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N. PAPAIOANNOU (N. ΠΑΠΑΪΩΑΝΝΟΥ), M. ZAVLARIS (M. ΖΑΒΛΑΡΗΣ), N. D. GIADINIS (N.Δ. ΓΙΑΔΙΝΗΣ), E. J. PETRIDOU (Ε.Ι. ΠΕΤΡΙΔΟΥ), V. PSYCHAS (Β. ΨΥΧΑΣ)

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■ A case of kidney infection by *Corynebacterium pseudotuberculosis* in sheep

Papaioannou N.¹, DVM, PhD, Zavlaris M.¹, DVM, Giadinis N. D.², DVM, PhD,
Petridou E. J.³, DVM, PhD, Psychas V.¹, DVM, PhD

¹Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

²Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

³Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

■ Κλινικό περιστατικό μόλυνσης του νεφρού προβάτου από *Corynebacterium pseudotuberculosis*

N. Γ. Παπαϊωάννου¹, DVM, PhD, M. Ζαβλάρης¹, DVM,
N. Δ. Γιαδίνης², DVM, PhD, E. Ι. Πετρίδου³, DVM, PhD, B. Ψύχας¹, DVM, PhD

¹Εργαστήριο Παθολογικής Ανατομικής, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

²Κλινική Παραγωγικών Ζώων, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

³Εργαστήριο Μικροβιολογίας & Λοιμωδών Νοσημάτων, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

ABSTRACT. A three year-old female sheep was presented to the Clinic of Farm Animals, School of Veterinary Medicine, Aristotle University of Thessaloniki, with mild nasal seromucous discharge and progressive weight loss, while rectal temperature was normal. Hematological examination revealed mild leukocytosis, while traces of hemoglobin and protein were found in urinalysis. The animal was euthanized and forwarded for necropsy. An extremely enlarged, nodular right kidney, whose renal parenchyma was completely replaced by multiple abscesses, was the remarkable finding of the necropsy. Microbiological and molecular examinations were performed to establish the diagnosis and rule out Maedi/Visna infection. Tissue samples from the kidney and other organs were, also, examined histologically. *Corynebacterium pseudotuberculosis* was isolated in microbiological tests. A kidney infection by *C. pseudotuberculosis*, presented as a clinical case with scant symptoms, is discussed in this case report.

Keywords: sheep, *Corynebacterium pseudotuberculosis*, kidney, abscess

ΠΕΡΙΛΗΨΗ. Στην Κλινική Παραγωγικών Ζώων της Κτηνιατρικής Σχολής του Α.Π.Θ., προσκομίστηκε θηλυκό πρόβατο Φρισιλανδικής φυλής, 3 ετών, με ιστορικό απώλειας βάρους επί δίμηνο. Στην κλινική εξέταση διαπιστώθηκαν θερμοκρασία απευθυσμένου 39°C, όρεξη φυσιολογική, ελαφρό ορώδες ρινικό έκκριμα, υγρός βήχας, ενώ κατά την ακρόαση της τραχείας ακούγονταν ρόγχοι. Οι αιματολογικές και βιοχημικές παράμετροι ήταν σε φυσιολογικά επίπεδα, εκτός από μια ήπια λευκοκυττάρωση, ενώ ίχνη αιμοσφαιρίνης και πρωτεϊνών ανιχνεύθηκαν στην εξέταση του ούρου. Λόγω της προοδευτικά επιδεινούμενης εξέλιξης του περιστατικού, πραγματοποιήθηκε ευθανασία και το ζώο προσκομίστηκε για νεκροτομική εξέταση. Το κύριο

Correspondence: Papaioannou N.
Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki,
541 24 Thessaloniki, Greece
Tel.: +30 2310 999976, Fax: +30 2310 999979, E-mail: nikpap@vet.auth.gr

Αλληλογραφία: Ν. Παπαϊωάννου
Εργαστήριο Παθολογικής Ανατομικής, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο
Θεσσαλονίκης, 541 24 Θεσσαλονίκη
Τηλ.: 2310 999976, Fax: 2310 999979, E-mail: nikpap@vet.auth.gr

