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Case report

CLINICAL, PATHOLOGICAL, AND CT SCAN FINDINGS OF MULTIPLE *COENURUS CEREBRALIS* CYSTS IN A NON-NATIVE LAMB IN IRAN

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Summary

Gholami, N., P. Dehghan Rahim Abadi, M. H. Naji Zadeh, D. Golchin, H. Ildar Abadi & H. Rasmi Atigh, 2022. Clinical, pathological, and CT scan findings of multiple *Coenurus cerebralis* cysts in a non-native lamb in Iran. *Bulg. J. Vet. Med.,* **25**, No 4, 672–680.

Coenurus cerebralis is the larval stage of *Taenia multiceps* which involves particularly the central nervous system of humans and ungulates. A six-month-old Charollais lamb was presented with cortical blindness, dullness, convulsion, head deviation, reluctancy to move, drooling of saliva and ataxia. Haematological findings revealed an inflammatory process. CSF analysis showed leukocytosis associated with increased total protein. By CT imaging, nine cysts were detected in the cerebrum and one in the cerebellum. Hounsfield units were below 10 in all these regions. CT images perfectly revealed multiple *Coenurus* cysts in various parts of the brain, which was confirmed by necropsy. At necropsy, cysts of variable size were noted at different anatomical sites of the brain. In the cerebrum, atrophy, liquefactive necrosis and mild demyelination were presenty adjacent to the cystic structures. Perivascular and perineuronal oedema, neuronal degeneration, hyperaemia and thrombosis were also seen in the cerebrum. The meninges revealed oedema, thrombosis, and mononuclear meningitis. Based on authors' knowledge, this is the first study in Iran on *Coenurus* cysts in a lamb brain utilising CT scan.

Key words: Charollais lamb brain, clinicopathological manifestation, CT scan, Iran, natural *Coenurus cerebralis* infestation

Coenurus cerebralis is a bladder-like cyst known as the larval stage of *Taenia multiceps* (Avcioglu *et al*., 2011). The disease associated with *C. cerebralis* has been named as gid or sturdy which particularly involves central nervous system of human and ungulates including sheep, goat, cattle, buffalo, deer, yak, horse and pig and

results in space occupying lesion in nervous tissue (Abera *et al*., 2016; Mokhber Dezfouli *et al*., 2019). *T. multiceps* has a worldwide distribution and lives in small intestine of carnivores as definitive hosts (Afonso *et al*., 2011; Scott, 2012). Coenurusis occurs in Asia, Africa, America and some area of Europe (Gazioglu *et al*., 2017). Its prevalence is about 1.9–9.8% in Asia and Africa (Scala *et al*., 2007). In iran, the disease has been reported in sheep, goats and wild goats (Tavassoli *et al*., 2011; Mokhber Dezfouli *et al*., 2019). Cysts can occur in any part of brain and spinal cord but are mostly seen in cerebral cortex. In sheep, the cysts are more often present in subarachnoid space, Because they can easily feed from cerebrospinal fluid (Haridy *et al*., 2013).

Coenurosis in sheep is manifested in three consecutive stages: acute, quiescent and chronic. In acute form, onset of illness is usually 10 days after parasite egg ingestion and is a result from immigration of oncospheres thorough brain tissue. Maturation of *C. cerebralis* may take 7–8 months and within this period, no signs can be detected and the cysts get larger following increased fluid volume (quiescent phase) (Gonzalo-Orden *et al*., 2000; Scala *et al*., 2007; Miran *et al*., 2015; Abera *et al*., 2016). Chronic form is more common in older sheep and is caused by mature cysts. At this stage, affected animals show symptoms including depression, head pressing and deviation, blindness, ataxia, uncontrolled and circular movements which may proceed to paralysis and convulsion (Scala *et al*., 2007; Miran *et al*., 2015; Mokhber Dezfouli *et al*., 2019). Diagnosis is usually based on clinical findings and interpretation of these signs can be helpful to locate the cyst (Gazioglu *et al*., 2017). Although confirmation of diagnosis is by necropsy and observation of fluid-filled cyst containing numerous scoleces surrounded by a thin and transparent wall, radiography and ultrasonography are mentioned as supplementary tools. There are a few reports about confirmation of cyst presence in brain by magnetic resonance imaging (MRI) and computed tomography (CT) scan methods (Gonzalo-Orden *et al*., 2000; Afonso *et al*., 2011; Gazioglu *et al*., 2017).

The aim of this study was to describe clinical, histopathological and CT findings of coenurusis caused by multiple *C. cerebralis* in a non-native lamb in Iran.

