; enzyme-linked immunosorbent assay – methods; desert climate; sheep; goats

## Introduction

Peste des petits ruminants (PPR), an *office international des epizooties* list A disease of sheep and goats caused by morbillivirus is characterized by high morbidity and high mortality rates resulting into heavy economical losses. The disease has been regularly reported from various parts of the world but mainly from Africa, Arabia, the Middle and Near East and the Indian subcontinent (1, 2, 3, 4).

Rajasthan state situated in the north-west part of India is the biggest state in regards to its geographical area and shares a very long international border with Pakistan. The inland borders are shared with other states: Punjab in the north, Haryana and Uttar Pradesh in the north-east, Madhya Pradesh in the south-east and Gujarat in the south-west. The arid region of western part of Rajasthan constitutes famous Thar desert, where ambient temperatures vary widely from subzero in winter to as high as 50 °C in summer in some places. In this area, the livestock remains the mainstay of desert economy over agricultural produce. The sheep are reared mainly for wool and meat, and goats for milk and meat. The migration of small ruminants, especially of sheep, to other adjoining states, is a regular feature during famine and during times of scarcity of feed and fodder in Thar desert. The migration of various sized flocks (usually of few hundreds to several thousands) normally starts in March which is the time of onset of summer in this region. The flocks return back by October, the time of onset of winter, but early return of animals is possible if rain is experienced in the area. The arrival of animals is often accompanied and/or followed by various dis-

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ease outbreaks in the unprotected flocks.

Though PPR is considered endemic throughout India (5) this region had never experienced the outbreaks in the past similar to that in the present report involving a wider geographical area with very high morbidity and mortality.

This paper puts on record some field investigations in regards to epidemiology, clinical signs, pathological findings, and laboratory investigations encompassing haemato-biochemical changes and histopathology in sheep and goats affected with PPR during natural outbreaks in the Thar desert.

## Material and methods

A series of PPR outbreaks were observed in the northwest Rajasthan affecting both sheep and goat populations between November 2005 and April 2006. Sheep were mostly Magra, Marwari and Rambouillet breeds and their crosses with local breeds in the organized farms and goats belonged to Marwari breed. The epidemiological observations were recorded in several outbreaks in 21 villages and one organized sheep breeding farm. The clinical signs were observed and post-mortem examination of 58 carcasses was carried out at various places.

The blood samples were drawn from 30 sheep (15 adults and 15 lambs) and 30 goats (15 adults and 15 kids) affected with PPR and 20 healthy sheep (10 adults and 10 lambs) and 20 healthy goats (10 adults and 10 kids) for haemato-biochemical analyses. The most of the adult animals were females but young ones included both male and female. Various haemato-biochemical parameters were determined by standard techniques (6, 7, 8) and serum cortisol by radioimmunometric method using $^{125}I$ radioimmunoassay kit (RADIM, Spain). The statistical significance for a given parameter was determined between healthy and PPR affected animals. Comparison between healthy and infected herds was made by student's t-test with significant difference considered at $p < 0.05$ (9).

## Results

*Field Investigation*

Epidemiology

The disease first appeared in November 2005 in migratory sheep flocks in Sardarshahar area of Churu district adjoining to Haryana state and later spread to other adjoining districts. The widespread outbreaks were encountered in different flocks untll April 2006. The highest morbidity and mortality rates were recorded during the periods when the lowest night temperatures dropped below zero in January 2006 with high humidity. Because of involvement of very large animal population in huge geographical area in the desert, the exact figures could not be gathered but the available data revealed death of few thousand animals with a case fatality of 70% in sheep and 80% in goats. The young animals of both species had higher case fatality rates than their respective adults. The disease was also recorded at an organized sheep farm where mortality was observed mostly in young animals below 3 months of age which were not vaccinated against PPR, however, vaccinated adult animals also died albeit with less severe clinical and pathological findings.