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εύρημα της νεκροτομής ήταν ο δεξιός νεφρός, που εμφανιζόταν διογκωμένος, οζώδης, ενώ το παρέγχυμά του είχε αντικατασταθεί από πολυάριθμα αποστήματα. Από δείγματα ιστών παρεγχυματικών οργάνων πραγματοποιήθηκαν μικροβιολογικές, μοριακές και ιστοπαθολογικές εξετάσεις, με σκοπό την απομόνωση του αιτιολογικού παράγοντα, τον αποκλεισμό της Maedi/Visna και τη μελέτη των ιστολογικών αλλοιώσεων, αντίστοιχα. Στη μικροβιολογική εξέταση από δείγματα νεφρού και πνεύμονα, απομονώθηκε το *Corynebacterium pseudotuberculosis*. Η περίπτωση ενός ενδιαφέροντος κλινικού περιστατικού, που προσκομίστηκε με ασαφή συμπτωματολογία από το οποίο απομονώθηκε το *Corynebacterium pseudotuberculosis* από το νεφρό, συζητείται στο άρθρο αυτό.

Λέξεις ευρετηρίασης: πρόβατο, *Corynebacterium pseudotuberculosis*, νεφρός, απόστημα

Case report

Corynebacterium pseudotuberculosis infections in sheep are associated with the formation of lymph node pyogranulomas and this accounts for the name "caseous lymphadenitis", (CLA). The lesions occur in two main forms; external, known as superficial or cutaneous, and visceral, which may co-exist within the same animal. The superficial form is characterized by abscessation of lymph nodes, palpated externally, consisting of a central mass of thick and sometimes dry greenish-white necrotic material surrounded by a connective tissue capsule. Any of the superficial lymph nodes of the body may be affected (Ayers 1977, Lofstedt 2002, Baird and Fontaine 2007). Visceral CLA, due to the haematogenous and lymphogenous spread (Batey 1986a), is followed by lesions in the lung, mediastinal lymph nodes, liver, kidneys or udder, and rarely in other organs (Stoops et al. 1984, Batey 1986a, Baird and Fontaine 2007, Radostits 2007, Valli 2007, Grant and Shelley 2007). *C. pseudotuberculosis* can be transmitted by animal to animal contact. The main source of infection are infected animals with lung lesions, which usually spread aerosols of infectious organisms (Baird and Fontaine 2007). The rupture of superficial abscesses releases huge numbers of bacteria to the skin and fleece and it contaminates the immediate environment. As a result, chronic infections may last for most or all of the animal's life, although they are rarely fatal (Baird and Fontaine 2007). Although the possibility of kidney infection has already been reported as a random finding, mainly in slaughtered sheep at abattoirs during routinely inspection (Stoops et al. 1984, Paton et al. 2003, Valli 2007, Grant and Newman 2007), according to our knowledge, clinical cases of kidney infections have not been reported. The present case report article presents a clinical case of a sheep with kidney infection and describes ante-mortem and post-mortem clinicopathological findings.

The case originated from an intensively reared dairy sheep flock located in Northern Greece, consisting of 230 Chios and East Friesian dairy sheep. The animals consumed alfalfa hay and straws ad libitum, while concentrates were fed according to their nutrient requirements. A three year-old female East Friesian sheep of the above flock was forwarded to the Clinic of Farm Animals at the School of Veterinary Medicine of Aristotle University of Thessaloniki. After two months, weight loss was reported, while appetite remained normal. Rectal temperature was recorded at 39°C and mucous membranes were slightly congested, while slight bilateral mucous nasal discharge was observed. The animal had wet cough, while trachea auscultation revealed ronchi. Blood, urine and fecal samples were collected. Due to the progressive worsening of the general condition of the sheep, the animal was forwarded for euthanasia and necropsy. Sampling of all parenchymal tissues was performed for histopathology, including kidney, liver, spleen, mediastinal lymph nodes, lung and intestines. All tissues were fixed in 10% formalin, embedded in paraffin, while series of sections in 4µm thickness were taken. The routine haematoxylin and eosin (H&E) stain was used in all histological sections. Samples from kidney and lung abscesses were aseptically collected and forwarded for microbiological examination. All samples were plated into blood base agar with 5% defibrinated sheep blood and MacConkey agar. Enzyme-linked immunosorbent assay (ELISA) and semi-nested Polymerase Chain Reaction (PCR) were performed in serum and in the whole blood, respectively, to rule out the Maedi/Visna virus. The hematological findings were normal, with a single exception of a mild leukocytosis (WBC was 13,500/µl, reference range 4,000-12,000/µl), (Kramer 2000, Panousis et al. 2007). The blood biochemical profile was within the normal limits cited in the literature

