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Case Report

CONCURRENT SCRAPIE AND CHRONIC COENUROSIS IN TWO CHIOS SHEEP

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Summary: Chronic coenurosis and Scrapie are two of the most common diseases of the central nervous system in the small ruminants of Greece and other countries, and both can cause severe financial losses in affected flocks. Clinicopathological examination of animals *in vivo* is not pathognomonic and so these conditions are usually confirmed by necropsy. This study reports, for the first time in the veterinary literature, two cases of the simultaneous presence of Scrapie and chronic coenurosis in two sheep.

Two Chios ewes aged 1.5 and 2 years, respectively, presenting chronic neurological symptoms, were presented to the Farm Animal Clinic of Aristotle University in Thessaloniki. Sheep 2 also had pruritus on the hindlegs. The haematological and biochemical profiles of the two animals were within the normal limits cited in the literature. Both animals were euthanised and necropsied, and were found to have two and one *Coenurus cerebralis* cyst in their brains respectively. As the two animals belonged to flocks reared in Scrapie-affected areas, the two brains were examined both histopathologically and immunohistochemically and were found to be positive for Scrapie. It can therefore be concluded that Scrapie could be present in sheep with coenurosis, especially in those from areas with a high rate of occurrence of Scrapie.

Key words: sheep; Scrapie; chronic coenurosis; diagnosis

Introduction

Chronic coenurosis and Scrapie are two of the most important chronic ovine encephalopathies, causing losses in sheep flocks in Greece and other countries, with severe financial consequences. In addition, Scrapie belongs to the disease-group of transmissible spongiform encephalopathies (TSEs) and thus has a possible zoonotic impact; any diagnosis is thus required to be reported to the appropriate public health authorities of the European Union (1, 2, 3, 4).

Coenurosis is a parasitic disease caused by *Coenurus cerebralis*, the larval stage of the taenia *Multiceps multiceps* (*Taenia multiceps*) that inhabits the small intestine of dogs and wild canids, the definitive hosts. Two clinical forms of the disease have been described in sheep; the chronic form, which is the most common and is usually observed in animals aged between 6 and 18 months, although it has been described in older animals (1, 4) and the acute form, which is more rarely observed and usually affects animals younger than 1 year (5). Chronic coenurosis presents with an insidious onset and slow progressive focal lesion of the brain (4).

Scrapie is a chronic, progressive and invariably fatal neurodegenerative disorder naturally affecting sheep and goats. It is caused by a prion and is usually observed in animals older than 2 years old, but it has also been diagnosed in younger animals.

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The first signs are usually behavioural changes that are most readily evident to shepherds. These early signs progress to a more definite neurological illness frequently characterized by signs of pruritus and ataxia, one of which usually dominates the clinical course (3, 6).

Clinically, these two encephalopathies are not easily distinguishable other than their different age predilection and the fact that Scrapie is often accompanied by pruritus. As such, diagnosis is based upon necropsy findings or the successful response to surgical treatment in chronic coenurosis or brain histopathological examination in cases of Scrapie. Neither of these diseases can be diagnosed ante-mortem with routine paraclinical examinations *in vivo* (3, 4, 7).

To the best of our knowledge, there has been no report to date describing the concurrence of coenurosis and Scrapie infections in sheep exhibiting neurological symptoms. This study describes the first known cases of coenurosis and Scrapie occurring simultaneously in two sheep belonging to separate flocks.

Materials and Methods

Case history

Two sheep of the Chios breed were presented with chronic neurological symptoms to the Farm Animal Clinic of the Faculty of Veterinary Medicine at the Aristotle University, Thessaloniki, Greece. They came from two different flocks in Northern Greece, neither of which had a history of coenurosis or Scrapie, although both were located in areas of high Scrapie prevalence.

Clinical examination

A thorough clinical and neurological examination of both animals was conducted (8, 9).

Haematological examination

Haematological examinations were conducted using an automated hematology analyser (ADVIA 120, Siemens).

Blood biochemistry

Blood serum samples were examined for glucose, blood urea nitrogen (BUN), creatinine, creatine kinase (CK), total and direct bilirubin, as well as calcium and phosphate, using a clinical chemistry analyser (Flexor E, Vital Scientific).

Maedi-Visna antibodies

Serum samples were examined for the presence of SRLV specific antibodies using the CHEKIT-CAEV/MVV ELISA test kit (IDEXX, Switzerland).

Post-mortem examination

The two animals were euthanased and a full necropsy was conducted.