Clinical signs

The course of the disease was acute and subacute, few of the animals died even in 36 hours of onset of the disease. The affected animals initially were severely depressed with a sudden rise in body temperature reaching almost 42 °C in some cases, and the fever persisted for 7-8 days. From the onset of fever, most animals had a serous nasal discharge which progressively turned into mucopurulent discharge, leading to severe respiratory distress. Areas of erosions were most commonly seen on the visible nasal mucous membranes and muco-cutaneous junctions with inflammation around the mouth (Fig.1). In many of the animals, lesions similar to orf developed at mucocutaneous junction of mouth. The erosive and necrotic stomatitis started as areas of hyperemia at gums, cheeks, dental pad and / or anterior dorsal part of tongue with frothy salivation. The areas later developed into irregular non-haemorrhagic lesions (Fig.2) and in some of the cases circular raised but flat non-bleeding lesions were present on the tongue (Fig.3). There was a great amount of necrotic debris on the older lesions (Fig.4). The individuals with severe oral lesions had visible swelling around mouth. A non-haemorrhagic diarrhoea was observed in all affected animals, developing 2-3 days after onset of the disease. Conjunctivitis was recorded with lachrymal discharge which became mucoid resulting in sticky eyelids. Abortion in pregnant animals was a consistent feature and vulvar mucous membranes had erosive lesions very similar to that in intestinal mucosa.. A subnormal temperature preceded death in animals with severe diarrhoea for few days.

**Figure 1:** Erosions at muco-cutaneous junction with inflammation around the mouth (PPR, kid)

**Figure 2:** Irregular non-haemorrhagic oral lesions (PPR, sheep)

**Figure 3:** Raised and flat circular lesions on tongue (PPR, goat)

**Figure 4:** Necrotic debris on oral lesions (PPR, sheep)

Pathological findings

Grossly, the carcasses were dehydrated with apparent swelling around the mouth. The lesions in mouth were consistently present in all animals except those who died within short period of 1-2 days. The lesions of respiratory tract included necrotic areas on the mucosa of nostrils and turbinates, severely congested tracheal mucous membrane and white froth in trachea. Lungs were congested and consolidated especially involving antero-ventral parts. Hydrothorax was recorded in few cases.

The oral cavity was full of white necrotic debris where oral lesions were severe. The lesions in gastrointestinal tract consisted of few erosions in the mucosa from where blood oozed out, large intestines were congested, especially at the caeco-colic junction with streaks of blood on mucosal crests (Zebra-stripes) (Fig.5) though the zebra-stripes were not seen in all the carcasses.

The lymph nodes, especially from mesenteries were severely oedematous, congested and enlarged (Fig.6). In some cases haemorrhages on the internal walls of gall bladder were recorded (Fig.7). Spleen was slightly enlarged in few cases. In some animals liver was studded with necrotic foci. Vaginitis was observed in many animals and vulvar mucus membranes were inflamed and had erosive lesions.

Though the types of lesions were more or less similar in all the animals, there was a variation in the severity and involvement of the organs. The vaccinated animals had less severe lesions on tongue.

## Laboratory Investigations

Haemato-biochemical parameters

The mean values of haemato-biochemical parameters in healthy animals and PPR affected sheep and

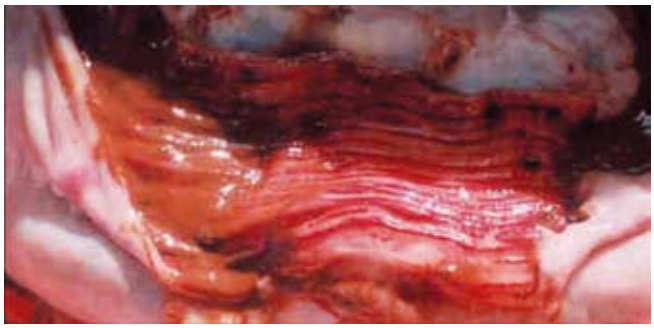

**Figure 5:** Zebra-stripes in large intestine (PPR, sheep)

**Figure 6:** Oedematous, congested mesenteric lymph node (PPR, goat)

**Figure 7:** Haemorrhages on the internal wall of gall bladder (PPR, sheep)

goats are presented in table 1 through 4. All parameters included in the investigation significantly differed between healthy and infected animals in both species in both young and adult animals ($p \leq 0.05$): total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin (Hb), packed cell volume (PCV), differential leucocyte count (DLC), relative viscosity, specific gravity and erythrocytic sedimentation rate (ESR).

In PPR affected animals of both species and age groups significant ($p \leq 0.05$) changes were recorded in serum biochemical parameters for sodium, potassium, glucose, total serum proteins, albumin, globulin, creatinine, urea and cortisol.

Histopathological changes

Histopathologically in lungs syncytial cells were present in alveolar lamina and inclusion bodies in cytoplasm and nucleus of alveolar macrophages and epithelial cells of bronchioli and bronchi. There was severe congestion of mucosa and submucosa and degeneration and necrosis of intestinal epithelium.

Serology

The sera collected from representative animals in different areas who survived the disease were subjected to c-ELISA which confirmed the presence of specific antibodies.