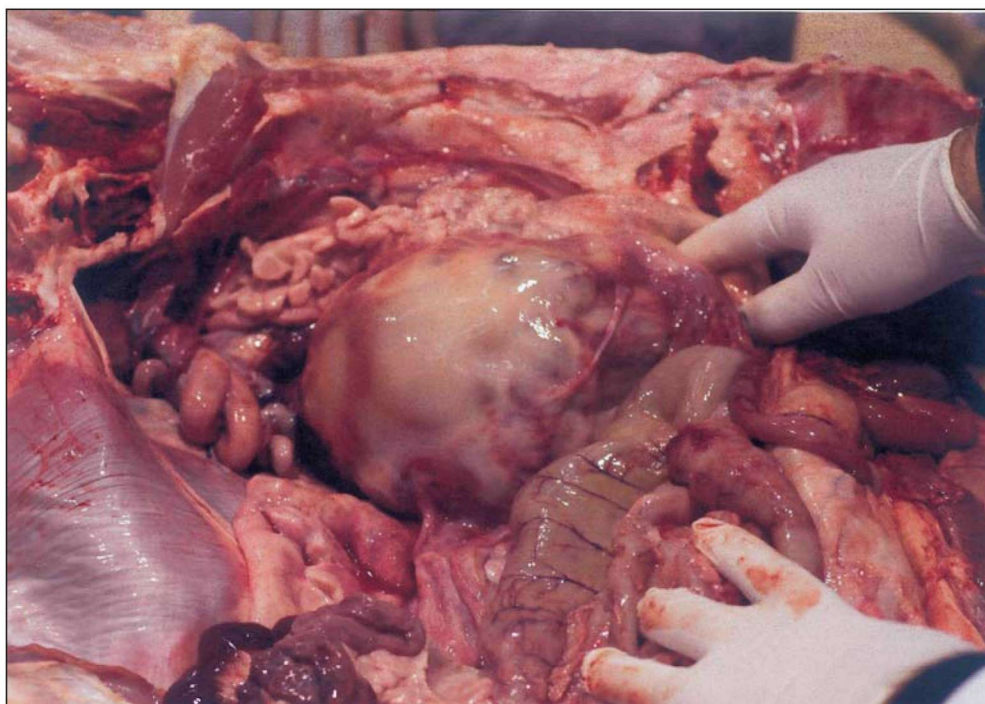


Figure 1

Remarkably enlarged and nodular appearance of the right kidney covered by a thick fibrous capsule and adhesions with abdominal cavity organs.

(Roubies et al. 2006). In urinalysis, traces of hemoglobin and protein were detected (urine dipsticks), while urine specific gravity measured with refractometry was 1.016. Parasitological examination of fecal sample was found negative for trematodes, cestodes and nematodes.

Gross findings during post-mortem examination of the carcass revealed a slightly poor general nutritional status and a small amount of seromucous nasal discharge. During necropsy, the most remarkable finding was the enlargement of the right kidney (18 x 14 cm), which occupied at least 1/3 of the abdominal cavity. Adhesions between kidney and intestines were also noted (Figure 1). The external surface of the kidney was nodular and the capsule observed was very thick (Figure 2). The longitudinal section of the kidney revealed the replacement of renal parenchyma by numerous well-encysted encapsulated abscesses. The abscesses contained greenish-yellow pus that had lamellar “onion-ring” appearance; these were particularly prominent when calcareous granules were deposited in successive layers at the margins of the expanding lesion due to the long lasting infection (Figure 3). Finally, purulent material was found in the dilated kidney pelvis. The left kidney was found macroscopically normal. In the thoracic cavity, both lungs were slightly distended and edematous, while two abscesses were detected in the

