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# COMPARATIVE PATHOMORPHOLOGICAL INVESTIGATIONS ON COLIBACILLOSIS AND CRYPTOSPORIDIOSIS IN CALVES

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**Summary**

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The aim of the studies was to report the specific gross and microscopic changes in the different intestinal compartments in clinical cases of gastroenteritis in neonate and juvenile calves. The survey comprised 17 cattle farms from 9 regions of the country with gastrointestinal problems in neonate and juvenile calves. The population of the latter included 2,560 animals at the age from 24 h to 30 days. A total of 170 faecal samples were collected from diarrhoeic calves as well as 75 necropsy samples from intestinal content and mesenteric lymph nodes for histopathological examination. Qualitative and quantitative antigenic tests detecting 5 of main agents of neonatal diarrhoea in calves – *Rainbow calf scour 5 BIO K 306 Detection of Rota, Corona, E.coli F5, Crypto and Clostridium perf. in bovine stool* (*BIOX Diagnostics, Belgium*), and quantitative ELISA *(BIOX Diagnostics, easy digest ,4 Belgium) sandwich test for feces*, *Rota, Corona, E.coli F5, Crypto* were used. The main identified etiological agents were *E. coli F5* and *Cryptosporidium parvum.* The used antigenic, gross anatomy and histopathological examinations of intestinal tract tissues in calves are reliable and are used for diagnostics and differential diagnostics of other common diseases at farms as *Rotavirus, Coronavirus, Salmonella, Clostridium perfringens,Coccidiosis* etc.

**Key words:** calves, *Cryptosporidium parvum, E.coli F5,* neonatal diarrhoea, pathology

## INTRODUCTION

Gastrointestinal diseases in newborn calves is a persisting important problem in dairy and beef lot cattle farms. The main cause of their occurrence is improper feeding and immunoprophylaxis of pregnant cows, hence birth of poorly developed and premature calves. At a global scale, this group of diseases is termed *neonatal calf diarrhoea* (*NCD*). The etiology of NCS is associated with both non-infectious and infectious agents. This includes compromised herd health, inadequate microclimate and rearing sites. Even minor flaws in feeding and ration composition could entail unoffending digestive tract disorders and replication of microbial pathogens increasing the risk of disease occurrence (Lyutskanov, 2013, Lorenz *et al.,* 2011).

The commonest agents of NCD in calves during the first 2 weeks of age are *Cryptosporidium parvum*, *E. coli K99,* followed by bovine rotaviruses (*GPA BRV*) and bovine coronaviruses (*BCoV*). Often, complicated NCD cases are presented as mixed infections with participation of *Cryptosporidium parvum, GPA BRV, BCoV* and *E. coli K99* (Lorenz *et al*., 2011). *E. coli* infections are of substantial economic significance, as they affect neonate calves, foals, piglets, lambs and chickens inducing enormous economic losses. Colibacillosis affects neonate calves in the first days after their birth and is manifested with enterocolitis and septicaemia signs. The characteristic clinical signs of the diseases are the severe profuse diarrhoea, rapid emaciation, lack of fever and high mortality rates. Endotoxins produced by *E. coli K99* are affirmed to cause pathological damage of organs and systems as hyperaemia, haemorrhages, vasomotor shock, local and general Shwartzman reaction, fever, increased pulmonary blood pressure, specific dermal reaction, placental damage followed by abortion (Chigerwe *et al.,* 2009; Izzo *et al.,* 2011).

*Cryptosporidium parvum* is outlined as one of the commonest agents of neonatal gastroenterites in calves up to 3 weeks of age (Castro-Hermida *et al.,* 2002; Chartier *et al.,* 2013; Cacciò & Widmer, 2014). Profuse diarrhoea is reported as the most important clinical sign. Its duration and intensity correspond to the site of invasion and extent of morphological changes of intestinal mucosa (Joachim *et al.,* 2003). Colonisation of distal small intestine compartments are histologically seen as villous atrophy, metaplasia and desquamation of surface epithelium (Dean-Nystrom *et al.,* 2008; Cacciò & Widmer, 2014). The authors specified that such changes could be observed in the duodenum, caecum and colon. Cellular infiltrate in *lamina propria* in their view comprised mainly neutrophils. Other researchers suggested *Cryptosporidium parvum* and *Giardia spp*. as the main protozoa involved in NCD etiology (De Graaf *et al*., 1999; Chigerwe *et al.,* 2009). Observed macroscopic changes in cryptosporidiosis include dehydration with profuse diarrhoea for a couple of days. Small and large intestines are distended, filled with gas and watery yellow fluid, while mesenteric lymph nodes are enlarged. The intestinal mucosa is strongly hyperaemic and covered with yellowing intestinal content (De Graaf *et al.,* 1999; Joachim *et al*., 2003). Microscopic changes are indicative for severe atrophy of ileal, caecal and colonic villi. Numerous *Cryptosporidium* development forms associated with epithelial cells lining ileal villi are observed (Gay *et al*., 2011; Duda-madej & Gosciniak, 2013).

