ABSTRACT. The research has been conducted on Karakul and Turcana crossbred lambs of 1-3 days of age; the purpose of this investigation was to reveal the development of lymphoid tissue associated of intestinal mucosa, local reactivity and the risk of apparition of parasitic enteritis.

For the histological examination, samples of tissue were drawn from small intestine (duodenum, jejunum, and ileum) and from mesenteric lymph nodes, which were fixed in formaldehyde 10%. Subsequent, the samples were specific processed, included to paraffin, sectioned at 5µm and coloured through the HEA and PAS methods. The examination and microphotography were accomplished at MC5 with 10 oc., 10, 20, 40, ob. and 63, 100 immersion ob.

Histological examination revealed the integrity of the epithelium with prominence of the brush border like and the taking over of the immunoglobulins as vacuole, through clathrin-coated vesicles, in a passage from the apical pole to the basal pole of the intestinal epithelial cell, the vacuole were surprised in lamina propria, with distension of central lacteal; diffuse lymphoid infiltration in lamina propria of villosity and Peyer’s patches with immature polymorphic cellulary population with outlining of the germinative centers and a dome zone, in submucosa; anizocaria, reflecting a polymorphic mezenchymal population. In lymph nodes, there were observed primary nodules in forming, without germinative center, and in subcapsulary sinus a low cellular population. The lambs are extremely vulnerable to the parasitic intestinal aggression due to the fact that the local defensive system is in forming and the reactive capacity is down, at this age.

Key words: lambs, newborn, small intestin, lymphoid tissue, reactivity.

Study purpose: to point out the particularities of the local intestine reactivity in newborn lambs, the immunoglobulin transfer role and to signal the risk of parasitic enteritis appearing.

Material and methods

The studies have been conducted on half-breed lambs of the Karakul and Turcana, being 1-3 days old. After slaughter for economic purposes (use of coat) thin intestine fragments (duodenum, jejunum, ileum) and mesenteric lymph nodes were extracted from lambs and fixed in formaldehyde, 10%. Later the fragments were processed specifically for histology study, immersed in paraffin wax, sectioned at 5µm and colored trough the HEA and PAS methods. Examination and microphotography have been performed on with the MC5 microscope using 10 oc., 10, 20, 40 ob. and immersion, 63, 100 ob.

Results and discussions

The histological examination of the small intestine mucosa has revealed the integrity of the intestine epithelia highlighting the brush border of the enterocytes (Fig. 1, 2) and obtaining the immunoglobulins as vacuoles, endocytosis by clathrin-coated vesicles, while passing from the apical pole to the basal of the enterocytes (Fig. 3).
The vacuoles were also detected in the *lamina propria*, with distension of central lacteal; diffuse lymphoid infiltration in *lamina propria* of villosity and submucosa (Fig. 3, 4, 5).

The Peyer’s patches immature polymorph cellular population with outlining of the germinative centers and the dome zone visible (Fig. 6, 7). Anizocaria was observed, reflecting a polymorphic mezenchymal population.
Intestinal villosity. We found vacuoles and clots in lamina propria also, with dilatation of the central lacteal duct. PAS x 200.

Fig. 3.

Fig. 4. One day newborn lamb. Jejunum, apex of villosity. Apical epithelium is fully loaded with spheres-vesicles who passed in lamina propria also. HEA x 1000.

Fig. 5. Jejunum. The apex of villosity: the enterocyte is occupied by the little spheres-vacuoles. We observe the cell in passing. HEA x 1000.
The Peyer’s patches, located in the ileum, are involving over the whole fetal life but regresses after birth. This structures stretch over one meter in length and count 100,000 follicles (8).

In lambs, the lymphoid follicles (in the ileum) in the Peyer’s patches are places of intense B lymphopoiesis. Over 95% of the B cells produced in follicles die through apoptosis and 5% of the cells migrate in circulation, contributing to the necessity of B cells.

