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Short Communication

Fatal *Eimeria gilruthi*-Induced Abomasal Coccidiosis: a still Neglected Parasitosis?

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Abstract

Abomasal coccidiosis was diagnosed in two cross-breed adult sheep and one adult goat which died after a 3-week period of weight loss, anorexia and diarrhoea. Necropsies revealed an extremely edematous abomasal mucosa with a nodular surface and multiple focal areas of haemorrhagies. Histological examinations of affected abomasal mucosa showed numerous giant (up to 600 µm) coccidian macromeronts containing myriads of merozoites. These macromeronts were thick-walled and associated with hyperplasia of mucous neck cells, parietal cell atrophy, and moderate to intense lymphoplasmacytic inflammation that was centered on degenerated macromeront walls. Based on morphological characteristics, the macromeronts were identified as stages of *Eimeria gilruthi*, formerly known as *Globidium*, a coccidian parasite of uncertain taxonomic status historically associated with incidental coccidian abomasitis in sheep and goats. The current cases and the significant *E. gilruthi*-induced mucosal inflammation suggest that heavy abomasal *E. gilruthi*-infections should be considered as etiological cause of weight loss, anorexia, diarrhoea, and proliferative/haemorrhagic abomasitis in sheep.

ABBREVIATION

H & E: Haematoxylin and Eosin Staining

INTRODUCTION

Small ruminant coccidiosis is an apicomplexan disease caused by different species of the genus *Eimeria*, which causes severe enteritis and/or typhlocolitis resulting in worldwide economic losses in the small ruminant industry [1-4]. In contrast to enteric coccidian infections, still very little is known on abomasal coccidiosis attributed to the species *Eimeria* (*Globidium*) *gilruthi* although the disease has been known for two centuries in small ruminants [5-9]. In general, *E. gilruthi* infections were reported as incidental finding. The lack of knowledge on the *E. gilruthi* life cycle, route of infection, pathogenesis and immune reactions in addition to an uncertain taxonomic classification [10] complicate the understanding of coccidian abomasitis in sheep and goats [11]. Necropsy findings on *E. gilruthi*-induced abomasal coccidiosis include so-called giant "globidium cysts" which are usually found as whitish foci (up to 1.5 mm of diameter) in the abomasal mucosa of infected sheep or goats corresponding to *E. gilruthi* macromeronts [11-14]. These macromeronts are localized within an extremely enlarged infected host cell and show an outer amorphous layer of 30-50 µm in width frequently being supported by a "secondary cyst wall" [11,12,14]. The cytoplasm of *E. gilruthi* macromeront-carrying host cells is highly modified and contains numerous fibrillary elements surrounding the parasitophorous vacuole membrane (PVM) at ultrastructural level [11, 14]. Within macromeronts, fully formed merozoites as well as cytomeres can be frequently found, depending on the maturation status of the macromeronts. Detailed ultrastructural analyses of the surface of *E. gilruthi* macromeront-harbouring host cells demonstrated large laminar protrusions [11] corresponding well to morphological features that are observed in other ruminant macromeront forming *Eimeria* species, such as *E. bovis* in cattle [15,16]. Interestingly, several electron microscopic investigations proposed the existence of different *Eimeria* species forming giant macromeronts in the abomasum/duodenum of small ruminants [13,14,17-21] and in cattle [9], thus demonstrating the importance of continuous investigations on these neglected coccidian parasites. In this context, Sénaud et al. [14], postulated, that at least some "globidium cysts" occurring in the abomasum of sheep must originate from a life cycle different from that of other enteric *Eimeria* species as the

highly motile merozoites being released from those "cysts" failed to enter ovine host epithelial cells *in vitro*.

The present study reports on three fatal cases of *E. gilruthi*-induced abomasal coccidiosis in sheep and goats being associated with proliferative haemorrhagic abomasitis, parietal cell atrophy and mucosal oedema. The distinct abomasal lesions of heavily *E. gilruthi*-infected mucosa should be considered as differential diagnosis to other frequently occurring parasitoses, such as teladorsagiosis and haemonchosis, in small ruminants.

MATERIALS AND METHODS

Necropsy and histological preparation of tissue samples

Two female cross-breed ewes (14 months and 2 years of age) and a two-year old goat originating from the northern region of Macedonia, Greece, were presented to the clinics for the investigation of severe weight loss, anorexia, diarrhoea and general bad condition. Due to rapid deterioration in clinical signs and poor diagnosis, euthanasia was elected and the animals were submitted for a complete *post mortem* examination at the Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece. The content of abomasum was washed through a sieve (pore size of 250 µm). The remnants of the sieving were transferred to Petri dishes and examined macroscopically. Additionally, the abomasal mucosa was carefully macroscopic analyzed for the presence of metazoan parasites. Tissue samples from all relevant organs were taken, fixed in neutral buffered 10 % formalin, embedded in paraffin, routinely processed, sectioned, stained with haematoxylin and eosin (H & E) and examined by light microscopy.