The analysis of literature data on gastrointestinal diseases of newborn and juvenile calves revealed their high prevalence and importance in contemporary cattle farms. This places the emphasis not only on their prevention and control, but also on gathering information on the possibility of gross pathological and histopathological diagnostics on the basis of macro- and microscopic changes respectively, and differentiation of conditions from other disorders of both infectious and non-infectious origin.

## MATERIAL AND METHODS

The study included 17 cattle farms from 9 regions of the country, with detected gastrointestinal problems in newborn and juvenile calves. The newborn and juvenile calves population was 2,560 for both categories, whose age ranged from 24 h to

30 days. At all farms, clinical-epizootiological and etiological investigations were performed, and morbidity and mortality rates were calculated. A total of 170 faecal samples from diarrhoeic calves as well as 75 necropsy samples from intestinal content and mesenteric lymph nodes for histopathological examination were collected. Qualitative and quantitative antigenic tests detecting 5 of main agents of neonatal diarrhoea in calves – *Rainbow calf scour 5 BIO K 306 Detection of Rota, Corona, E.coli F5, Crypto* and *Clostridium perf. in bovine stool* (*BIOX Diagnostics, Belgium*), and quantitative ELISA *(BIOX Diagnostics, easy digest ,4 Belgium) sandwich test for feces*, *Rota, Corona, E.coli F5, Crypto* were used for detection of amount of antigens of indicated enteropathogens in studied faecal samples. A total of 75 carcasses of calves, all with gastrointestinal diseases, were submitted to routine necropsy using the standard protocol. Tissue samples (size 2.5 cm) were collected from the affected proximal and distal gastrointestinal tract areas – abomasum, duodenum, jejunum with mesenteric lymph nodes, ileum, caecum, colon, rectum) for histopathological examination. Samples (2.5×2.5×1 cm) were also obtained from parenchymal organs – liver, lungs, kidneys, spleen and heart. Specimens for histological examination were fixed in 10% neutral buffered formalin for 48-72 h and embedded in paraffin. From paraffin blocks, 4 μm cross sections were cut on a Leica RM 2235 microtome and conventionally stained with haematoxylin-eosin (H/E).

## RESULTS

So far, during the dry period, no prophylaxis of pregnant cows against diseases as rotaviral infection, coronaviral infection, colibacillosis as well as prevention of cryptosporidiosis in neonate calves had been carried out in 13 out of the 17 surveyed farms. B In the other 4 farms, cows were vaccinated against infectious bovine rhinotracheitis (*IBR*) and mucosal disease-bovine viral diarrhoea (*BVD*), but not against agents causing gastroenteritis in neonate and juvenile calves. Epidemiological studies on farms where calves were affected with enteritis demonstrated the highest morbidity of 65.13% and mortality 33.70% in calves aged 4 to 8 days.

Clinically, affected calves showed deteriorated general condition, dehydration and profuse diarrhoea. Calves were with pronounced enophthalmos with pale conjunctivae, and some animals – with ruffled haircoat and refused eating or drinking. Faeces were diarrhoeic, of yellow to yellow-greenish colour, mixed with mucus, blood and gas bubbles, and occasionally – entirely bloody.

The analysis of data from field tests (qualitative) and laboratory (quantitative) antigenic diagnostic tests showed that in 60% of cases the predominating etiological agent was *Cryprosporidium parvum,* followed by *E. coli F5 K99* with 35%, while co-infections with more than one etiological agents comprised 5%.

Gross anatomy necropsy findings of carcasses with colibacillosis (15 bodies from 24 h to 4 days of age) showed dehydration, enophthalmos, polyarthrites of carpal and stifle joints, and omphalitis in relation to septicaemic form the disease. The perianal area and tail base were stained with yellow-greenish diarrhoeic faeces. The mucosa lining the oral and nasal cavities were diffusely hyperaemic and oedematous, spattered with petechiae. Cloudy mucus mixed with feed particles were found stuck to the pharyngeal and oesophageal mucosae. The abdominal