The factors that influence the selection and death of the B cells are unknown. “In vitro” studies have shown that only B cells, from the Peyer’s patches in the ileum, die trough apoptosis within 24 hours. In fetuses injected with IgM monoclonal antibodies it was noted that the dome zone of the follicles in the Peyer’s patches (in the ileum) was significantly larger than in fetuses not injected. In the Peyer’s patches of the jejunum and ileum, there were few IgM+ found, that would react with the monoclonal antibodies of the B cells. Big populations of TCD₈⁺ and TCD₅⁺ cells were observed in the Peyer’s patches. Populations of stromal cells were also observed (5, 8).

In lymph nodes, there were observed primary nodules in forming, without germinative center and in subcapsulary sinus a low cellular population (Fig. 8, 9).
In sheep, the lymphatic lymph nodes are specific and are named hemic lymph nodes. They are formed through confluence of blood vessels, more than the lymphatic ones. The sinusoid spaces of the hemic lymph nodes are sanguine sinuses. The forming hemic lymph node is colonized by T lymphocytes that migrate from the embryonic circulation and by B lymphocytes originated in the bone marrow. The migrated lymphocytes (T) have the tendency of installing themselves in the periphery region of the lymph node. Colonization of the hemic lymph nodes with lymphocytes is done at the same time and the same way as in general lymph nodes. This way, in adults, the lymph circulation is afferent from the periphery and efferent through the hile.

The *humoral immunity* in sheep is characterized through the existence of three major classes of immunoglobulins: IgG, IgA, IgM. The existence of *homocitotrop antibodies* has been described, which have antigenic specificity and physiochemical properties similar to IgE from other species.

Sheep immunoglobulin’s are characterized through great originality:

- there exists two subclasses IgG1 and IgG2 that have very different physiochemical and biological properties;
- the selective transfer of a single subclass of immunoglobulins and more precisely IgG1 in large quantities in mammary gland as well as

**Fig. 8.**
3 Days newborn lamb. Mesenteric lymphnode: is observed bands much thicker infiltrates by lymphocytes and plasmacytes, and in perifollicular sinus there is a small cell population. HEA x 100.

**Fig. 9.**
Mesenteric lymphnode: perifollicular sinus, cell population made by rare lymphocytes, reticular cells, macrophages. HEA x 1000.
other exocrine secretions, is a phenomenon not present at other species. This particular characteristics have led to the designation of infraclasses for IgG1 and IgG2 and of subclasses, for the two varieties of IgG2 (IgG2a, IgG2b).

In serum, the concentration of IgG (IgG1, IgG2) is very high and represents around 30% of the total proteins. The individual variations are however significant and depend on genetic, physiological, or pathogen factors. The serial concentration of IgA is 2-4 times higher than IgM and 10 times higher than in other species.

In the colostrum secretion IgG1 is in concentration of 60-80 mg/ml, representing 80% of the immunoglobulins. This rate persists in milk, but in time the concentration of IgG1 will be diminished 100 times. It is to be emphasized the role IgG1 has in mucous protection in sheep. The preparation of a serum has permitted the manifestation of isotypical characteristics (homocitotrop) with affinity for the same cells antibodies, different from those of other immunoglobulin classes. The physiochemical and biological properties allow their assimilation with IgE, involved in the resistance to parasites (8).

Cellular immunity. The phagocytosis activity of the polymorphonuclear cells and mononuclear leukocytes in sheep blood have been measured using two different types of ingested inert particles. The proportion of granulocytes was 67,83% and 3,74% monocytes. The determined phagocytosis activity was 30-50% (1).

Two subpopulations of lymphocyte T have been identified: SBUT4+ and SBUT8+ (counterpart of human CD4+ and CD8+).

The SBUT4+ lymphocytes (CD4+), represent 20% of the mononuclear in periphery blood, 80% in timus and 50% as lymphocytes in the efferent lymphatic vessels. These cells recognize the antigen associated with second class CMH and works as a helper.