## Discussion

*Field Investigations*

Epidemiology

The disease first appeared in the sheep flocks which returned back after migration to other states during periods of famine in this desert area. The arrival of flocks was coincided with sudden lowered ambient temperatures. It is expected that the sheep on migration to other states came in contact with native small ruminant population where endemicity of the disease is much higher. The stress of migration and environmental temperature might have precipitated the onset of disease (10). The animals during feed scarcity often become nutritionally deficient resulting into increased susceptibility to infection (5). The spread of disease to other adjoining districts was due to unrestricted movements of the animals as they are reared on free range system of feeding and management covering long distances during the day (10, 11). The serial occurrence of dis-

**Table 1:** Haematological values (Mean ± SEM) in healthy and PPR affected sheep

| S. No. | Parameter | Sheep | | | |
|---|---|---|---|---|---|
| | | Adults | | Lambs | |
| | | Healthy (n = 10) | PPR affected (n = 15) | Healthy (n = 10) | PPR affected (n = 15) |
| 1. | TEC (x$10^{12}$/ l) | 7.92±0.23 | 8.99±0.31* | 8.91±0.41 | 10.0±0.39* |
| 2. | TLC (x$10^{9}$/ l) | 9.98±0.31 | 6.0±0.37* | 11.11±0.22 | 7.11±0.41* |
| 3. | Hb (g/dl) | 10.41±0.61 | 13.11±0.31* | 13.34±0.51 | 15.12±0.31* |
| 4. | PCV (%) | 29.33±0.61 | 34.12±0.39* | 34.12±0.58 | 37.22±0.34* |
| 5. | DLC (%) | | | | |
| | Neutrophil | 35.3±0.30 | 61.8±0.30* | 36.3±0.22 | 60.9±0.25* |
| | Lymphocyte | 55.2±0.21 | 35.2±0.21* | 54.8±0.31 | 35.8±0.20* |
| | Monocyte | 5.3±0.05 | 2.3±0.01* | 5.4±0.01 | 1.9±0.01* |
| | Eosinophil | 3.1±0.03 | 1.1±0.01* | 3.0±0.01 | 1.1±0.01* |
| | Basophil | 0.6±0.02 | 0.2±0.001* | 0.5±0.001 | 0.15±0.001* |
| 6. | Relative Viscosity | 4.3±0.03 | 4.9±0.04* | 4.6±0.05 | 5.0±0.03* |
| 7. | Specific gravity | 1.050±0.003 | 1.059±0.001* | 1.055±0.002 | 1.060±0.001* |
| 8. | ESR (mm/4Hr.) | 1.93±0.04 | 0.20±0.04* | 1.48±0.02 | 0.30±0.03* |

1. Figures in parenthesis indicate number of animals.
2. In adults and lambs mean comparison has been made between respective healthy and PPR affected animals for each parameter. All the differences were significant ($p \leq 0.05$) as indicated by asterix.
3. PPR = *Peste des petits ruminants*
4. TEC = Total erythrocyte count
5. TLC = Total leucocyte count
6. Hb = Haemoglobin
7. PCV= Packed cell volume
8. DLC= Differential leucocyte count
9. ESR = Erythrocytic sedimentation rate

**Table 2:** Biochemical parameters (Mean ± SEM) in healthy and PPR affected sheep

| S. No. | Parameter | Sheep | | | |
|---|---|---|---|---|---|
| | | Adult | | Lambs | |
| | | Healthy (n = 10) | PPR affected (n = 15) | Healthy (n = 10) | PPR affected (n = 15) |
| 1. | Sodium (mmol/L) | 130.11±1.89 | 140.12±2.00* | 133.2±1.34 | 144.2±3.0* |
| 2. | Potassium(mmol/L) | 5.3±0.1 | 8.0±0.10* | 5.5±0.10 | 9.12±0.09* |
| 3. | Calcium(mmol/L) | 2.40±0.025 | 2.25±0.022$^{NS}$ | 2.45±0.022 | 2.32±0.017$^{NS}$ |
| 4. | Phosphorus(g/L) | 0.043±0.0034 | 0.040±0.0007$^{NS}$ | 0.045±0.0007 | 0.043±0.0003$^{NS}$ |
| 5. | Glucose (mmol/L) | 2.48±0.072 | 1.65±0.055* | 2.56±0.055 | 1.54±0.049* |
| 6. | TSP ( g/L ) | 71.2±1.0 | 61.0±3.1* | 78.0±1.1 | 63.0± 1.1* |
| 7. | Albumin( g/L ) | 39.4±1.2 | 23.1±0.9* | 41.2±0.9 | 23.8±0.9* |
| 8. | Globulin( g/L) | 36.0±0.9 | 36.9±0.8* | 36.8±0.9 | 40.1±0.7* |
| 9. | Creatinine(µmol/l) | 97.24±3.53 | 185.64±2.65* | 79.56±2.65 | 185.64±1.76* |
| 10. | Urea(mmol/L) | 3.83±0.018 | 4.99±0.021* | 3.33±0.021 | 4.69±0.019* |
| 11. | Cortisol(mmol/L) | 0.165±0.011 | 0.259±0.019* | 0.173±0.008 | 0.312±0.019* |

