

FINDINGS OF POST-MORTEM LIVER INSPECTION IN TRADITIONAL REARED SHEEP – CASE REPORT

REZULTATELE INSPECȚIEI POST-MORTEM A FICATULUI DE LA OVINE CRESCUTE ÎN SISTEM TRADIȚIONAL PASTORAL – STUDIU DE CAZ

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ABSTRACT | REZUMAT

In addition to the already existing traditional customs in certain areas, there has recently been a noticeable growth of interest in ovine meat in the European Union, mostly due to the unrestricted access to international cuisine and the appeal of the tasty oriental sheep meat dishes and also due to the growing Muslim population. As the liver holds a core significance during post-mortem inspection of meat, we investigated the post-mortem findings in ovine livers, in a batch of sheep carcasses obtained from private producers in a limited mountain area in Romania. All investigated liver samples revealed parasitic modifications: hydatid cyst in 9 livers out of 10, cysticercosis in 1 liver sample, and fascioliasis in 6 samples, which were confirmed by the histological examination of the modified areas of liver tissue. The high prevalence of parasitic infestation calls for a more extended analysis involving a larger sample pool, for statistical significance, while still supporting the need to increase the awareness of farmers in the pastoral mountain areas, and a better standard of care for herd health management.

Keywords: sheep, traditional pastoral rearing, post-mortem inspection, liver, parasitic infestation

Pe lângă obiceiurile tradiționale deja existente în anumite zone, a fost înregistrată recent o creștere notabilă a interesului pentru carnea de oaie în Uniunea Europeană, mai ales datorită accesului fără restricții la bucătăria internațională și a aprecierii generale a preparatelor orientale din carne de oaie, la care se adaugă creșterea populației musulmane în UE. Deoarece ficatul are o importanță crucială în cadrul inspecției post-mortem a cărnii, am investigat rezultatele inspecției post-mortem a ficatului de ovină pentru un lot de carcase obținute de la producători privați într-o zonă montană din România. Toate probele de ficat investigate au evidențiat modificări date de prezența formațiunilor parazitare: chist hidatic în nouă probe din 10, cisticercoză în o probă de ficat și fascioză în șase probe, care au fost confirmate prin examinarea histologică a zonelor modificate. Prevalența ridicată a infestării parazitare, evidențiată prin acest studiu, relevă necesitatea efectuării unei analize mai extinse care implică un grup de probe mai mare, pentru semnificație statistică. Rezultatele obținute subliniază necesitatea creșterii gradului de conștientizare a fermierilor din zonele de munte pastorale și a menținerii unui standard mai bun de îngrijire pentru managementul sănătății efectivelor de ovine crescute în sistem tradițional.

Cuvinte cheie: oi, creștere pastorală tradițională, inspecție post-mortem, ficat, infestare parazitara

The post-mortem inspection of organs and carcasses remains a core objective of the meat inspection and control system despite the shift of current food legislation towards a risk-based approach, which minimizes invasive slaughterhouse examination techniques (20). During post-mortem organ inspection, the liver holds a preeminent significance due to its role in drainage from all abdominal visceral sites. The main hazards that focus the attention of the food inspectors assessing the liver during post-mortem examination

are the biological ones. A vast majority of bacterial, fungal, and viral analyses require liver samples which are harvested aseptically immediately after opening the abdominal cavity, while all other organs are still in situ. Nevertheless, the routine inspection of the liver aims at the identification of visible modifications related to additional hazards, such as parasitic infestation, relevant for animal health, herd health, and public health. Liver disorders usually reveal a disrupted metabolism and may be indicative of either acute or chronic disease, with different relevance for public health. The liver can also be damaged by infectious processes (1) or by poisoning related to heavy metals or mold toxins (6, 19). In addition, breeding systems can make

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animals susceptible to certain lesions, such as the fatty liver syndrome in caged chickens (10).