Case description

In late summer 2019, a six-month-old Charollais lamb was examined with history of dullness, reluctancy to move, drooling of saliva, head deviation, frequent convulsion, ataxia, and reduced appetite. Clinical examination revealed cortical blindness, tachycardia (160/minute), tachypnea (60/minute) and increase in body temperature (40.5 ^{o}C). A 5-mL blood sample was collected in EDTA coated tubes from the jugular vein and sent to the laboratory for haematologic examination, which showed increased white blood cell count, elevated plasma fibrinogen concentration, and higher neutrophil count compared to normal values (Table 1).

Two mL of cerebrospinal fluid (CSF) were collected from the lumbosacral region with a 18 gauge needle and a syringe, following sedation. CSF volume was normal and it appeared clear. CSF analysis showed total protein concentration of 75 g/L and WBC of 0.65 G/L (Table 2). CSF culture resulted in no bacterial growth.

After the lamb was anaesthetised (intravenous ketamine at a dose rate of 22 mg/kg was administrated for induction of

Table 1. Haematological characteristics of the affected lamb with *C. cerebralis* comparing with the normal range

Parameters	Affected lamb	Normal range*
Haemoglobin (g/L)	97	90–150
Haematocrit (%)	33–40	27–45
RBC (T/L)	14.0	9.0–15.0
MCV (fL)	35.00	28–40
MCH (pg)	9.50	8–12
MCHC (g/L)	335	310–340
WBC (G/L)	14.4	4.0–12.0
Segmented neutrophils (G/L)	9.2	0.7–6.0
Band neutrophils (G/L)	–	rare
Lymphocytes (G/L)	4.5	2.0–9.0
Eosinophils (G/L)	0.7	0–1.0
Fibrinogen (g/L)	6	1–5

* Constable *et al*. (2017).

anaesthesia, providing 10–15 minutes of anaesthesia for imaging purposes), CT images were obtained in transverse plane from cephalic region using a multidetector CT scanner (Somatom spirit 2, Siemens, Germany) aiming evaluation of brain structure (X-Ray tube potential: 130 kV, pitch: 1, rotation time: 1 second, slice thickness: 1 mm). CT scan images revealed presence of ten cysts in the brain; nine in the cerebrum and one in the cerebellum. These cystic structures were distinguishable from surrounding tissue by their lower density: below than 10 Hounsfield units (Fig. 1).

The lamb was euthanised for welfare reasons by intravenous injection of sodium pantobarbital at a dose rate of 150 mg/kg according to AVAM guidelines for the euthanasia, three days after CT imaging. Multiple unilocular *C. cerebralis* cysts were observed at necropsy. The variably sized cysts, 1.5 to 5.5 centimeters in diameter, contained clusters of scolices attached to cyst walls. *Coenurus* cysts were scattered in different parts of the cerebrum and the cerebellum; one cyst in each of the frontal and occipital lobes, two cysts in parietal and temporal lobes, three cysts in cerebral white matter, and one cyst in the cerebellum. Adjacent brain tissue was compressed by the cysts, so that gyri and sulci were obliterated. Hyperaemia was apparent in the cerebrum and the cerebellum (Fig. 2). No cystic structures were seen at the periphery of the spinal cord and in other parts of the body.

Table 2. CSF analysis of the affected lamb with *C.cerebralis* comparing with the normal range

Parameters	Affected lamb	Normal range*
Total protein (g/L)	75	23
WBC (G/L)	0.65	0.11
Segmented neutrophils (G/L)	0.25	0.05
Band neutrophils (G/L)	0	0

* Scott (1992; 2010).

The brain was immersed in 10% neutral buffered formalin and submitted for histopathological evaluation. Representa-