Histopathology and immunohistochemistry

Tissue samples were taken from the brains of both sheep for histopathological evaluation. The samples were fixed in 10% formol saline and, using standard methods, the tissues were blocked in paraffin and cut into 4–5-μm thick sections. These tissue sections were stained with haematoxylin-eosin (HE), Kluver-barrera stain and periodic acid Schiff stain (PAS) for histopathological examination. Moreover, in sections from the brainstem, at the level of the obex, immunohistochemical staining for PrP^{Sc} detection was performed using the labeled streptavidin avidin-biotin peroxidase method (LSAB kit, DAKO) and applying the monoclonal antibody anti-PrP 2G11. An antigen unmasking pretreatment was carried out in a bath of 87% formic acid for 10 min and hydrated autoclaving at 121 °C for 20 min at 2 atm (12). The staining pattern was revealed by applying 3-3' diaminobenzidine (DAB) as a chromogen.

Parasitological examination

The cysts were examined and identified according to the protocol used in previous studies (13, 14).

Results

Clinical findings

Sheep 1 was an 18-month-old female displaying weight loss, although it had a normal appetite and was afebrile, and its mucosal membranes were normal. It presented with ataxia and epileptiform seizures for 35 days that had gradually worsened. Sheep 2 was a 2-year-old female with a normal temperature, mucosal membranes and appetite. It presented with ataxia, blindness and pruritus on the hindlegs for 30 days that had gradually worsened.

Haematological findings

Haematological findings were within the normal limits cited in the literature for Chios sheep (10).

Biochemical findings

Serum biochemical findings were within the normal limits cited in the literature for Chios sheep (11).

Maedi-Visna examination

Sheep 2 was found to be seropositive for Maedi-Visna infection, while sheep 1 was seronegative.

Gross lesions

On postmortem examination of sheep 1 a cyst containing clear fluid and numerous scolices was detected. The cyst covered part of the brainstem, the cerebellum and the left cerebral hemisphere. Coronal sectioning of the brain revealed that part of the cyst was detected within the dilated left lateral ventricle. A 3 cm-diameter, fluid-filled superficial cyst with white clusters of scolices was also found. A parasitic cyst was also found in the left cerebral hemisphere of sheep 2.

Histology and imminohistochemistry

Histologically, the brain tissue of the affected hemisphere adjacent to the cyst revealed typical pressure atrophy lesions of the cerebral grey and white matter such as degeneration and atrophy of neurons, neuronophagia, satellitosis,

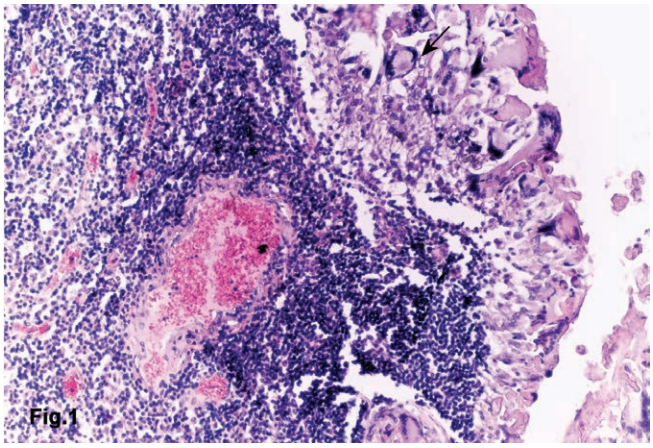

Figure 1: Cerebellum. Periparasitic area characterized by granulomatous inflammatory reaction and necrosis. Presence of foreign body giant cell (arrow). HE x 200

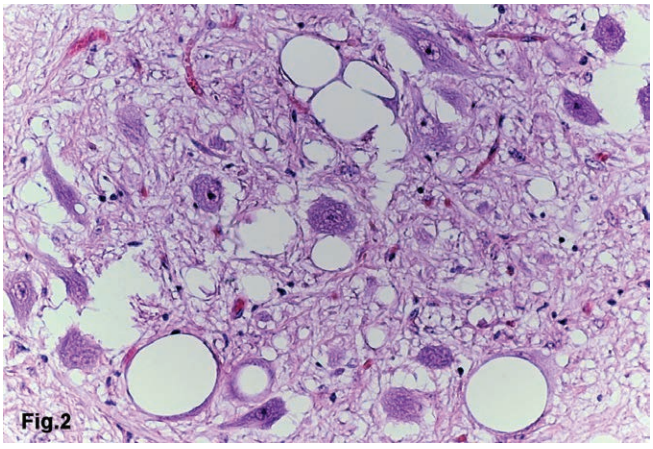

Figure 2: Obex, dorsal motor nucleus of the vagus nerve. Typical pattern of neuropil and neuronal vacuolation. HE x320