1. Figures in parenthesis indicate number of animals.
2. In adults and lambs mean comparison has been made between respective healthy and PPR affected animals for each parameter. All the parameters except calcium and phosphorus showed significant differences ($p \leq 0.05$) as indicated by asterix.
3. NS= Non significant difference ($p > 0.05$)
4. PPR = *Peste des petits ruminants*
5. TSP = Total serum proteins
6. NS = Non-significant

**Table 3:** Haematological values (Mean ± SEM) in healthy and PPR affected goats

| S. No. | Parameter | Goats | | | |
|---|---|---|---|---|---|
| | | Adults | | Kids | |
| | | Healthy (n = 10) | PPR affected (n = 15) | Healthy (n = 10) | PPR affected (n = 15) |
| 1. | TEC ($x10^{12}$/ l) | 8.22±0.41 | 0.11±0.51* | 9.00±0.38 | 11.31±0.39* |
| 2. | TLC ($x10^{9}$/ l) | 9.31±0.81 | 5.93±0.38* | 10.99±0.31 | 6.43±0.22* |
| 3. | Hb (g/dl) | 10.12±0.31 | 13.12±0.41* | 11.88±0.21 | 13.88±0.34* |
| 4. | PCV (%) | 28.22±1.0 | 33.12±0.91* | 32.34±1.01 | 37.12±0.93* |
| 5. | DLC | | | | |
| | Neutrophil | 36.1±0.12 | 62.4±0.21* | 37.0±0.22 | 66.3±0.31* |
| | Lymphocyte | 55.3±0.16 | 34.2±0.20* | 54.0±0.15 | 32.8±0.41* |
| | Monocyte | 5.1±0.03 | 2.1±0.02* | 5.0±0.01 | 2.10±0.01* |
| | Eosinophil | 3.0±0.04 | 1.0±0.01* | 3.0±0.01 | 1.12±0.01* |
| | Basophil | 0.5±0.001 | 0.1±0.001* | 0.4±0.001 | 0.15±0.001* |
| 6. | Relative Viscosity | 4.0±0.04 | 4.8±0.03* | 4.25±0.06 | 4.9±0.02* |
| 7. | Specific gravity | 1.050±0.001 | 1.055±0.001* | 1.054±0.001 | 1.059±0.001* |
| 8. | ESR (mm/4Hr.) | 1.4±0.03 | 0.27±0.03* | 1.53±0.04 | 0.30±0.04* |

1. Figures in parenthesis indicate number of animals.
2. In adults and lambs mean comparison has been made between respective healthy and PPR affected animals for each parameter. All the differences were significant ($p \leq 0.05$) as indicated by asterix.
3. PPR = *Peste des petits ruminants*
4. TEC = Total erythrocyte count
5. TLC = Total leucocyte count
6. Hb = Haemoglobin
7. PCV= Packed cell volume
8. DLC= Differential leucocyte count
9. ESR = Erythrocytic sedimentation rate