cavity was filled with 800 to 2500 ml reddish non-transparent fluid. The serosa of the omasum and abomasum exhibited numerous petechial and ecchymotic haemorrhages and enlarged regional lymph nodes. The abomasum was strongly dilated and enlarged, filled with milk coagula mixed with grayish fluid. Its mucosa was oedematous and hyperaemic, with striated haemorrhages among the plicae. In two of cases, single ulcers 0.5–1 cm in diameter were found out. Beneath the spleen capsule, petechiae could be seen. Along the entire length of the gastrointestinal tract, multiple subserosal haemorrhages and diffuse hyperaemia were observed. In 4 necropsied calves, there were macroscopic changes typical for catarrhal-haemorrhagic enteritis (Fig. 1), while other suggested a gastroenterocolitis. The mucosal changes were the most pronounced in the proximal and middle parts of the small intestine. The mucosa was diffusely hyperaemic, and intestinal content was yellowish, with bad odour and mixed with whitish particles. Mesenteric lymph nodes in all calves were hyperaemic, enlarged 1.5 to 2 times and juicy, accompanied with overfilling of mesenteric blood vessels. The liver was cyanotic, and its surface: spattered with pale yellow areas. The gallbladder in all calves was very enlarged. Beneath the kidney capsule, haemorrhages could be observed, and medullary and papillary zones were intensively reddened. The lungs of 9 animals was cyanotic-haemorrhagic at some areas (bronchopneumonia), with emphysema and oedema in the periphery of pneumonic foci. The trachea and bronchi demonstrated a large amount of non-transparent foamy fluid – exudate. Bronchial lymph nodes were enlarged, juicy with hyperaemic cross section.

**Fig. 1.** Catarrhal haemorrhagic enterocolitis, calf affected with colibacillosis.

Six calves showed serous pericarditis, the pericardial sac was filled with yellowish fluid, there were not adhesions with the epicardial surface. With no exception, epicardial and endocardial surfaces were spattered with numerous petechiae and sugillations. All calves exhibited single haematomas on bicuspid valves (Fig. 2).

**Fig. 2.** Haematomas on bicuspid valves of the heart, calf affected with colibacillosis.

Necropsied bodies of calves with cryptosporidiosis were 60, at the age from 3 to 10 days. Gross findings during carcass inspection revealed severe dehydration and emaciation resulting from the profuse diarrhoea. Eyeballs showed strong opacity and were very sunken, without discharge. The perianal region and tail base were stained with yellow-brownish faeces with soft consistency, mixed with undigested milk particles. No changes were found out on the skin, haircoat and hooves.

**Fig. 3.** Hyperaemic serosa and gas-filled small and large intestines, calf affected with cryptosporidiosis.

The abdominal cavity of three of examined calves contained approximately 1000 ml cloudy reddish fluid. Pathoanatomical alterations were found mainly in digestive organs. Small and large intestine were with diffusely hyperaemic serosa and filled with gas (Fig. 3). Intestinal wall was very thinned with prominating intestinal content, and multiple petechiae could be observed beneath the serosa. The intestinal content was light brown, mixed with undigested feed particles and gas bubbles. The intestinal mucosa was diffusely hyperaemic, spattered with striated haemorrhages in the area of the ileum, caecum and colon. Mesenteric lymph nodes were enlarged and juicy, with hyperaemic cores. The abomasum was strongly enlarged by gas, milk coagula and cloudy fluid. The mucosa was diffusely hyperaemic and oedematous in the area of plicae and lined with transparent mucus.

In cases of confirmed colibacillosis, microscopic lesions were found out in all studied samples collected from the abomasum, small intestine, mesenteric lymph nodes, kidneys, liver, spleen, heart and lungs. The abomasum showed surface epithelium desquamation and hyperaemia of *propria mucosae*, along with inflammatory oedema of the submucosa and muscles. The proximal small intestine had lesions of inflammatory degenerative type and specific neutrophilic and lymphocytic infiltration of the mucosa. Intestinal villi of small intestinal mucosa (duodenum, jejunum and ileum) were thickened at a various extent due to inflammatory proliferative changes, which were most prominent in the jejunum. Desquamated epithelial cells were shed in crypts and intestinal lumen (Fig. 4). Some of capillaries and arterioles in the submucosa demonstrated

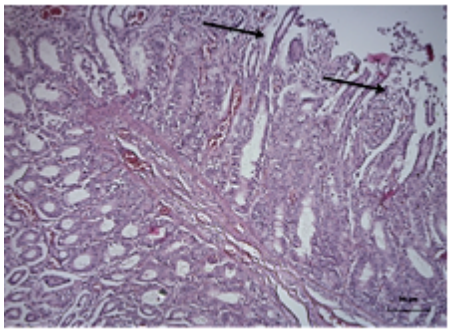

**Fig. 4.** Catarrhal desquamative inflammation of the mucosa and intensive vascular hyperaemia in the submucosa (arrows) in the duodenum of a calf with, colibacillosis H/E, Bar=10 μm.

bacterial thrombi. A particularly pronounced inflammatory oedema was found out in submucosal and subserosal spaces in the distal small intestinal compartment.

