

## ETIO-PATHOGENETICAL RESEARCHES IN COCCIDIOSIS OF THE LAMBS BRED IN BIG GROUPS, IN AN UNIT FROM THE WEST OF ROMANIA

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**Key words:** coccidiosis, lamb

**Abstract.** The diarrheic syndrome can be attributed to various causes: biotic agents (bacteria, parasites, fungi and viruses), alimentary, inobservance of the hygiene conditions, intervention of some maternal, genetic, immunity and human factors.

An important place in producing this syndrome in lambs devolves upon the *Eimeria*.

In this study we had in view the qualitative estimation of the value of the biotic agents which intervene in the beginning of the diarrheic syndrome in lambs, from the neonatal period till to the age of 3 months. Observations concerning the share of the coccidian infection in producing this syndrome in lambs were performed. Clinical exam were accomplished, specifying the incidence of the diarrheic syndrome, the losses by mortality in lambs, also anatomo-pathological exams by necropsy, histo-pathological exam (hematoxilin-eosine method). The coproscopical exam was done, using the Blagg modified by Şuteu, McMaster, sedimentation by centrifugation and Baermann methods. The coccidian species were determined by micrometry, according to the morphological features cited by various authors. For determining the bacterial charge in faeces samples from lambs, selective (Istrate-Maitert) and differentiation (MIU, TSI, MILF, citrate Simmons) mediums were used for enterobacteria, Chapmann medium for enterococc and agar with blood for the investigation of the hemolytic capacity. The presence of Corona and Corona viruses were investigated by electrophoresis in poliacrylamide gel, respectively the hemagglutination assay with mouse red cells.

As a result of the etio-pathogenetical and diagnostic investigations performed, we came to the conclusions: the diarrheic syndrome evolved in the lambs aged from 4 weeks to 3 months, with an incidence of 26%. The mortality occurred in 16% of the sick lambs; the clinical aspects were represented by diarrhea with aqueous yellow-chocolate colored feces, with abundant mucus, anomaly, then anorexia and dehydration; anatomo-pathologically, catharal enterocolitis, with punctiform hemorrhages, whitish foci on the intestinal mucous membrane, hypertrophy of the mesenteric lymph nodes were observed. As a histopathologic result, the necrotic catharal enteritis, caused by coccidian evolution forms, and mesenteric hyperplastic lymph adenitis were observed. The diarrheic syndrome in lambs took place by the intervention of some unior multiple biotic agents: protozoa like *Eimeria* (extensivity 95%, intensivity 17500 EPG); *Cryptosporidium* (17.82%) and *Giardia* (22%); and the suprainfection with bacterial germs, unpathogen, firstly *E. coli* (45%) and *Proteus* (65%). We didn't find Rota and Corona-viruses by the usual methods. The specific structure of the *Eimeria* population in the lambs with diarrheic syndrome is: *E. ovinoidalis* (42%); *E. crandallis* (28%); *E. faurei* (10%); *E. parva* (17%) and *E. pallida* (3%).

### INTRODUCTION

The higher incidence in the neonatal period and the one after, the difficulty of determining the etiology, the therapeutic successes or failures in the diarrheic syndrome continue to arouse the interest of the researchers.

The diarrheic syndrome can be attributed to various causes: biotic agents (bacteria, parasites, fungi and viruses), alimentary, inobservance of the hygiene conditions, intervention of some maternal, genetic, immunity and human factors (10, 9, 19).

An important place in producing this syndrome in lambs devolves upon the *Eimeria* (5, 11, 16, 20).

In this study we had in view the qualitative estimation of the value of the biotic agents which intervene in the beginning of the diarrheic syndrome in lambs, from the neonatal period till to the age of 3 months. Observations concerning the share of the coccidian infection in producing this syndrome in lambs were performed.

## MATERIAL AND METHOD

The etio-pathogenetical observations in the coccidiosis of the lambs bred near their mothers in big collectivities for sheep breeding were performed in an unit from the west region of the country, on an effective of 1850 lambs, Transylvania Merinos breed, of the age between 4 weeks and 3 months.

Clinical exam were accomplished, specifying the incidence of the diarrheic syndrome, the losses by mortality in lambs, also anatomo-pathological exams by necropsy on 15 lambs. Fragments from the intestinal mucous were drawn for the histo-pathological exam. The sections were colored by hematoxilin-eosine method (HE).

The coproscopical exam was done on 100 samples, 50 from lambs and 50 from sheep mother, using the methods McMaster, and sedimentation by centrifugation and Baermann. For making evident the protozoa cysts and oocysts, inclusive for *Cryptosporidium*, we used the Blagg modified method. The coccidian species were determined by micrometry, on 100 oocysts, according to the morphological features cited by various authors (4, 5, 6, 8, 13, 17, 18).