Specific literature (8) shows that pathological lesions are most commonly detected in the respiratory system of animals (approx. 35% of slaughtered animals), closely followed by liver damage affecting 34% of slaughtered animals as revealed during post-mortem slaughterhouse inspection. The pathological findings in the liver of livestock animals are referred to in a series of studies investigating the causes of livestock carcass condemnation. However, most of these studies are species-specific: cows (17), pigs, rabbits, chicken broilers, laying hens, and turkeys (3, 18). However, major economic loss due to liver condemnation in sheep, associated with a lack of appropriate veterinary care is pointed out by other authors in different parts of the world (14).

Sheep was selected for this study in the light of traditional consumption in Romanian households throughout the entire year, associated with increased consumption of lamb during Easter Holiday, all over the country. In addition, a recent international study (12) reveals the relevant motivations of sheep meat consumers, which perceive this type of meat as being "tasty", "natural" and "healthy" due to its lower environmental impact and reduced fat content compared to other meats. Moreover, there has been a recent growth of interest in this type of meat in the European Union, mostly due to the unrestricted access to international cuisine and the appeal to the tasty oriental sheep meat dishes. At the same time, the growing Muslim population requiring Halal certification emphasizes the focus on the official inspection of sheep and goat meat with special attributes related to specific slaughtering techniques.

The aim of this study was to investigate the occurrence of post-mortem findings in ovine livers, as well as to evaluate the prevalence of specific lesions in sheep from private producers in a limited mountain area in Romania.

MATERIALS AND METHODS

The study was performed on sheep raised and slaughtered in a traditional system, in the mountain areas of Bran (Braşov county). The obtained meat was meant for domestic consumption only, mostly processed as pastrami. The sample group was represented by 10 sheep, of either Țigaie or Țurcană breeds, aged between 5 months and 9 years, slaughtered mostly in autumn 2021, but also in April 2022 (Table 1). Of the total number of sheep in Romania, the sheep of the Țigaie breed have a share of 5.34%, the second largest after the Țurcană breed.

The liver inspection was initiated with an examination of the liver *in situ*, after opening the abdominal cavity,

in order to achieve an evaluation of the liver in context with other changes in the abdominal cavity. The next step consisted of gentle washing out of any excessive fluid accumulation or haemorrhage in the abdomen to provide a clear *in-situ* picture of pathology (Fig. 1). The water was run as a gentle soak and not under pressure in order to avoid removing and/or destroying any critical lesions such as fibrinous deposits, hematoma, adhesions, etc.

Table 1
Cases of sheep included in the study

No.	Slaughter	Breed	Age	Gender
1	20/10/21	Țigaie	5 y	F
2	20/10/21	Țigaie	8 y	F
3	20/10/21	Țigaie	9 y	F
4	3/11/21	Țurcană	6 y	F
5	3/11/21	Țigaie	8 y	F
6	3/11/21	Țigaie	9 y	F
7	24/11/21	Țigaie	8 y	F
8	24/11/21	Țigaie	9 y	F
9	24/11/21	Țigaie	8 y	F
10	21/04/22	Țurcană	5 m	M



Fig. 1. *In situ* liver pathology before evisceration, revealed for carcass 3

The liver inspection followed the requirements of Commission Implementing Regulation (EU) 2019/627 of 15 March 2019, laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and the Council:

- For the young domestic sheep and sheep with no eruption of permanent incisors (carcass of animal no.10 as indicated by table no.1) (Art. 20.1 (e)): a visual inspection of the liver and the hepatic and pancreatic lymph nodes (*Lnn. portales*).
- When there were indications of a possible risk to human health, animal health, or animal welfare (Art. 20.2 (d)): palpation of the liver and its lymph nodes; incision of the gastric surface of the liver to examine the bile ducts.
- For the category indicated as Other domestic sheep and goats, namely Carcasses and offal of sheep having a permanent incisor erupted or 12 months of age or more (carcasses of animals no. 1-9 as indicated by table no.1) (Art. 21.1 (e)): a visual inspection of the liver and the hepatic and pancreatic lymph nodes (*Lnn. portales*); palpation of the liver and its lymph nodes; incision of the gastric surface of the liver to examine the bile ducts (20).