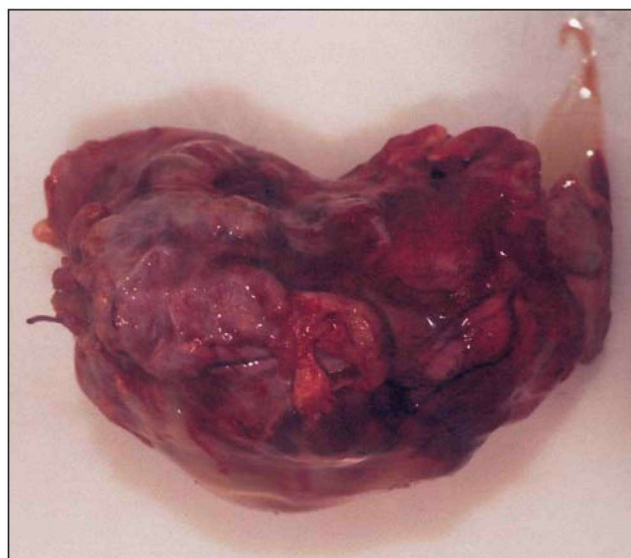


Figure 2. Same kidney, as in figure 1, showing nodular appearance of the organ.

cranial lung lobe and the mediastinal lymph node, respectively. These abscesses had diameter 2 cm each. The lung abscess was encapsulated and circumscribed by a narrow zone of inflammation. No bronchopneumonia, pleuritis, adhesions, serous fluid or fibrin were noted. Nasal cavities had a small amount of mucous discharge and the trachea was partially filled by a foamy material. Histological appearance of lung lesion was characterized

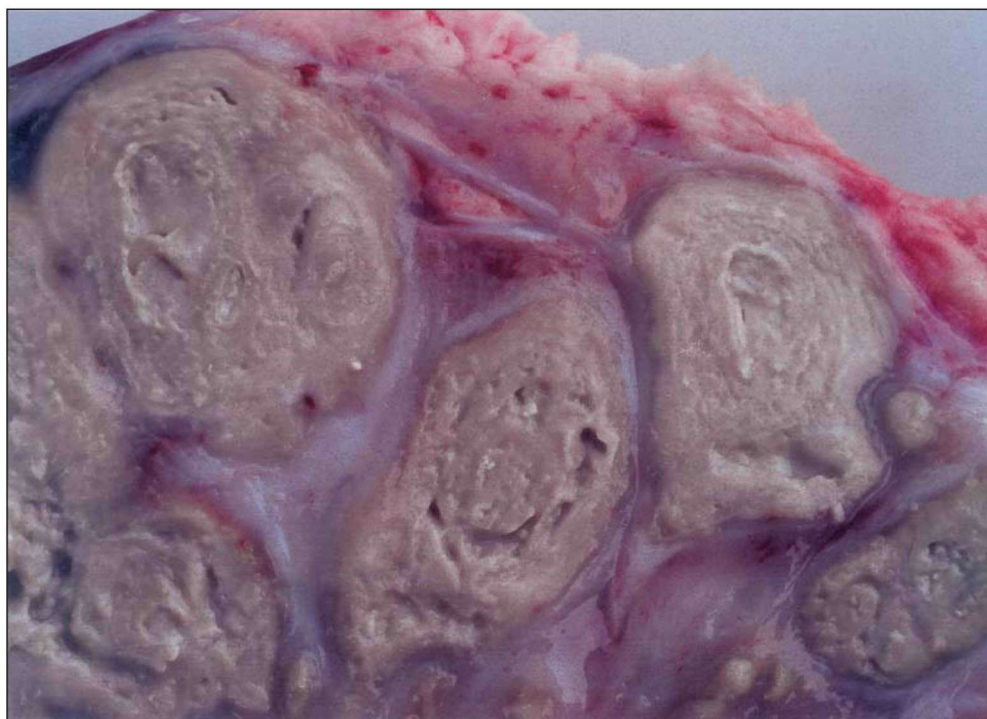


Figure 3
Abscesses with concentric lamellations of yellow pus. Note the calcareous granules deposited in successive layers.