For determining the bacterial charge of 20 feces samples from lambs, selective (Istrate-Maitert) and differentiation (MIU, TSI, MILF, citrate Simmons) mediums were used for enterobacteria, Chapmann medium for enterococ and agar with blood for the investigation of the hemolytic capacity (3).

The virus diagnostic for rotaviruses was performed from 18 samples and for Corona, from 15. In order to make evident the rotaviruses, the feces samples were put to a sodium dodecyl sulfate and phenol extraction, then the vertical electrophoresis in poliacylamide gel and silver stain were performed, using the technique described by Herring et al. (1982). The presence of the Corona viruses in the diarrheic feces was investigated by the hemagglutination assay with mouse red cells (3).

## RESULTS AND DISCUSIONS

In the studied unit, sheep, in the winter months, both in ewe and in multiparturient, the salmonella abortion evolved (abortion in the last part of the gestation, miscarriages of unviable lambs), with losses of 5-7%.

In the lambs of age between 4 weeks and 3 months, the diarrhea syndrome appeared with 26% incidence. Sixteen percentages of sick lambs died.

The lambs with diarrhea syndrome had watery faeces, yellow-brown colored, with abundant mucus, followed by misbehavior, inappetence, and after 3-4 days, anorexia and dehydration.

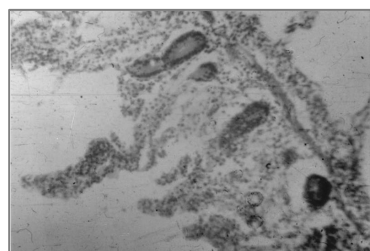
At the necropsy, in all died lambs catarrhal enteritis with spot hemorrhages in duodenum and jejunum, and mesenteric lymph nodes hypertrophy was observed. In 66% six weeks old lambs, was noted the presence of white spots in the intestinal mucosa. At microscopically examination, the presence of the necrotic catharal enteritis was noticed, caused by various coccidian evolution forms (Fig. 1-11). The sections through the mesenteric lymph nodes showed the presence of the hyper plastic lymph adenitis (Fig. 12). Moreover, tonsillitis, esophagitis and necrotic purulent pneumonia were registered in two lambs.

Coprospectral examination in lambs using McMaster method distinguished the presence of *Eimeria* spp. in 95% lambs and  $17.5 \times 10^3$  oocysts per gram faeces. Concomitantly infection with *Giardia* (22%) and *Cryptosporidium* (17.82%) was detected. The ewes were asymptotically carrier, 60% of them being positive for *Eimeria* spp., with oocyst per gram faece 500. Also, they were infected with *Trichostrongylidae* (40%, with 1500 OPG), *Nematodirus* spp. (20%, with 150 OPG), *Dictyocaulus filaria* (30%, with 10 LPG) and *Moniezia* spp. (20%).

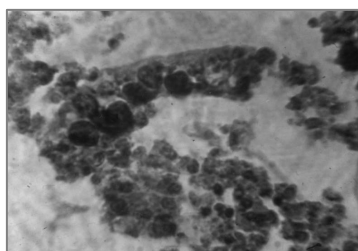
The coccidian species in lambs with diarrhea syndrome, from the studied unit, detected by size and morphology of the oocysts, in agreement with Dulceanu (1980) and Euzéby (1987) (Table 1), were the following: *E. ovinoidalis* (42%), *E. crandallis* (28%), *E. faurei* (10%), *E. parva* (17%) and *E. pallida* (3%).

Also, at bacteriological examination was detected the presence of high levels of *E.coli* (45%), *Proteus* (65%), staphylococci (30%), streptococci (25%) and fungi spore (55%). Also other bacteria were observed: *Serratia* (5%), *Klebsiella* (5%), *Pseudomonas pyocianea* (5%) and *Clostridia* (10%).

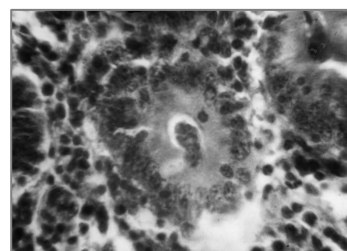
The virus exam by the electrophoresis of the viral genome demonstrated that none of the samples examined contained rotaviruses. By hemagglutination assay, we obtained the titre "0" in 7 samples, "1/2" in one sample, "1/4" in 2 samples and "1/64" in one sample. The hemagglutinant titres which are very low or the absence of the hemagglutination suggest also the absence of the coronaviruses.