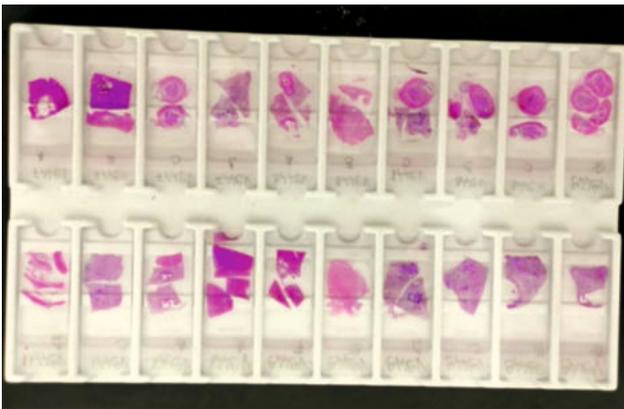


Fig. 2. HE- coloured histological sections

For the purpose of this study, the post-mortem inspection of the ovine liver was performed according to Georgescu M. (2018): the visual inspection of the liver aimed to assess the general appearance, colour, and lesions of the liver tissues, according to the liver conformation:

- The external fibrous coat, and the connection ligaments - general appearance, transparency, and colour of the fibrous coat;
- The diaphragmatic, visceral faces of hepatic lobes - surface appearance, size, shape, and lesions. In ovine, an incision of the gastric surface of the caudate lobe;

- The dorsal and ventral margins - size, shape, appearance;
- Gall bladder and bile ducts;
- Lymph nodes of the liver - visual examination and incision (5).

For the identified lesions, additional histopathological examination was performed, for confirmation purposes. The samples were sectioned with a scalpel with a sharp blade and then fixed in 10% buffered formaldehyde solution% (Fig. 2).

RESULTS AND DISCUSSIONS

For the sampled carcasses considered for this study, the liver was the organ most affected by the changes. Of the 10 sampled carcasses, all showed modifications of the liver and lungs and 8 also revealed modifications in other organs. All modifications revealed by this study were of parasitic nature. As the lesions were confined to the affected organ, the official decision implies confiscation of only the livers, while the rest of the carcass showing no additional modifications being accepted for consumption.



Fig. 3. Liver, sheep, overview. Multiple parasitic cystic formations

The visual inspection revealed hydatid cysts in 9 livers out of 10, cysticercosis in 1 liver sample, fascioliasis in 6 samples. No other lesions or modifications were noticed. The histological / microscopical evaluation confirmed:

- Hydatid lesions in 9 livers out of 10 - round, cystic formations, mostly hard at palpation, difficult to section, white-yellow, mostly variable in size, up to 1 inch. The liver showed normal colour (Fig. 3, 4, 5);
- Cysticercosis in 1 liver sample;
- Hepatitis due to larval migration in 6 samples (Fig. 6, 7, 8).

In sample no. 10 the visual observation revealed several transparent parasitic cysts on the diaphragmatic face of the liver (Fig. 9), which had a clear liquid, with a hanging pedicle (Fig. 11,12), which at the histopathological examination was concluded to be consistent with *Cysticercus ovis*. They were attached to the liver capsule (Fig. 10).

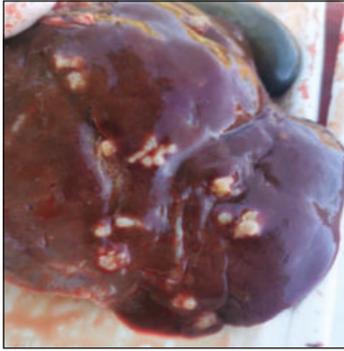


Fig. 4. Liver, sheep. Cystic parasitic formations, calcified



Fig. 5. Sheep liver detail, compact formation, white-grey-yellow, with a granular and sandy appearance at sectioning