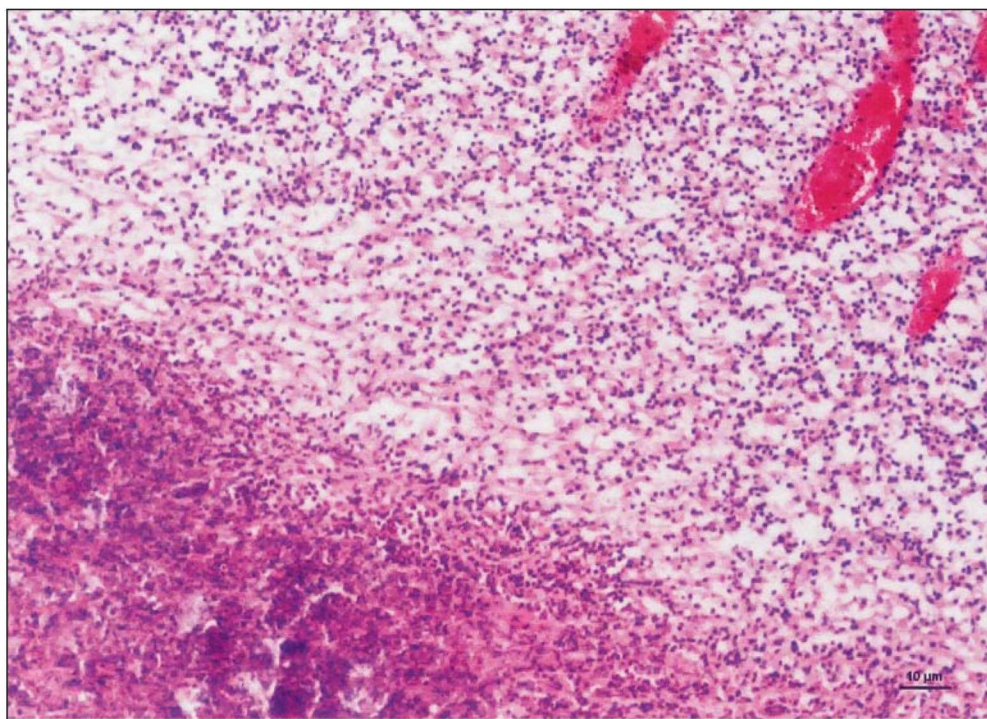


Figure 4
Histopathological appearance of the lung abscess. The central necrotic tissue is infiltrated and demarcated by neutrophils and lymphocytes. (H&E x 20), Bar = 10µm.

by a central area of necrotic tissue, which was demarcated with a clearly visible inflammatory zone of lymphocytes, neutrophils, macrophages and, rarely, eosinophils (Figure 4). Concerning the right kidney lesions, renal parenchyma was replaced by necrotic tissue, whereas foci of degeneration and necrosis of the tubular

epithelial cells and renal corpuscles were detected (Figure 5). The intermediate connective tissue was infiltrated by neutrophils, lymphocytes and macrophages, whereas hemorrhages were relatively often. The left kidney was found normal in histological examination. The rest of the organs, examined histologically, didn't