RESULTS AND DISCUSSION

Post mortem examination revealed that all animals were in poor body condition with strongly reduced body fat stores. Major internal lesions were restricted to the abomasum of the animals. The abomasum was distended and contained approximately 500-700 ml of turbid reddish-brown, watery fluid. The abomasal wall was oedematous with a nodular surface and multiple focal areas with patchy mucosal haemorrhagies (Figure 1A, B).

No juvenile/adult nematode, cestode or trematode parasites were detected in abomasal/intestinal contents. Trematodes were not found in the liver of the animals.

The abomasal mucosa contained macroscopically visible cyst-like whitish nodules up to 1 mm in diameter. Based on morphological findings previously reported [11,14,22] and based on their abomasal localization, these coccidian cyst-like structures were identified as macromeronts of *E. gilruthi.* Histological analyses revealed that several *E. gilruthi* macromeronts were localized in deep zones of the abomasal mucosa. Inflammatory cellular infiltrations were commonly detected around the macromeronts (Figure 2A-E). Some of the immune cells appeared in direct contact with external protrusions of the infected host cell (Figure 2C). The presence of red blood cells (Figure 2E) confirmed mucosal bleeding as already observed macroscopically. The macromeronts were situated in significantly enlarged host cells and reached a size of up to 600 µm in length. Most macromeronts showed an extremely thick outer amorphous layer of about 30-40 µm in width (Figure 2A, B), whilst some of them had thinner wall structures (Figure 2C). The cytoplasm of *E. gilruthi*-infected host cells was hardly visible and the host cell nucleus was hypertrophic and revealed several nucleoli (Figure 2B, E). The outer surface of infected host cell always showed long laminar protrusions of 20-25 µm length (Figure 2B). Overall, the macromeronts showed different maturation stages with some of them still being immature (Figure 2C). Others were fully developed and contained thousands of merozoites (Figure 2B, D). These had a slender appearance and measured 6 ± 0.2 µm in length and 1.6 ± 0.2 µm in width (in fixed samples, Figure 2F).

The apicomplexan parasite *E. gilruthi*, formerly known as *Globidium gilruthi*, belongs to the order Eimeriida (class Coccidea) and mainly affects small ruminants. Although abomasal coccidiosis has been reported for almost two centuries in small ruminants and cattle [5-8] there is still an enormous knowledge gap concerning life cycle, route of transmission, pathogenesis, immune reactions and taxonomic classification [10]. Furthermore, it is suspected that not only *E. gilruthi* but also other two or three more species might affect the abomasal and duodenal

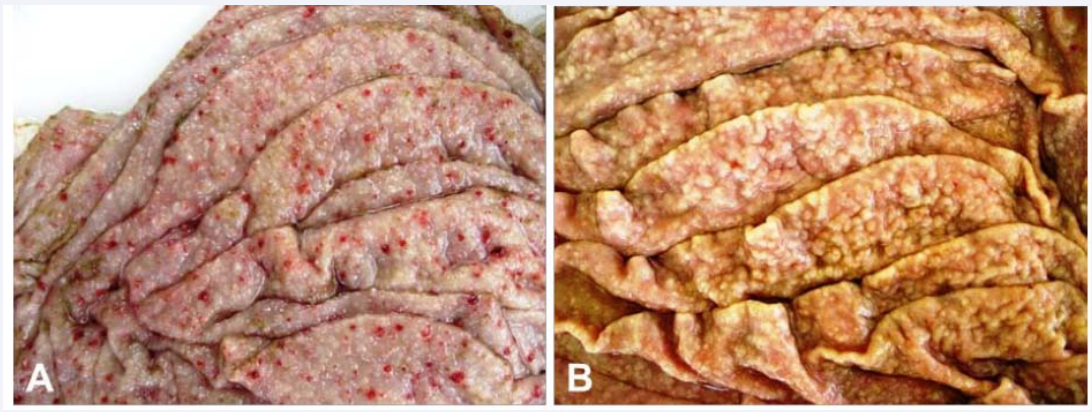

Figure 1 Abomasal mucosa of naturally *Eimeria gilruthi*-infected sheep (A) –*Eimeria gilruthi*-induced patchy haemorrhagies in the abomasal mucosa (B) – severely oedematous abomasal mucosa showing a characteristic nodular surface.