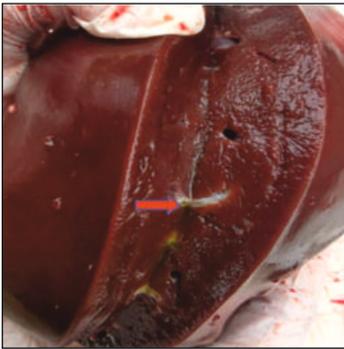


Fig. 6. Liver, sheep. Parasitic angiocholitis, severe fibrosis of the walls of the bile ducts (arrow) after sectioning on the transverse axis of the liver

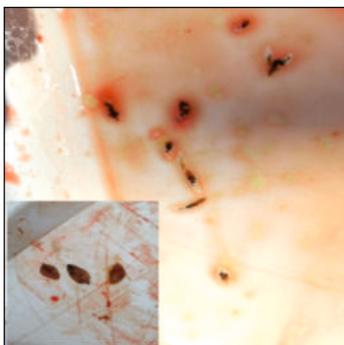


Fig. 7. *Fasciola hepatica* expressed after liver sectioning



Fig. 8. Liver, sheep. Parasitic angiocholitis, severe fibrosis Liver, sheep. *Fasciola* spp. Parasites visualized when sectioning bile ducts



Fig. 9. Liver sample, sample no. 10



Fig. 10. Parasitic cyst, with the observation of the scolex (arrow)



Fig. 11. Parasitic cyst with pedicle highlighting



Fig. 12. Samples taken for histopathological examination for diagnosis

For histopathological analysis, tissue samples of approximately 2 x 2 cm, were collected. The microscopic examination revealed hepatitis of possible parasitic migration in 6 samples (*Fasciola spp.*). Other findings consisted of chronic lesions of haemorrhagic-necrotic hepatitis, with a fibrosed pathway and abundant cellularity represented by eosinophils and mononuclear cells (mainly lymphocytes) (Fig. 13, 14).

The hydatid cyst was also confirmed by histopathological examination (Fig. 15). The three layers of the cyst were observed: (1) the outer pericyst; (2) the middle-laminated membrane; and (3) the inner germinal layer. Inside, determined by the first layer, the inner germinal layer, are the septa that make up "daughter cyst", consisting of protoscoleces. In the outer pericyst, inflammatory cells such as eosinophils, macrophages, and lymphocytes were observed (Fig 16).

In sample no. 10 the presence of cysticercosis was confirmed, with degeneration of the hepatic parenchyma, but also the cellular infiltrate with numerous inflammatory cells present (Fig.17): macrophages, lymphocytes, epithelial cells, rare foreign-body giant cells, and eosinophils. Fig. 18 reveals the chronicity of the lesion, as the formation of the double-layer parasitic cyst could be noticed. On the outside, the fibrous capsule and the adjacent inflammatory reaction were noticed.

For sample no. 4, which presented parasites in the bile ducts as observed during post-mortem inspection, parasitic fibrous angiocolitis was identified by histopathological examination, along with the granulomatous inflammatory reaction. Also, the epithelial regenerative hyperplasia of the bile ducts and the fibrosis of the portobiliary space, with moderate eosinophilic infiltrate were revealed (Fig. 19, 20).

Similar studies which focused on the incidence of liver damage in various species of cattle revealed chronic changes to be more frequent ($p < 0.05$) than acute and parasitic modifications in most species and animal categories: cows, heifers, fattening bulls, calves, sows, finishing pigs, piglets (18). However, when it

comes to small ruminants, such as ewes and lambs, the same authors reveal similar results to the ones presented in our case report, as for sheep and lambs, the most frequent liver modifications were parasitic.