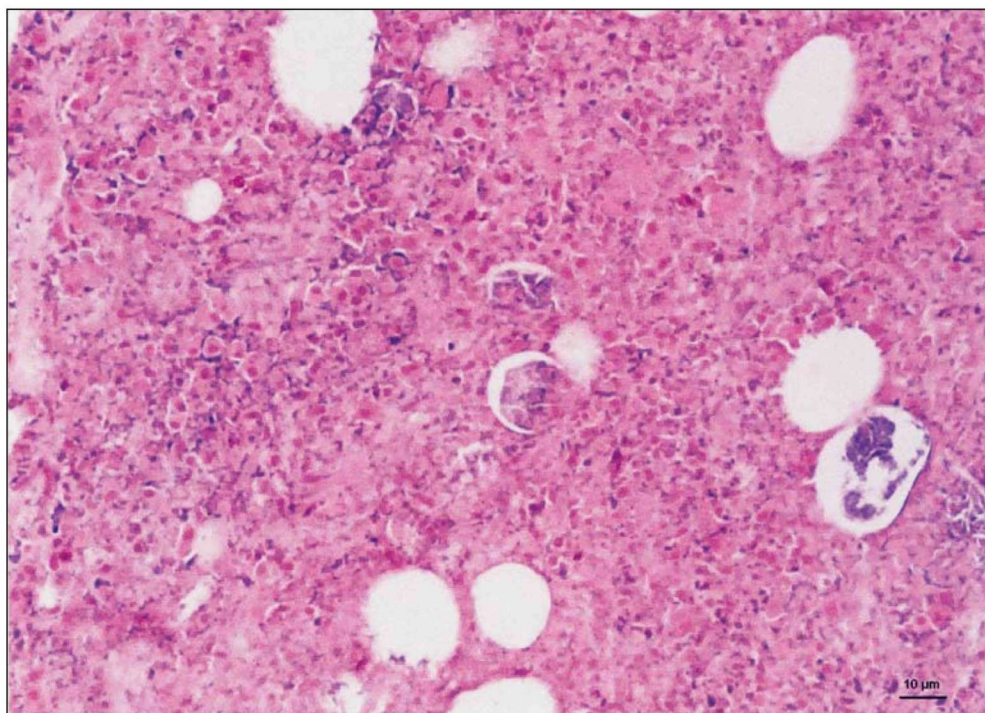


Figure 5

Cortical area of the kidney, showing replacement of renal parenchyma by necrotic tissue. Renal corpuscles are clearly degenerated. (H&E x 10), Bar = 10µm.

show any pathological lesion.

After aerobic incubation at 37°C for 72 hours, small white and dry colonies were observed. Gram staining revealed the presence of gram positive small pleomorphic rods, catalase negative, oxidase positive. The isolate was identified as non-nitrate reducing biotype of *Corynebacterium pseudotuberculosis*, according to biochemical properties (Quinn et al. 1999). The presence of Maedi/Visna virus was not detected in both ELISA and semi-nested PCR.

Discussion

In this report, we describe a case of pseudotuberculosis infection in a sheep with unilateral kidney localization. To our knowledge, previous reports describe few cases of kidney infection, sporadically seen in abattoirs during meat inspection, and not separate clinical cases (Stoops et al. 1984, Batey 1986a, Jeckel et al. 2009). A clinical diagnosis of CLA in urine culture is just recently mentioned (Ferrer et al. 2009). In our case, the mild clinical signs and mild leukocytosis couldn't establish the diagnosis of *C. Pseudotuberculosis* infection, from a clinical point of view. Clinical appearance of the case suggested a chronic, perhaps generalized, infection of the lower respiratory tract. Several reports mention that weight loss could add the possibility of *C. Pseudotuberculosis*

infection in differential diagnosis (Lofstedt 2002, Baird and Fontaine 2007, Radostits 2007, Valli 2007, Grant and Newman 2007), but the typical symptom of external abscesses was not noticed in our case. Radiography and ultrasonography could be helpful for the diagnosis of this case (Scott et al. 1997), but the clinical findings were not suggestive for these specific examinations. Necropsy was the most reliable examination, which suggested the diagnosis, since the finding of internal abscesses confirmed the hypothesis above. This method benefits veterinarians who survey the flocks to differentiate the infection from other diseases with no specific symptoms. Accumulation of leukocytes, macrophages and eosinophils at the periphery of the necrotic lesions in kidney and lungs examined histologically, share similar histopathological profile of pseudotuberculosis infections mentioned in other studies (Ayers 1977, Lofstedt 2002, Radostits 2007, Valli 2007, Grant and Newman 2007, Jeckel et al. 2009). It is known that phospholipase D toxin is an important aspect of pathogenicity in natural infection of sheep and, when the infection is generalized, it gives scant clinical evidence of intoxication (Batey 1986b, Baird and Fontaine 2007), as in our case, and it is the probable initiator of this lymphadenitis. Early pyogranulomas contained clumps of bacteria and cellular debris with a relatively high proportion of