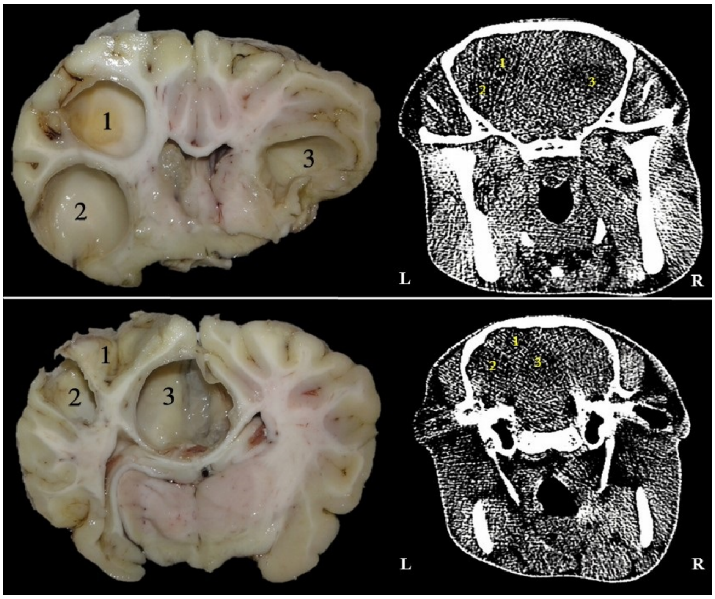

Fig. 1. Multiple *C.cerebralis* cysts in CT images. Presence of three cysts in white and grey matter in the same location depicted by CT scanning and confirmed at necropsy.

tive sections were collected from various anatomical sites of the brain adjacent to the cysts. Specimens were processed, embedded in paraffin blocks and cut in 5 µm thickness and then stained by the routine haematoxylin and eosin method.

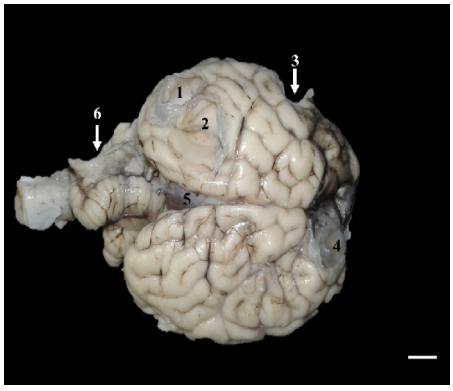

Fig. 2. Multiple *C.cerebralis* cysts. Presence of six cysts in cortex of cerebrum and cerebellum (bar=1 cm).

Histopathological examination revealed thick eosinophilic cyst walls, with numerous protoscolices protruding from the inner aspect. Compressive atrophy was seen in both gray and white matter underneath the cysts, in addition to focally extensive liquefactive necrosis and mild demyelination. Mild multifocal perivascular and perineuronal oedema, diffuse hyperaemia, neuronal degeneration and thrombosis were noted in the affected cerebral tissue. Multifocal meningeal oedema, thrombosis and mononuclear meningitis with perivascular mononuclear infiltration were present in the overlying meninges of the affected area (Fig. 3–5).

Coenurosis is a major problem in sheep and goat industry and has a worldwide distribution (Avcioglu *et al*., 2011; Miran *et al*., 2015; Mokhber Dezfouli *et al*., 2019). The disease is endemic in the Middle East region. Its prevalence ranges between 0.09% and 18.65% in different

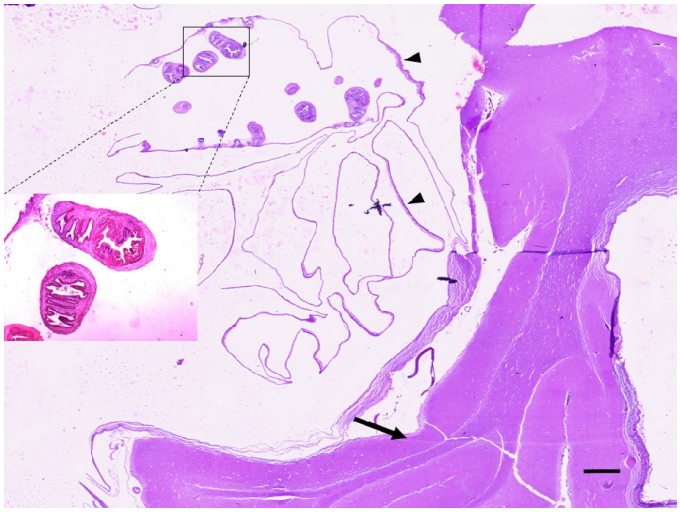

Fig. 3. Subgross photograph of a cross section of a coenurus cyst and the surrounding cerebri gyri, revealing numerous protoscolices protruding from the inner aspect of the cyst wall (arrowhead), and compression atrophy of gray and white matter (arrow) (bar=500 µm).

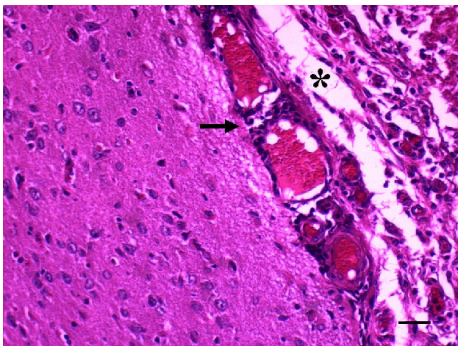

Fig. 4. Meningeal infiltration of mononuclear cells (*), newly formed vessels, perivascular mononuclear infiltration (arrow) and mild neuronal degeneration; H&E (bar=100 µm).