For our study group of carcasses, the overwhelming incidence of parasites detected at post-mortem inspection was related to the traditional rearing system, the housing type, and the proximity of the herds with the sheep dogs, which maintain the life cycle of the parasites. In addition to these features, which resemble the ones in other studies (2, 4, 9, 13), Romanian breeders in the mountain areas are using a traditional pastoral system, the so-called "short-distance transhumance", targeting mountains with high alpine pastures grazed by sheep flocks during summer. This system is mentioned in the literature (7) with deep implications in the national history and the habits of the Romanian people, using the term "pendulation" to describe short-distance transhumant pastoralism. Sheep flocks graze during the summer on mountain pastures and return to the village during late autumn, they graze in arable fields, meadows, pastures, or forests, depending on the ecological conditions. During winter, all animals are kept either in barns or free-range, as the local sheep breed, Țurcană, is resistant to the cold climate of Brașov County (7). This system, even though the most appreciated as being strongly related to the Romanian history and culture, is prone to lacking proper sanitary management (as indicated also by the missing ear tags for 4 out of ten animals, which would alone stand as sufficient reason for confiscation of the whole carcass, according to the current legislation), timely deworming and controlled hygiene conditions, including species separation (from sheep dogs), leading to the high prevalence of parasitism. The associations of similar factors with liver and gastro-intestinal parasitism are also mentioned by other authors (15), which also point out the climate factor related to increased humidity as favourable for parasitic transmission in sheep herds. This factor can also be related to the rainy mountain areas of Bran-Brașov considered in our case report. In agreement with other authors (11), a more in-depth evaluation of the farmers' perception and animal management practices for parasite prophylaxis and treatment in sheep is needed. This aspect should be considered particularly relevant, as very recent studies published by scientists from Timisoara (16), revealed extremely high prevalence of parasitic infestation (76.78%) in the lungs of pastoral reared sheep from Western Romania. Therefore, a deeper involvement of the inspectors in the management of the animal health of the sheep population reared under the pastoral traditional system is required, as sheep production is still mainly handled by less educated and insufficiently skilled farmers, in an unfavourable economic system.

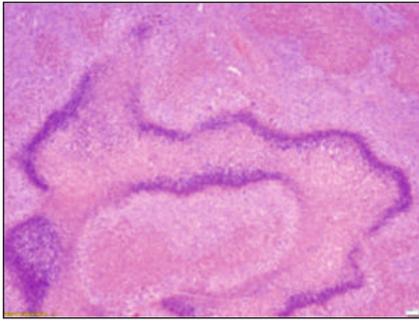


Fig. 13. Sheep liver, parasitic traumatic hepatitis. Haemorrhagic-necrotic outbreak delimited by a strong cellular infiltration; col. HE, ob. 4x

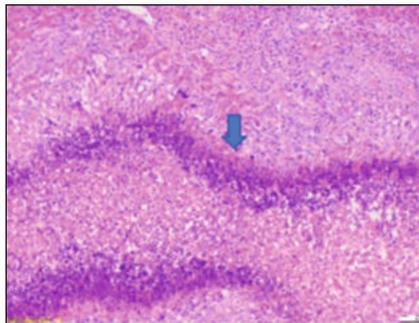


Fig. 14. Sheep liver, larval migration hepatitis, with necrosis and haemorrhage (arrow), severe infiltration with inflammatory cells; col. HE, ob. 10x

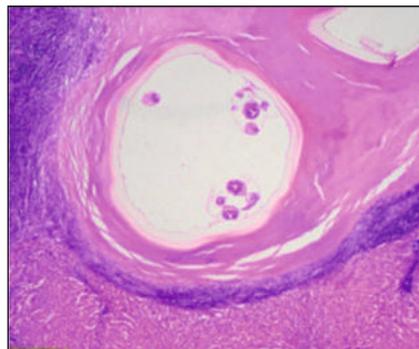


Fig. 15. Liver, hydatid cyst, overview; col. HE, ob. 4x

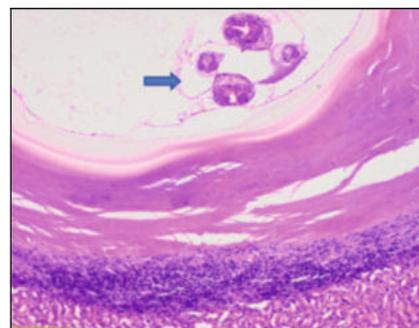


Fig. 16. Liver detail, with the highlighting of the three layers and the presence of protoscolices (arrow); col. HE, ob. 10x