**Table 4:** Biochemical parameters (Mean ± SEM) in healthy and PPR affected goats

| S. No. | Parameter | Goats | | | |
|---|---|---|---|---|---|
| | | Adults | | Kids | |
| | | Healthy (n = 10) | PPR affected (n = 15) | Healthy (n = 10) | PPR affected (n = 15) |
| 1. | Sodium (mmol/L) | 131.12±1.31 | 141.1±0.71* | 133.0±1.7 | 145.2±0.61* |
| 2. | Potassium(mmol/ L) | 5.6±0.09 | 7.9±0.05* | 5.9±0.09 | 8.1±0.13* |
| 3. | Calcium(mmol/ L) | 2.46±0.032 | 2.42±0.005$^{NS}$ | 2.475±0.05 | 2.42±0.05$^{NS}$ |
| 4. | Phosphorus(g/L) | 0.0583±0.0004 | 0.056±0.0009 $^{NS}$ | 0.057±0.0009 | 0.055±0.0009$^{NS}$ |
| 5. | Glucose (mmol/L) | 2.45±0.044 | 2.101±0.038* | 2.59±0.038 | 2.15±0.028* |
| 6. | TSP ( g/L ) | 70.7±0.9 | 60.0±1.0* | 77.1±1.0 | 61.0± 0.2* |
| 7. | Albumin( g/L ) | 36.2±0.1 | 21.0±0.3* | 36.3±0.3 | 22.0± 0.3* |
| 8. | Globulin( g/L) | 34.1±0.1 | 39.0±0.6* | 34.3±0.2 | 38.0±0.8* |
| 9. | Creatinine(µmol/l) | 97.24±7.70 | 167.96±3.53* | 114.92±2.65 | 238.68±7.95* |
| 10. | Urea(mmol/L) | 2.49±0.049 | 3.83±0.019* | 2.05±0.066 | 3.33±0.0166* |
| 11. | Cortisol(mmol/L) | 0.168±0.009 | 0.284±0.022* | 0.165±0.008 | 0.259±0.008* |

1. Figures in parenthesis indicate number of animals.
2. In adults and lambs mean comparison has been made between respective healthy and PPR affected animals for each parameter. All the parameters except calcium and phosphorus showed significant difference ($p \leq 0.05$) as indicated by asterix.
3. NS= Non significant difference ($p > 0.05$)
4. PPR = *Peste des petits ruminants*
5. TSP = Total serum proteins
6. NS = Non-significant

ease outbreaks had also been recorded for a long duration in different flocks in various adjoining districts in west Bengal (10). The highest morbidity and mortality rates were recorded during the periods when the lowest night temperatures dipped below zero in January 2006 with high humidity. In the present outbreaks humidity might have played the role in disease occurrence as it may be one of the factors allowing thriving and multiplication of PPR virus in nature (10). The environmental stress particularly hot and humid is also held responsible for precipitation of the disease (12).

Because of involvement of very large animal population in huge geographical area in desert the all figures could not be gathered regarding morbidity and mortality nevertheless the available data suggested a case fatality of 70% in sheep and 80% in goats. The PPR antibody prevalence is low (10- 30%) in goat population in the northern parts of the country suggesting higher risk of infection in goats (5). Moreover, the recovery rate of goats infected with PPR virus is less in comparison to that in sheep (13). In the Thar desert sheep and goats are reared in unorganized sector by small and marginal farmers and these animals are used as cash crops without any inputs. Neither these animals are supplemented with feed additives nor usually vaccinated against the diseases in which cost is incurred. This area never experienced PPR before hence all of the animals in these outbreaks were also not protected against it. The non-vaccination of these animals explains the very high morbidity and mortality rates. In the present serial outbreaks both species of small ruminants were affected whereas in some earlier reported outbreaks only goats died and sheep were not affected at all (10, 14). In contrast to the present observation a significantly higher mortality rate was recorded in sheep than in goats in Cameroon (15).

The disease was also recorded at an organized sheep farm where mortality was observed mostly in young animals below 3 months of age which were not vaccinated against PPR. It has been suggested that new born animals become susceptible to PPR virus infection at three to four months of age (16). However, few vaccinated adult animals also died albeit with less severe clinical and pathological findings.

Clinical signs

Though the course of the disease was acute and subacute with appearance of clinical signs and development of typical lesions, in cases where animals died within 1-2 days of onset of disease no lesions were recorded. Most of the affected animals had very high body temperatures for long periods. The circular raised, but flat lesions on dorsum of tongue were seen only in few cases. The development of lesions on muco-cutaneous junction of mouth very similar to that in orf or contagious ecthyma needs to be differentiated. The presence of mucopurulent discharge and froth in trachea led to respiratory occlusion and development of a typical obstructive sound during breathing. The amount of necrotic debris in oral cavity depended on the extent and age of the lesions. Unlike in other outbreaks typical overt oral lesions were recorded in the present outbreaks in sheep as well as in goats (17).

A continuous profuge diarrhoea caused severe dehydration in the affected animals. The kids were more severely affected due to dehydration and showed prostration for longer duration as compared to lambs. These animals had more subnormal temperatures before death.

Pathological findings

The mouth lesions were present in all of the animals except those who died within 1-2 days of appearance of disease. Even the vaccinated animals at the organized sheep breeding farm had lesions on tongue. Lungs in all the animals showed congestion and consolidation but hydrothorax was recorded in few cases only. No animal had any kind of lesion in abomasums (17) and heavy haemorrhages in large intestines (18) were not observed in any of the animals.