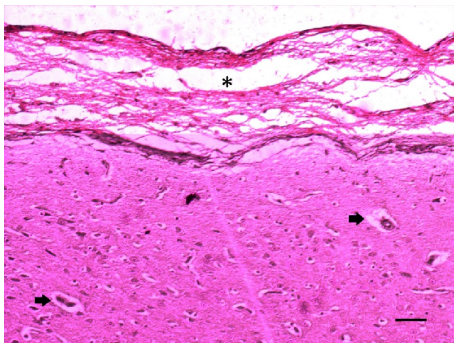

Fig. 5. Liquefactive necrosis (*), mononuclear infiltration and perivascular oedema (arrows) of the cerebral parenchyma adjacent to the parasitic cyst; H&E (bar=150 µm).

parts of Iran (Tavassoli *et al*., 2011; Rahsan *et al*., 2018; Mokhber Dezfouli *et al*., 2019). Some authors suggested season of the year as a risk factor for occurrence of the disease. At the beginning of spring, pastures start to grow in Iran and abundant forage is available for the herds. This may explain increased occurrence of coenurosis at this time. On the other hand, high moisture and temperate weather at this season may have a contributory role for survival of parasite's egg at pasture. It is assumed that coenurosis usually infects sheep (especially lambs) more than goats (Tavassoli *et al*., 2011; Soundararajan *et al*., 2016; Mokhber Dezfouli *et al*., 2019). There may be age-related resistance against the disease, although, it's causal

mechanism is not known yet (Afonso *et al*., 2011). *Coenurus* cysts have a high tendency to infect the central nervous system and masses result in increased intracranial pressure being space occupying (Mokhber Dezfouli *et al*., 2019). Clinical manifestations can be different depending on cyst's size and location an extent of brain tissue destruction (Scala *et al*., 2007; Avcioglu *et al*., 2011). Clinical signs of described lamb matched those mentioned in the literature references. In a study conducted by Achenef *et al*. (1999), tachypnea and tachycardia were recorded in 20 percent of infected animals. They also believed that head deviation only can be seen in the early stages of the disease and circling appeared as the disease progressed. Some researchers assumed that investigation of head tilt was more useful as an aid to locating *Coenurus* cyst in brain tissue, than circling movements (Gazioglu *et al*., 2017). In this case, head tilt was observed but recumbency and circling were not seen. Circling results from cyst presence in one of the cerebral hemispheres (El-Neweshy *et al*., 2019), but imaging and necropsy findings in this lamb suggested the presence of multiple cysts in both cerebral hemispheres. This may explain why circling was not evident in this case.

Haematological findings including leukocytosis, neutrophilia and elevation of plasma concentration of fibrinogen were indicative of an inflammatory process which resulted in a marked increase in body temperature. Possibly, extensive lesions in brain tissue have caused recall of inflammatory cells and leukocytosis. On the other hand, besides other clinical signs, analysis of CSF can arouse suspicion of coenurosis. Increased total protein and leukocytes in CSF are indicators of inflammatory response. As two separate study by Forjani Kish *et al*. (2013) and Zobba *et al*. (2014) have shown, these two analytes were elevated in animals infected with coenurosis, whereas in another research by Mokhber Dezfouli *et al*. (2019), CSF leukocyte count was normal in a wild goat infected with coenurosis yet total protein was increased.

By the way, there is no pathognomonic sign for this disease, and other space occupying lesions like abscesses, intracranial haemorrhage and even infection with *Oestrus ovis* larvae can produce similar signs (Achenef *et al*., 1999; Zobba *et al*., 2014; Amer *et al*., 2017). Therefore, necropsy and other additional diagnostic tools like ultrasonography, radiography, CT scan and MRI can be used for differential diagnosis. CT imaging is an effective practice for *ante mortem* diagnosis and precise localisation of the cysts in the brain tissue.

By CT imaging of the lamb, multiple cysts were detected as hypodense areas in different parts of the brain. Gazioglu *et al*. (2017) had described similar CT findings but in contrast to our study, they reported that cyst wall appeared as a hyperattenuated line because of calcification, a lesion that was not seen in histopathologic examination of the present case. Skull bone can be softened by pressure of the growing cysts (Achenef *et al*., 1999; Evangelisti *et al*., 2016) but in the present study, no change in skull bone at necropsy was noticed, however, thinning of bone diameter was apparent on CT images. *Coenurus* cysts often develop in both cerebral hemispheres (96%) and less frequently in the cerebellum (4%). Most common sites for *C. cerebralis* cyst formation are the subarachnoid space and brain parenchyma respectively (Haridy *et al*., 2013). The cysts have a thin and translucent wall and usually contain several scolices which

form cluster shape aggregation and are adherent to cyst's wall (Haridy *et al*., 2013). The cysts found in the brain of this case, had similar specifications.