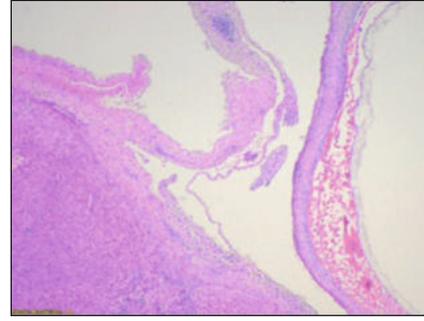


Fig. 17. Lamb liver, hepatoperitoneal cysticercosis; col. HE, ob. 4x

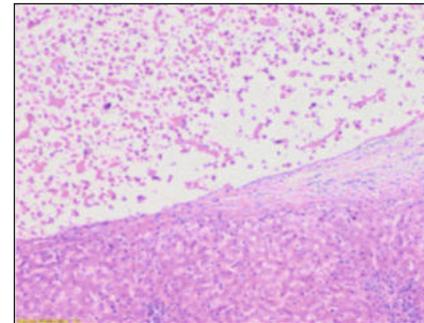


Fig. 18. Lamb liver, detail. The structure of the tissue capsule around the cysticercus, leukocyte infiltrate, represented by lymphocytes, epithelial cells, peripheral fibrous capsule; col. HE, ob. 10x

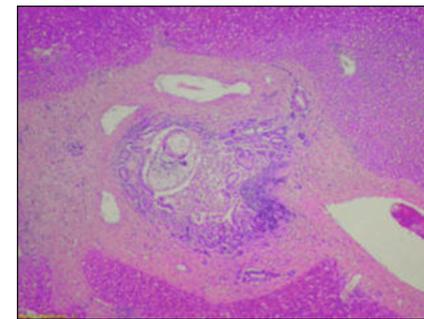


Fig. 19. Liver, sheep. Parasitic fibrous angiocolitis, with regenerative hyperplasia of the bile ducts and fibrosis of the portobiliary space; col. HE, ob. 4x

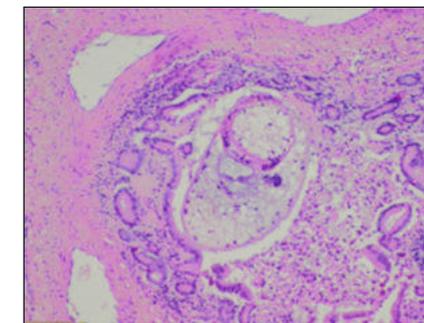


Fig. 20. Liver detail, sheep. Presence of the granulomatous inflammatory reaction. Epithelial regenerative hyperplasia of the bile ducts is observed; col. HE, ob. 10x

CONCLUSIONS

All investigated liver samples revealed parasitic modifications: hydatid cyst in 9 livers out of 10, cysticercosis in 1 liver sample and fascioliasis in 6 samples. All post-mortem findings were consistent with the results of the histological evaluation which was carried out for confirmation. The prevalence of parasitic infestation was higher than expected and justifies the need for a more extended study of the sheep population reared under a traditional pastoral system in the mountain area of Braşov county which would add statistical significance to our findings. However, this case report is not anecdotal, as it highlights a significant public health problem, as the identified parasitic infestations reveal a dangerous gap in the herd health management, for sheep reared under the pastoral traditional system. The exposed parasitic infestation highlights the lack of awareness of the farmers, associated with and leading to a lack of proper sanitary veterinary prophylactic and therapeutic care, as indicated also by the missing ear tags in some of the animals included in the study. Considering the great significance of sheep and lamb meat consumption in Romania and the impact on the public health of all identified parasites, this case report calls for a more extensive evaluation of post-mortem investigation findings for traditionally reared sheep and also for the need to increase the awareness of farmers in the pastoral mountain areas, for a proper understanding and controlling of both biological hazards with the potential for foodborne infections, on one hand, and infectious agents which compromise the herd health, on the other hand.

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