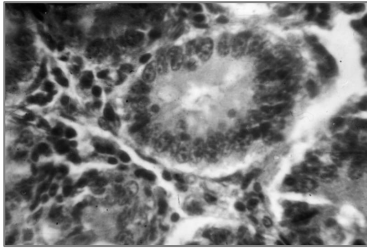
**Fig. 1:** Catharal necrotic enteritis and the presence of the coccidian morphologic forms (H-E, x100)



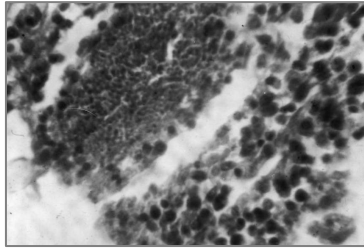
**Fig. 2:** Free coccidian morphologic forms in the desquamated cells (H-E, x400)



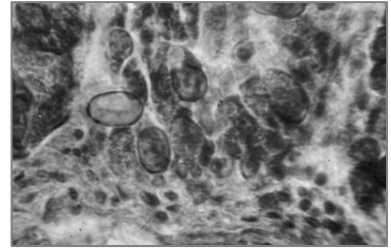
**Fig. 3:** Trophozoites in the lumen of the intestinal glands and the necrosis of the intestinal epithelial cells (H-E, x200)



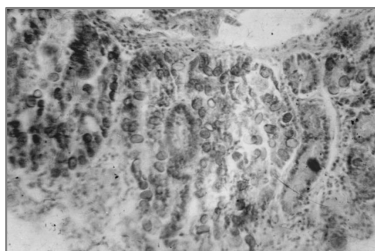
**Fig. 4:** Trophozoites of *Eimeria* spp. in the cytoplasm of the intestinal epithelial cells from the glandular zone (H-E, x200)



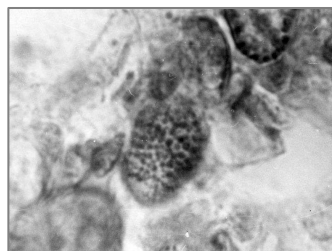
**Fig. 5:** Schizonts with merozoites localized in the superior half of the intestinal vili (H-E, x200)



**Fig. 6:** Different evolutionary stage of *Eimeria* spp. in the intestine: trophozoites, schizonts, macrogametes, zygotes (H-E, x400)



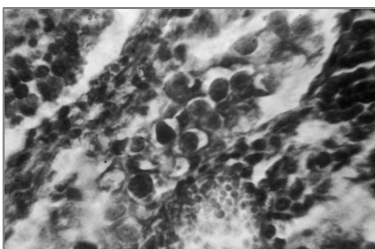
**Fig. 7:** Series of immature schizonts and gamete formations; necrotic enteritis (H-E, x100)



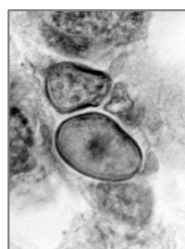
**Fig. 8:** Schizogonic stage in the epithelium of the mucous membrane of the jejunum (H-E, x1000)



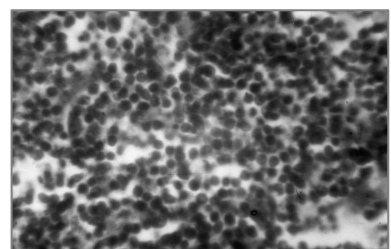
**Fig. 9:** Gamete stage in the mucous membrane of the jejunum (H-E, x1000)



**Fig. 10:** Series of *Eimeria* spp. zygotes in the epithelium of the intestinal mucous membrane in lambs (H-E, x400)



**Fig. 11:** Zygote – detail (H-E, x1000)



**Fig. 12:** Mesenteric hyperplastic lymphadenitis in lambs with coccidiosis (H-E, x200)

Table 1.

The morphologic characters of the coccidian oocysts from the lambs, by comparison with the characters described by other authors