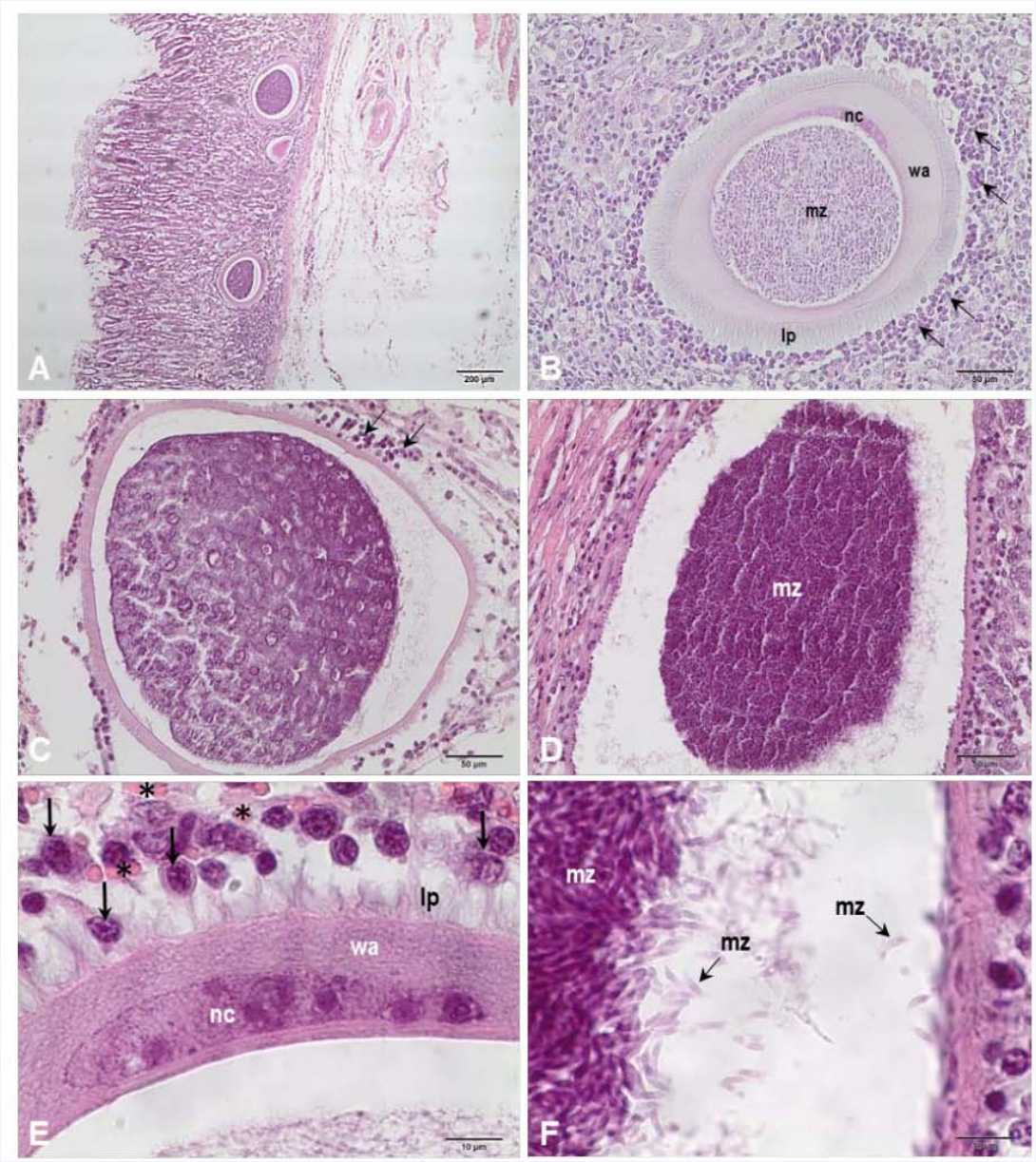

Figure 2 *Eimeria gilruthi* macromeronts in ovine abomasal mucosa (A) – thick-walled *E. gilruthi* macromeronts in deep zones of the abomasal mucosa (B) –single mature *E. gilruthi* macromeront containing fully developed merozoites (mz), showing a thick wall (wa), a hypertrophic host cell nucleus (nc) and long laminar protrusions (lp) on the surface of the infected host cell. Note the massive cellular infiltration (arrows) surrounding the macromeront (C) – immature *E. gilruthi* macromeront displaying a thinner wall and surrounded by lymphocytes (arrows) (D) – mature *E. gilruthi* macromeront containing fully developed merozoites (mz) (E) – magnified details of an *E. gilruthi* infected host cell with a hypertrophic host cell nucleus (nc), laminar protrusions (lp) on the surface, a thick macromeront wall (wa) as well as infiltrating immune cells (arrows) and erythrocytes (*) close to the macromeront (F) – *E. gilruthi* merozoites (mz) within a macromeront.