The haemorrhages recorded on the luminal wall of gall bladder in the present outbreak in some cases has not been reported to the best of our knowledge but presence of thick granular bile had been reported (14). The necrotic foci in liver of some animals could have developed due to some secondary bacterial infection of haematogenous route.

Though the types of lesions were more or less similar in all the animals but there was variation in the severity and involvement of the organs.

Treatment

The affected animals were given antibiotics to control secondary bacterial infections along with anti-inflammatory drugs. Many animals which received proper treatment in early stages of the disease were saved. It was observed that higher fatality rates in PPR affected animals was more due to secondary bacterial infections than the disease itself

which could have been due to immune suppression associated with morbillivirus infections (19).

*Laboratory Investigations*

Haemato-biochemical parameters

The mean values of haemato-biochemical parameters in healthy animals corroborated the earlier findings in sheep and goats of arid tract (20, 21, 22). The severe dehydration in the affected animals was evidenced by increased viscosity and specific gravity which led to polycythaemia (23). Severe leucopoenia could have been due to the inhibition of peripheral blood lymphocytes proliferation by PPR virus (19). A marked lymphocytopoenia, monocytopoenia, neutrophilia and eosinopoenia in present investigation could have been due to the combined effect of virus infection and stress as evidenced by elevated cortisol levels (24).

The increased mean values of sodium and potassium reflected haemoconcentration. The total serum protein values decreased but globulin concentration increased indicating immune response towards infection. The higher globulin concentration was achieved at the expense of compensatory fall in albumin levels.

Serum cortisol was recorded higher in PPR affected stock indicating stress. Cortisol causes increase in blood glucose levels due to glycogenolytic property but in present investigation, decreased glucose levels could have been due to animals not being fed since the onset of infection. The higher levels of cortisol causing muscle wasting resulted in increased serum creatinine levels and an increased urea concentration reflected protein breakdown and haemoconcentration simultaneously.

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# OBSEŽNI IZBRUHI KUGE MALIH PREŽVEKOVALCEV PRI OVCAH IN KOZAH V PUŠČAVI THAR V INDIJI

A. K. Kataria, N. Kataria, A. K. Gahlot

**Povzetek:** V puščavi Thar v Radžastanu (Indija) smo naleteli na resne izbruhe kuge malih prežvekovalcev (PPR). Bolezen je prizadela populacijo ovac in koz, ki živi na obsežnem geografskem področju, in je pokončala več deset tisoč živali. Izbruhe bolezni smo analizirali iz epidemiološkega, kliničnega, patološkega, hematološkega in biokemičnega vidika. Izbruhi so se najprej pojavili v nomadskih čredah ovac, ki so se vračale s sosednjih področij z endemično prisotno kugo malih prežvekovalcev, kasneje pa se je razširila še na koze. Ocenjena smrtnost pri ovcah je 70-odstotna in pri kozah 80-odstotna. Mlajše živali so bile bolj prizadete kot odrasle. Naglo širjenje bolezni je pogojeval stres zaradi migracije v kombinaciji z nizkimi temperaturami in visoko vlago v okolju ter pomanjkanjem hrane. Ugotovili smo tipične klinične in patološke najdbe, pri večini hematoloških in biokemičnih parametrov pa smo ugotovili statistično značilne spremembe ($P \leq 0.05$). Zaradi sistema proste paše je bilo prizadetih veliko čred tudi na oddaljenih predelih, bolezen pa se je tam bistveno dlje zadrževala. Število obolenj se je zmanjševalo v odvisnosti z naraščanjem temperature v okolju.
Trenutna situacija v zvezni državi Radžastan zahteva strog nadzor bolezni PPR in serološko pregledovanje v kombinaciji z neprekinjenim cepljenjem nomadskih čred na ogroženih področjih in državnih mejah.

**Ključne besede:** ovce, bolezni – diagnostika – virologija; koze, bolezni – diagnostika – virologija; epidemije; peste-des-petits-ruminants – diagnostika – virologija; klinična patologija; kri, kemične analize; protitelesa, virusna – kri; encimsko vezan imunosorbentni test – metode; puščave; ovce; koze

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# PROF. DR. MILAN POGAČNIK – *DOCTOR HONORIS CAUSA*

Prof. Dr. Milan Pogačnik (left) receiving honorary doctorate from the rector of Ss. Cyril and Methodius University in Skopje Prof. Dr. Gjorgji Martinovski

Not many members of the University of Ljubljana had received the title of honoris causa in the last few years. Therefore, the news that professor Pogačnik, full professor of the veterinary pathology, forensic and administrative veterinary medicine, received this honour on September 26th is much more favourable.