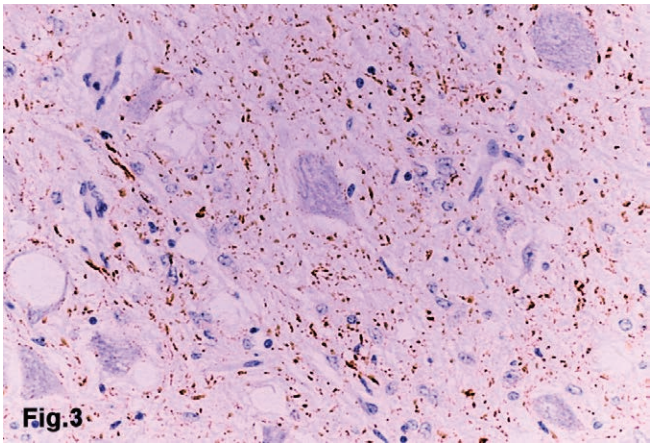

Figure 3: Obex, dorsal motor nucleus of the vagus nerve. Intraneuronal immunolabelling of PrP^{sc} as well as fine punctuate and linear deposits in the neuropil. IHC, LASB, x 320

demyelination, perivascular cuffing or infiltration of mononuclear inflammatory cells, non purulent meningitis, diffuse microgliosis and astrocytosis. The cyst was surrounded by granulomatous inflammation (Figure 1). Blood vessels of medulla oblongata, mesencephalon and choroid plexus in the fourth ventricle were infiltrated by lymphocytes. Neuropil vacuolation and neuronal perikaryonal vacuolation were also found in the dorsal motor nucleus of the vagus nerve at the level of the obex (Figure 2). Immunolabeling of the brainstem, at the level of the obex revealed the following patterns of PrP^{Sc} accumulation: intaneuronal, fine punctuate and linear in the neuropil of the dorsal motor nucleus of the vagus nerve (Figure 3).

Parasitological findings

The cysts that were found in the two sheep brains were identified as *Coenurus cerebralis*.

Discussion

In this study, typical clinical, macroscopic and microscopic findings of both cerebral coenurosis and Scrapie were found occurring simultaneously in the same animal. The differential diagnosis included the three main chronic sheep encephalopathies: chronic coenurosis, Scrapie and Visna, as well as other rarely observed conditions, such as brain abscess or neoplasms that occupy the space within the cranium and can have similar clinical manifestations (4, 7). Based on the clinicopathological findings of both of the living animals before necropsy, sheep 1 was most likely suffering from chronic coenurosis, as it was young and also non-pruritic (4), while sheep 2 at 2 years was probably affected with Scrapie, as it had pruritus possibly complicated by the presence of Visna, as it was also seropositive for this lentivirus. The diagnosis of coenurosis was confirmed by the necropsy findings; histopathological and immunohistochemical examination of the brain excluded the presence of Visna and confirmed Scrapie infection (4, 7).

It is notable that coenurosis may be treated relatively easily surgically and usually has a good prognosis with a high percentage of affected animals recovering quickly (1). Scrapie, in contrast, is an incurable condition and whole flocks should be destroyed where prevalence is high, as required by the EU regulation (3, 7). As such, the suggested course in cases of the co-existence of these two conditions is euthanasia of the affected animal.

In conclusion, in areas or herds with a high prevalence of Scrapie, it is useful to take into account its possible co-existence with chronic coenurosis, and to consider Scrapie as a possible cause of the failure of chronic coenurosis treament.

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ISTOČASNA PRISOTNOST PRASKAVCA IN KRONIČNE CENUROZE PRI DVEH OVCAH PASME CHIOS

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Povzetek: Kronična cenuroza in praskavec sta dve izmed najbolj pogostih bolezni centralnega živčnega sistema pri drobnici v Grčiji in mnogih drugih državah, obe povzročata velike finančne izgube v prizadetih čredah. S kliničnim patološkim pregledom živali je bolezen težko diagnosticirati, diagnosticira se šele z obdukcijo. V tej študiji prvič v veterinarski literaturi poročamo o prisotnosti obeh bolezni pri dveh ovcah.

Na kliniko Farm Animal Clinic na Aristotle University v Solunu sta bili pripeljani dve ovci pasme chios, stari 1,5 in 2 leti, s kroničnimi nevrološkimi simptomi. Pri eni je bilo prisotno tudi srbenje na zadnjih nogah. Hematološki in biokemijski profil sta bila pri obeh živalih v normalnih mejah, navedenih v literaturi. Obe sta bili evtanazirani in z obdukcijo je bila v možganih ugotovljena prisotnost cist *Coenurus cerebralis*. Ker sta bili iz čred, vzrejenih v območju praskavca, smo možgane analizirali s histološkimi in imunohistološkimi preiskavami na prisotnost praskavca in ga v obeh primerih tudi potrdili. Zato je mogoče sklepati, da je pri ovcah s cenurozo, še zlasti v območjih z visoko stopnjo pojavljanja praskavca, možno pričakovati tudi prisotnost praskavca.

Ključne besede: ovce; praskavec; kronična cenuroza; diagnoza