mucosa of sheep and goats [11,14,23] causing the formation of large deeply situated mucosal "cysts" (macromeronts) [11,14]. The establishment of suitable *in vitro* culture systems for the propagation of *E. gilruthi* merozoites as unsuccessfully intended by Sénaud et al. [14], would clearly provide helpful tools for studying detailed molecular parasite-host cell interactions as previously demonstrated for other ruminant intestinal *Eimeria* species displaying macromeront formation [15,24-30].

Since the current report shows a fatal outcome of *E. gilruthi* infections, the economic importance of abomasal coccidiosis may have been underestimated in the past due to unawareness among clinicians. In agreement to other reports [22,31,32], this infection can cause clinical abomasitis being accompanied by severe diarrhoea, acidosis, anorexia and weight loss [22,32]. As described in this study, occasionally even fatal outcomes might occur during *E. gilruthi* coccidiosis as reported for sheep and goats [22,33]. The fatal outcome is most probably related to the massive replication capacity of *E. gilruthi* in affected abomasal or duodenal mucosa and subsequent host immune response against

these parasites as already described for other closely related ruminant *Eimeria* [34,35].

Since sheep and goats are herbivores, they presumably acquire *E. gilruthi* by the oral uptake of exogenous sporulated oocysts. Clinical signs of *E. gilruthi* abomasal coccidiosis can include abomasitis with low pH (acidosis) and duodenitis [9,22,31-33] leading to malabsorption, diarrhoea, anorexia, dehydration and weight loss. In contrast to intestinal small ruminant *Eimeria* coccidiosis which mainly affect young animals [2,4,36,37], *E. gilruthi*-induced coccidiosis can also affect older animals [17,18,22,38] which is in accordance to the current case. Similar to sheep and goats abomasitis [22,31,33], enteritis have been reported to be induced by *E. gilruthi*-like macromeronts also in cattle [9], but clinical cases are rare, so far.

A definitive clinical diagnosis of *E. gilruthi* abomasal coccidiosis *intra vitam* is difficult to confirm in the absence of classifiable oocysts in faeces and abomasum biopsies are rarely performed in small ruminants for diagnostic purposes. Therefore, diagnosis is mainly achieved by the histopathological evaluation of tissue samples at necropsy as true for the current case. Histological analyses here allowed for *E. gilruthi* diagnosis based on typical morphological characteristics of macromeronts as described by others [11,17,20].

Treatments against *E. gilruthi* infections in sheep and goats are difficult and not well studied. Until now, reports on successful ovine or caprine abomasal coccidiosis treatment are lacking, despite the relatively common administration of effective anticoccidial drugs (e. g. toltrazuril, diclazuril) in ovine and caprine coccidiosis [39,40]. The pathogenicity of *E. gilruthi* was clearly demonstrated in this report. Thus, abomasal coccidiosis should be considered as differential diagnosis in any clinical case of small ruminant diarrhoea or weight loss.

CONCLUSION

During the last decades few reports on ruminant abomasal coccidiosis have been published despite the fact that there are nowadays potent available diagnostic tools such as serological, biochemical and molecular techniques. Taking into account that *E. gilruthi*-derived abomasistis can result in fatal outcome, it becomes evident that efforts should be made to better understand the life cycle, epidemiology and pathogenicity of the disease. Suitable *in vitro* culture systems for this neglected parasite should also be addressed in the near future. Recent *in vitro* investigations have identified numerous pathways involved in ruminant *Eimeria*-triggered modulation of the host cells with macromeront formation. These detailed data should be interpreted as a first step toward a better understanding of global parasite-host cell interactions, and further investigations applying functional assays are urgently needed in order to elucidate the valence of the different mechanisms of *E. gilruthi* infection. The recent knowledge gained on ruminant *Eimeria* metabolic requirements as well as its predictive auxothrophy for cholesterol may result in novel pharmaceutical strategies for the development of new anticoccidial drugs against *E. gilruthi*. Thus, we call for extended basic research in the field of small ruminant abomasal coccidiosis.

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