The title was given to professor Pogačnik by the University of St. Cyril and Method of Skopje, Macedonia, as the recognition for his merits for the development of the veterinary medicine in the Republic of Macedonia, for his significant contribution to the development of the veterinary pathology, forensic and administrative veterinary medicine, for his important help in international research projects and his influence on the scientific, educational and professional development of the Skopje Faculty of Veterinary Medicine, especially for the renovation of the study programme in accordance with the European standards.

The ceremony, which took place in the solemn hall of the Skopje University, was attended by the rector, deans, vice deans, senators and managers of the university institutes, the president of EAEVE (European Association for the Establishments of Veterinary Education) professor Marcel Wanner, members of the executive board and deans of some European veterinary faculties.

Thirty six years of professor Pogačnik's work in the field of the veterinary medicine has always been connected with the veterinary pathology, forensic and administrative veterinary medicine and, consequently, with the animal care and protection, veterinary public health and environment protection. Interdisciplinary work, which he devoted his energy to, has been combined with different fields of the veterinary medicine, agriculture, husbandry, food and environment protection, medicine, pharmacy, economy and law. Professor Pogačnik is the principal investigator of the research project Animals' health, environment and safe food, as well as the holder of some target oriented and applied projects in the field of sustainable farming in which he successfully incorporated the interdisciplinary work of professionals from agriculture, forestry and veterinary medicine. The international projects he has conducted enabled Slovenian agricultural and veterinary medicine to establish its place in the Europe and worldwide. The repercussions of his work have resulted in the projects concerning the China Karst cultivation.

As the dean of Ljubljana Veterinary Faculty, professor Pogačnik has essentially contributed to the recognition of the faculty at home and abroad. For a long time he was a member of EAEVE executive committee, one of the founders and the president of VetNest (Veterinary Network of European Student and Stuff Transfer) which associates veterinary faculties of Budapest, Brno (Czech Republic), Košice (Slova-

kia), Wroclaw (Poland), Zagreb (Croatia) Ljubljana and Vienna (Austria) University of Veterinary Medicine. Moreover, professor Pogačnik conducted the international TEMPUS project, dealing with the veterinary education. He also coordinated the Slovenian part of the survey Vet 2020 which goal was to foresee the necessities of the veterinary profession in the next decade. He is also the Slovenian delegate in OIE (Office International de Epizootie), member of ECVAM scientific committee (European Centre for the Validation of Alternative Methods) and, lately, also the member of EFSA directorate (European Food Safety Authority).

Professor Pogačnik has mediated his knowledge and experiences achieved in the scientific, educational, organizational, professional, and management work to other European educational establishments. It is crucial to stress his advisory help to the veterinary faculties in the republics of former Yugoslavia. He has participated as the reviewer in the evaluation of study programmes. As an excellent scientist, lecturer and manager he has actually been the mentor of all faculties in the former common country. This part of the Europe has struggled with many difficulties in the last decade, but the professional connections and the relations have never been cut off.

At the ceremony, the dean of the Faculty of the Veterinary Medicine in Skopje professor Mišo Hristovski stressed the paramount value of the help given by professor Pogačnik to the Skopje Faculty, especially for its international enforcement. With professor's help, the Faculty of Veterinary Medicine in Skopje, together with Veterinary Faculty in Ljubljana, participates in different international research projects. With the endeavours of professor Pogačnik, the Skopje Faculty became the fully authorised member of EAEVE. Experiences with the Slovenian model of the organisation of the Veterinary Faculty were also introduced in the Skopje Faculty system. First contacts with the colleagues from Macedonia were established in the eighties of the last century. Important events which set up the successful collaboration were scientific meetings in Ohrid and the first embryo transfer in Macedonia, carried out by the Slovenian experts. After the establishment of the Faculty of Veterinary Medicine in the autonomous Republic of Macedonia 16 years ago, the connections became closer and stronger. The cooperation continued with the active bilateral projects, expert exchanges, evaluation of study programmes, organisation of the veterinary institute, introduction of quality assurance, accreditation of diagnostic laboratories, etc. Especially important is the introduction of Bologna process in the study of the veterinary medicine, which demanded a lot of new knowledge, experience and skills. Professor Pogačnik is very honourable and respected expert in many fields of the veterinary science at national and international level.

With his previous work and cooperation, professor Pogačnik essentially contributed not only to the development of the Faculty of Veterinary Medicine of the University St. Cyril and Method of Skopje, but also to the general development of the veterinary medicine in the Republic of Macedonia.