| <i>Eimeria</i><br>species                                    | Form                  | Color            | Micropile                   | Polar<br>capsule        | Average dimensions (µm) |                            |                        |
|--------------------------------------------------------------|-----------------------|------------------|-----------------------------|-------------------------|-------------------------|----------------------------|------------------------|
|                                                              |                       |                  |                             |                         | Euzéby<br>(1987)        | Dulceanu<br>(1980)         | Personal<br>parametres |
| <i>E. ovina</i><br>( <i>E. bakuensis</i> )                   | ellipsoidal<br>ovoid  | brown-<br>orange | yes                         | yes                     | 27 x 20                 | 39.7 x 19.7                | 25.0 x 18.0            |
| <i>Globidium</i><br><i>faurei</i><br>( <i>E. intricata</i> ) | ellipsoidal           | dark<br>brown    | yes                         | yes,<br>prominent       | 48 x 32                 | 42.9 x 32.9                | 43.6 x 32.4            |
| <i>E. crandallis</i>                                         | spherical<br>elliptic | pink tint        | yes,<br>lightly<br>marked   | yes                     | 23 x 19                 | 24.2 x 16.8                | 22.8 x 15.4            |
| <i>E. ahsata</i>                                             | ovoid                 | light<br>yellow  | yes                         | yes,<br>like an<br>arch | 38 x 25                 | 37.3 x 25.1                | 33.6 x 24.2            |
| <i>E. ovinoidealis</i>                                       | ellipsoidal           | yellow-<br>brown | yes,<br>a little<br>evident | no                      | 22 x 18                 | 24.3 x 18.8                | 20.4 x 18.4            |
| <i>E. faurei</i>                                             | ovoid                 | yellowish        | yes,<br>rarefied            | no                      | 31 x 23                 | 26.7 x 19.7                | 26.4 x 19.2            |
| <i>E. parva</i>                                              | sub-<br>spherical     | light<br>yellow  | no                          | no                      | 16 x 16                 | 16.9 x 14.1                | 15.2 x 14.0            |
| <i>E. weibridgensis</i>                                      | ellipsoidal<br>oval   | pale<br>yellow   | yes                         | yes,<br>large           | -                       | 28.5 x 19.9                | 26.5 x 18.8            |
| <i>E. pallida</i>                                            | oval                  | light grey       | no                          | no                      | -                       | 18.4 x 13.8<br>14.9 x 11.5 | 15.6 x 10.5            |

If coccidiosis is nowadays relatively frequent in numerous sheep breeding units, sometimes its importance is overestimated and the “presence of eimeria” – otherwise well tolerated by animals – is confounded with the “coccidiosis – disease”, which causes losses (23). For the estimation of the infection intensivity in the flock – number of oocysts per gram of feces (OPG), in lambs with diarrheic syndrome – is significantly higher (between 10 thousands and 40 thousands) than the healthy ones (20). This fact is shown also by the results of our researches.

Various authors pointed out that in the etiopathogeny of the diarrheic syndrome in lambs and kids, beside eimeria, the *E. coli* bacteria intervene, frequently associated with Rota and Corona viruses, with other protozoa – cryptosporidia and giardia (3, 12, 14, 21) and/or digestive helminths (1, 2, 7, 15, 16).

Euzéby (1987) shows that the association microflora of the eimeria plays a prominent part in the evolution of the disease; the coccidiosis evolves subclinic in the absence of the bacterial, viral and mycotic suprainfections. This situation makes obvious the epidemiologic importance of the suprapopulation from the breeding farms, generator of a synergic microbism with the coccidian infection (6).

Şuteu et al. (1978) emphasize that the results of the bacteriologic exam prove a significant increase both of the number of Gram-negative coliform bacteria, but particularly of the *Clostridium* germs. It is noticed the existence of an intestinal dismicrobism, which together with the parasitical infection determines gastro-intestinal troubles in the sick lambs (20).

Our investigations confirm the intervention of all these biotic factors in the lambs with diarrheic syndrome (from the studied unit), except the enteric viruses Rota and Corona.

The results obtained emphasize the importance of the complex investigations in determining the etiopathogeny of the diarrheic syndrome of lambs until the age of 3 months, also the major role of the eimeria in his generation, concomitantly with the bacterial suprainfection.

## CONCLUSIONS

As a result of the etio-pathogenetical and diagnostic investigations performed in a unit from the west of the country, on 1850 lambs Transylvania Merinos breed, aged 4-12 weeks we came to the conclusions:

1. The diarrheic syndrome evolved in the lambs aged from 4 weeks to 3 months, with an incidence of 26%. The mortality occurred in 16% of the sick lambs.
2. The clinical aspects were represented by diarrhea with aqueous yellow-chocolate colored feces, with abundant mucus, anomaly, then anorexia and dehydration. Anatomopathologically, catharal enterocolitis, with punctiform hemorrhages, whitish foci on the intestinal mucous membrane, hypertrophy of the mesenteric lymph nodes were observed.
3. As a histopathologic result, the necrotic catharal enteritis, caused by coccidian evolution forms, and mesenteric hyperplastic lymph adenitis were observed.
4. The diarrheic syndrome in lambs took place by the intervention of some unor multiple biotic agents: protozoa like *Eimeria* (extensivity 95%, intensivity 17500 EPG); *Cryptosporidium* (17.82%) and *Giardia* (22%); and the suprainfection with bacterial germs, unpathogen, firstly *E. coli* (45%) and *Proteus* (65%). We didn't find Rota and Corona-viruses by the usual methods.
5. The specific structure of the *Eimeria* population in the lambs with diarrheic syndrome is: *E. ovinoidalis* (42%); *E. crandallis* (28%); *E. faurei* (10%); *E. parva* (17%) and *E. pallida* (3%).

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