The SBUT8+ lymphocytes (CD8+), represent 12% in periphery blood, 80% in timus and 15% as lymphocytes in the efferent lymphatic vessels. These cells recognize the antigen associated with firs class CMH and works as a suppressor/cytotoxic cells (2, 10).

From the intraepithelial lymphocytes and intestinal lamina propria, of sheep, were isolated T lymphocytes with alpha, gamma receptors (TR-alpha, gama), cells CD4+, CD8+, CD5+ and second class CMH. This cells have been compared with TR-alpha, gamma+ cells in the periphery blood, intestinal lymph and Peyer’s patches in the jejunum, observing notable differences.

TR-alpha, gamma+ cells have a 18% presence of the intraepithelial lymphocytes and 20% of the lymphocytes in lamina propria. The population of CD8+ was 24% and the population of CD5+ cells was 54% of the intraepithelial lymphocytes and in lamina propria. In blood and intestinal lymph CD4+, CD8+, CD5+ cells had universal presence.

A particular aspect of intraepithelial lymphocytes was the distinct presence as a population of CD5+ and TR-alpha, gama+ cells. Among the intraepithelial lymphocytes and in lamina propria one finds CD2+ cells and second class CMH.

It is shown that 60-95% of the intraepithelial subsets and in lamina propria are with second class CMH, comparable to only 5-40% in the Peyer’s patches in the jejunum, lymph nodes, spleen and blood. It is possible that the circulation of the TR-alpha, gamma and CD8+ cells located in the small intestine mucous epithelium to be activated and stopped by CD5+, under the influence of the local microenvironment (4).

Absence of the placentar transfer of immunoglobulin material during the gestation, makes the newborn ruminant to be agamaglobulinic at birth (8). The survival of the newborn is owed to the particular abundance of immunoglobulin and essentially in IgG1 of the colostrum, as well as the intestinal capacity to intactly absorb this Ig in the first 24 hours of life (7).

In particular the mammary gland concentrates IgG1 serum trough a specific fixation mechanism, on the epithelial cell surface, having receptors with a high affinity for IgG. The colostrum ingestion protects the newborn ruminant from pathogen agents of the gastrointestinal tract. The colostral immunoglobulin attaches themselves to the surface of the bacteria and viruses present in the
organ lumen, preventing it to adhere to the intestinal epithelium, inflammation of the mucosa and diarrhea (6).

A part of the colostral immunoglobulin are transferred during the first hours of life, directly to the epithelial cells trough pinocytosis and later freed in the lymph vessels and the blood circulation (8). The colostrum is equally rich in IgA, IgM and antimicrobial substances (complement, lizosym, lactoferrin, transferring, enzymatic systems, growth factors, vitamins, oligominerals, etc.).

The proteolytic activity of the digestive tract being very weak and yet reduced trough the presence of trypsin inhibitors in the colostrum, colostrial Ig arrives intact in the small intestine. They are rapidly absorbed, due to the pinocytosis activity of the immature cells in the intestinal mucosa. After two hours from administrating the colostrum, the concentration of serial IgG is sufficient to insure an efficient start of the passive immunity.

The intestinal absorption capacity of macromolecules is rapidly diminishing in the hours following birth; it starts to diminish from the second hour and is completely gone after 24 hours. This absorption basically nonselective for molecules with a low molecular weight, while molecules with a high molecular weight like IgM, are absorbed harder (7, 8).

The quantity of maternal immunoglobulin transferred in the first 24 hours of life is the same as the mother’s serial Ig concentration. The general absorption, systemic of colostral immunoglobulins subsists from 6 to 36 hours. If IgG has not attained 4mg/ml serum in 24 hours, results that the colostral immunoglobulins transfer has been deficient (8).

The general immunity, accorded by the colostrum, permits the protection of the newborn against systemic infections. IgA will be in part reexcreted by the mucous as Ig secretion, in the digestive and respiratory tract (8, 9). The number of lymphocytes in the colostrum is important, yet their role in the immunity transfer is arguable (8).