At the event of the first nomination of doctor honoris causa in the veterinary profession in Slovenia, we sincerely congratulate professor Pogačnik for this great honour.

*Prof. Dr. Vojteh Cestnik,*
*Veterinary Faculty, Ljubljana*

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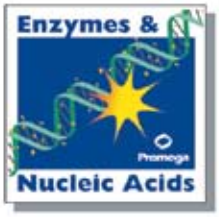







**IZDELKI ZA MOLEKULARNO BIOLOGIJO**

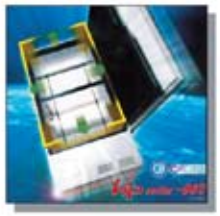
**DOKUMENTACIJA IN ANALIZA GELOV**

**PLASTIKA ZA CELIČNE KULTURE**

ELGA LABWATER

SANYO

Invitrogen life technologies



**ČISTA VODA ZA LABORATORIJ**

**SKRINJE IN HLADILNIKI**

**CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA**

BIOHIT

minerva biolabs

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**ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE**

**DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL**

**HPLC in GC POTROŠNI MATERIAL**

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Contributions should be written in English and should not exceed 12 pages (27 lines per page, approx. 75 characters per line). They should be submitted electronically (preferably to E-mail address, slovetres@vf.uni-lj.si), written in any word processor for Windows. Authors are requested to provide names of three potential reviewers. The text should be double spaced and the lines should be numbered on the left-hand side. The margin on the left-hand side of the page should be 4 cm.

The front page of a manuscript should start with the title, followed by the name and surname of the author(s). If there is more than one author, their names should be separated by commas. The next line ('Addresses of authors:') should contain the authors' full names and addresses (institution, street and number, postcode and place) after the colon. All the given data should be separated by commas. The name, address and E-mail and/or phone number of the corresponding author should be written in the next line.

The Summary of 16-20 lines (1000-1500 characters) should follow on the next page.

Under 'Keywords:' (after the colon), keywords should be given. Individual words or word combinations should be separated by semicolons. Scientific papers and papers which present the author's research and findings should also include the following obligatory headings assigned by the author to appropriate parts of the text: Introduction, Materials and methods, Results, Discussion, and References. Review articles should consist of an introduction, sections logically titled according to the content, and references. Information on fund-providers and other matters important for the paper (e.g. technical assistance) should be supplied under 'Acknowledgements', which should be placed before the references. Figure legends should follow the references.

Tables, graphs and diagrams should be logically incorporated in the text file. Original photographs or drawings should be sent as separate files in bmp, jpg or tif format. They should be referred to by type and using Arabic numerals (e.g. Table 1:, Figure 1:, etc.). The colon should be followed by the text or title. All references cited in the text should appear in the References. They should be numbered in the text in the order in which they appear, marked with Arabic numerals placed in parenthesis. The first reference in the text should determine the number and order of the respective source in the References. If the author refers again to a source which has already been used in the text, he should cite the number the source had when it was referred to for the first time. Only works which have been published or are available to the public in any other way may be referred to. Unpublished data, unpublished lectures, personal communications and similar should be mentioned in the references or footnotes at the end of the page on which they appear. Sources in the References should be listed in the order in which they appear in the text. If the source referred to was written by six authors or less, all of them should be cited; in the case of seven or more authors, only the first three should be cited, followed by 'et al.'.

Any errata should be submitted to the editor-in-chief in good time after publication so that they may be publishedin the next issue.

### Examples of references

**Book:** Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

**Chapterorar ticle in a book:** Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

**Article in a journal or newspaper:** Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

**Article in proceedings of a meeting or symposium:** Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

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Slovenski veterinarski zbornik (Slovenian Veterinary Research) objavlja izvirne prispevke, ki še niso bili objavljeni oz. poslani v objavo drugam. Za vse navedbe v prispevkih so odgovorni avtorji. Uredniška politika obsega publiciranje znanstvenih člankov, preglednih znanstvenih člankov, strokovnih člankov, povzetkov disertacij in drugih prispevkov, kot so kritične presoje o vsebini razprav, objavljenih v zborniku, kratke znanstvene prispevke, pisma uredniku in drugo. Avtorji pošljejo prispevke na naslov uredništva. Glavni urednik pregleda vse prispevke. Za vse članke je obvezna strokovna recenzija, za katero poskrbi uredništvo.

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Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

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Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

### Načini citiranja

**Knjiga:** Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

**Poglavje ali prispevek v knjigi:** Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

**Članek iz revije ali časopisa:** Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Č**lanek iz zbornika referatov:** Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

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