eosinophils, giving to the purulent core a slightly green hue, as in our report. At this point, it must be underlined that this typical lamellation is specific to the organism and only this pathological finding during necropsy could guide us to the diagnosis of pseudotuberculosis. The infiltration of neutrophils and monocytes/macrophages suggest inflammatory cell accumulation in necrotic tissue (Pepin et al. 1994, Baird and Fontaine 2007). It is, also, probable that similar events take place in kidney with entrapment of infected leucocytes or free bacteria by embolism, a fact that could be concordant with our case and might explain the unilateral appearance of kidney infection (Jolly 1965). Our case supports the notion that natural infection of sheep, even generalized, gives scant clinical evidence of intoxication (Radostits 2007, Valli 2007). There are no specific clinical signs indicative of CLA in sheep affected by the visceral form of CLA (Stoops et al. 1984). Although CLA lesions in the lungs may produce signs of respiratory infection, affected sheep may exhibit no specific clinical signs other than the occasional presence of detectable superficial abscesses to suggest a diagnosis of generalized CLA. Maedi/Visna was ruled out by semi-nested PCR performed in the whole blood, as well as serology (Pritchard and Dawson 2002), while *Corynebacterium pseudotuberculosis* isolation and presence of small rods positively stained in Gram staining, confirmed the diagnosis. Whilst isolation and identification of *C. pseudotuberculosis* remains the gold standard in CLA diagnosis, this may not always be advantageous or possible. Animals suffering from the visceral form of the disease may show no external lesions that can be sampled, but they remain a potential source of infection to others and that's why our case is worth being reported. Serological tests for CLA do not confidently detect infection in individual sheep (Baird and Fontaine 2007, Radostits 2007, Valli 2007). Other bacterial pathogens, such as *Actinobacillus lignieresii*, *Arcanobacterium pyogenes*, *Staphylococcus aureus* subsp. *anaerobius* are all capable of producing suppurative lymphadenopathy; however, these infections tend to be sporadic and are rarely seen as a flock problem (Baird and Fontaine 2007). Differential

diagnosis should, also, include melioidosis, tularemia, other causes of pneumonia in small ruminants and lymphosarcoma (Radostits 2007). The wide spread of the disease, the economic losses, the chronic infections in flocks and the possibility of human infection, due to *C. pseudotuberculosis* (Peel et al. 1997), led researchers to focus on treatment and prevention. Several reports support that the use of antibiotic therapy and surgical drainage techniques are not encouraging (Baird and Fontaine 2007, Radostits 2007), while the use of vaccines is under question (Baird and Fontaine 2007).

Caseous lymphadenitis in sheep is mainly the result of an infected wound. The organism can penetrate intact skin of freshly shorn sheep, however, it may, also, be transmitted by dipping fluids. Ingestion is a more rare way of infection and it is apparent only in lesions to nodes of the buccal cavity. Inhalation can produce lung abscesses, while parasitic wounds are considered to be extremely rare as a portal of infection (Batey 1986b, Baird and Fontaine 2007, Radostits 2007). CLA has been reproduced in sheep by intradermal, subcutaneous, intravenous, intratracheal, intravaginal and intralymphatic route (Pepin et al. 1994, Fontaine et al. 2006, Baird and Fontaine 2007). It was not easy to find the route of the infection in the present case, since no wound infection was reported. After the initial entry, the organism spreads rapidly to the local drainage lymph node, where multiple microscopic pyogranulomas develop, grow in size and coalesce to form larger abscesses. This is sometimes followed by a further extension of the infection via the blood or the lymphatic system, leading to similar lesions in other organs.

The possibility of haematogenous and lymphogenous spread of infection in internal organs is sporadically reported in sheep presented in abattoirs (Stoops et al. 1984, Batey 1986a, Jeckel et al. 2009). A clinical case with scant symptoms, described in our report, could lead veterinarians to misdiagnose. The extremely enlarged and replaced by an abscess right kidney found in necropsy is worth being noted. Necropsy, in comparison with microbiological examinations in flocks with weight losses, constitutes a method with preventional and diagnostic utility. ■

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