Like other studies, hyperaemia was evident in the superficial parts of the cerebrum and the cerebellum, adjacent to the cyst's wall (Amer *et al*., 2017). Either single or double cysts patterns are commonly present within brain tissue (Hobbenaghi *et al*., 2014). Simultaneous occurrence of multiple cysts in different sections of the brain is rare, but in the this case, ten *Coenurus* cysts were developed in various parts of the brain. Previously, Hobbenaghi *et al*. (2014) and Amer *et al*. (2018) had reported occurrence of several small *Coenurus* cysts in sheep brain. Presence of multiple cysts in the brain makes the disease course shorter. There are limited reports of cerebellum involvement with *C. cerebralis* cyst (Avcioglu *et al*., 2011; Soundararajan *et al*., 2016) whereas we detected the presence of one cyst in this organ. Histopathologic examinations revealed compressive atrophy in both gray and white matter, focally extensive liquefactive necrosis, and mild demyelination adjacent to the cystic structures. The same histological changes have been reported in the cerebrum by Haridy *et al*. (2013), Abera *et al*. (2016) and Gazioglu *et al*. (2017). Mild multifocal perivascular and perineuronal oedema, diffuse hyperaemia, neuronal degeneration, and thrombosis were presented in the studied samples. It has been stated that lymphoplasmacytic and eosinophilic infiltration of the necrotic area would be anticipated in addition to the presence of macrophages (Haridy *et al.,* 2013; Amer *et al*., 2017). The same cellular infiltration has been reported at the periphery of the cyst walls (Gazioglu *et al*., 2017) with preponderance of eosinophils (Haridy *et al*., 2013; Abera *et al*., 2016; Gazioglu *et al*., 2017). In contrast to previous studies (Haridy *et al*., 2013; Hobbenaghi *et al*., 2014; Farjani Kish *et al*., 2015; Abera *et al*., 2016; Amer *et al*., 2018; Gazioglu *et al*., 2017; Mokhber Dezfouli *et al*., 2019) gliosis, astrocytosis, and microgliosis were absent. That is while mild multifocal perivascular and perineuronal oedema and thrombosis are the novel histopathological findings in this study. Calcification and presence of giant cells at the periphery of the cyst walls that have been previously reported (Amer *et al*., 2018; Rahsan *et al*., 2018) were not observed in the present case. Meningeal hyperaemia and perivascular mononuclear infiltration were detected in earlier reports (Mokhber Dezfouli *et al*., 2019; Haridy *et al*., 2013; Rahsan *et al*., 2018) as well as in the present case. Multifocal meningeal oedema and thrombosis were additionally observed in the present lamb adjacent to the cyst walls. It is presumed that the large number of cystic structures and the significant extent of the affected cerebral tissue may have played a role in thrombosis.

Variation in occurrence of coenurosis in different regions of the world is influenced by several factors including the level of natural immunity of the hosts, presence of definitive hosts and difference in husbandry systems and deworming methods that are practiced (Gazioglu *et al*., 2017). Charollais sheep are recently imported from European countries to Iran for increasing meat production. Iran is identified as an endemic region for coenurosis but inffestation of native sheep with multiple *C. cerebralis* cysts is uncommon (Hobbenaghi *et al*., 2014; Farjani Kish *et al*., 2015). Antibody production and complement-mediated lysis of oncospheres during early developing stages, are two main immunologic re-

sponse against taeniid cestodes in body of the intermediate host. Presence of multiple cysts in various parts of the brain, confirmed by necropsy or CT findings, suggested extensive larval migration through encephalic vessels following ingestion of large numbers of eggs of *T. multiceps* by the lamb. It can be concluded that either incompetent immune system of the lamb was incapable of provoking a strong and adequate immunologic reaction, or migration of oncosphere was so intensive that the immune system could not defeat it. It seems that exotic breeds are more susceptible to severe infection with coenurosis and therefore management factors that facilitate infection should be controlled.

Although coenurosis can be diagnosed by clinical signs, imaging techniques are used in valuable animals to specifically locate the cysts for surgical treatment (Scott, 2012; Khosa *et al*., 2016) . Hence the present case was more precisely investigated to obtain more reliable data on the diagnosis, despite the costs.

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