Feeding of the lambs with milk substitutes before the administration of colostrum determines a significant decline in the Ig concentration in serum (P<0,05). The lambs fed only with milk substitutes had a very low Ig concentration (P<0,001), while the lambs fed with colostrum had a much higher Ig concentration compared to the control subjects. No significant differences (P>0,1) were reported between the serial Ig concentration, in lambs left with their mothers and nursed colostrum trough sucking, comparative to lambs separated from their mothers after getting colostrum (6).

During the first days of life, the lambs are extremely vulnerable to intestinal microbial san/or parasitic aggressions because the local defense system is forming and the reactive capacity is minimal. Lamb cohabitation with mother sheep in deficit hygienic conditions and alimentation, leads to a growing risk of infestations in lambs with invasive elements, (sporozoa oocysts, eggs and helmith larva), eliminated during winter by the adult sheep, carriers and eliminators of parasites (Strongyloides, Eimeria, Giardia, Bunostomum, etc.) with the appearance of digestive disorders and economic loses.

**Conclusions**

The histological exam of the small intestine mucosa in newborn lambs (1-3 days), has revealed aspect regarding the mucous integrity, the passive transfer of colostral immunoglobulins, aspect of Peyer’s patches in the ileum and aspects of the mesenteric lymph nodes.

The intestinal epithelium has been integral with highlighting of the enterocytes and the brush border. The passage of colostral immunoglobulins as clathrin vacuoles was detected, from the apical pole of the enterocytes towards their basal pole and in lamina propria.

The Peyer’s patches contain a immature polymorph cellular population with outlining of the germinative centers and the dome zone visible.

The mesenteric lymph nodes contain the forming primary nodes, without germinative center and in the subcapsular sinuses was observed a reduced cellular population.

Due to the immunological particularities and reactivexis, in the first days of life, the lambs are extremely vulnerable to microbial and parasitic aggressions, being exposed to serious illnesses.
REZUMAT

Aspecte histologice privind reactivitatea mucoasei intestinale la mieii nou-născuți și riscul apariției enteritelor parazitare

Cercetările s-au desfășurat pe miei de rasă metis Karakul x Târcană, în vârstă de 1-3 zile, cu scopul de relevare dezvoltarea țesutului limfoid asociat mucoasei intestinale, reactivitatea locală și riscul apariției enteritelor parazitare.

Pentru examenul histologic s-au prelevat fragmente din intestinul subțire (duoden, jejun și ileon) și din limfonodurile mezenterice, care au fost fixate în formaldehidă 10%. Ulterior au fost prelucrate specific, incluse la parafină, sectionate la 5µm și colorate prin metodele HEA și PAS. Examinarea și microfotografierea au fost efectuate la microscopul MC5 cu oc. 10, ob. 10, 20, 40 și imersie, ob. 63, 100.

Examenul histologic a relevat integritatea epiteliului cu evidențierea marginii în perie și preluarea imunoglobulinelor sub formă de vacuole, endocitate prin vezicule clatrine, în pasaj de la polul apical spre cel bazal al enterocitelor. Vacuolele au fost surprinse și în lamina propria, cu ectazia chiliferului central; infiltrarea limfoidă difuză în lamina propria a vilozițăților și în submucoasă, plăci Payer cu populație celulară polimorfă imatură cu schițarea centrului germinativi și zona domului; anizocarie, reflectând o populație mezenchimală polimorfă. În limfonoduri, au fost observați noduli primari în formare, fără centrul germinativ, iar în sinusurile subcapsulare, o populație celulară redusă. La această vârstă, mieii sunt extrem de vulnerabili la agresiunea intestinală parazitară întrucât sistemul de apărare locală este în formare iar capacitatea reactivă este minimă.

Cuvinte cheie: miei, nou-născuți, țesut limfoid, reactivitate.

Bibliography