Observed histopathological changes in the different digestive tract compartments in calves with cryptosporidiosis comprised mainly desquamative catarrh of intestinal mucosa and atrophy of part of intestinal villi. They were with rounded tips, some of them assumed a ball-like shape and were fused. The mucosa was diffusely hyperaemic, with extensive haemorrhages at some areas. Oedema and vascular hyperaemia were observed in the submucosa and muscles. An usual finding was the diffuse infiltration of both mucosa and submucosa with neutrophils in the distal parts of small intestine, caecum and colon. Ileal crypts contained numerous developmental forms of the agent (*Cryptosporidium parvum*) (Fig. 5). Mesenteric lymph nodes exhibited diffuse hyperaemia affecting the core and the medulla.

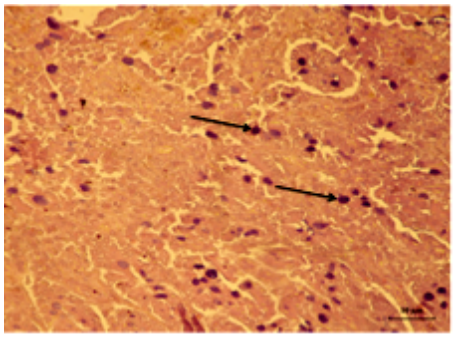

**Fig. 5.** Multiple developmental forms (arrows) of cryptosporidiosis in the ileum crypts, calf with cryptosporidiosis, H/E, Bar=10 µm.

## DISCUSSION

The analysis of data from epidemiological, etiological, gross anatomy and histopathological examinations in neonate and juvenile calves in 17 cattle farms in 9 regions of the country provided information in two research aspects. First, they showed the commonest causative agents of gastrointestinal diseases in calves at an early age and second, determined the morphogenesis of observed macro- and microscopic lesions of these disorders. In our opinion, the onset of diarrhoea in neonate calves is largely associated with the lack of direct transfer of maternal antibodies during dam's pregnancy, which makes offspring susceptible to various respiratory and digestive infections. The majority of cattle farms do not vaccinate cows during the dry period against the agents causing enteritis in calves, which is important for the immune status of animals at the farm and for provision of colostrum with rich antibody content for neonates (Lyutskanov *et al*., 2011; Castro-Hermida *et al.,* 2002). Thus, we agree with the statement of authors that NCD is a multifactorial disorder caused by numerous predisposing factors as hygiene at rearing premises, farm management, nutrition, herd immunological status and presence of microbial pathogens (Lyutskanov *et al*., 2011; Izzo *et al.,* 2011; Lyutskanov, 2013;). Established etiological agents *Cryptosporidium parvum* and *E. coli K99 (F5)* agree with other reported findings (Duda-madej and Gosciniak, 2013), having found the same enteropathogens in 80% of studied samples from diarrhoeic calves. This allowed assuming that the infectious factor was the leading one in gastrointestinal diseases in calves. We agree with the already reported data that *E. coli F5 (K99)* are responsible for more than one-third of NCD at the earliest age, as our investigations showed that *E. coli F5* were the only bacterial agent isolated from those causing diarrhoea in calves (Hur *et al*., 2013; Malik *et al*., 2013).

The results from gross anatomy and histopathological examinations on carcass material and tissue samples from calves with NCD deserve a special attention. Observed specific features of diarrhoeic staining of carcasses could be used as guideline in future pathoanatomical diagnostic work. In support of this, we could note some differences in the colour of diarrhoeic faeces – yellow-greenish in colibacillosis and brownish to bloody, mixed with food particles in cryptosporidiosis.

The extent of observed lesions varied both among the different gastrointestinal tract compartments, and between each of diagnosed disorders (colibacillosis, cryptosporidiosis, corona- and rotaviral infection). Macroscopic gastrointestinal changes in *E. coli* infections were manifested by catarrhal, sometimes catarrhal-haemorrhagic enteritis, most pronounced in proximal small intestine. Common findings in all carcasses with colibacillosis were sugillations and petechiae on the epicardium and endocardium, accompanied by haematomas on bicuspid valves of the left ventricle and hyperaemia in both renal medullae. These findings confirmed lesions described by other researchers in 41 calves with colibacillosis (Zhelev *et al*., 1968; Gerov *et al*., 1978; Angelov & Karadzhov, 1978). Observed macroscopic digestive tract changes in cryptosporidial enteritis allowed outlining some differences vs colibacillosis. They comprised mainly diffuse hyperaemia of small intestinal serosa along the entire length, with pronounced caecal and colonic meteorism while the other organs and systems were only slightly affected. The caecal content in cryptosporidiosis had a specific brownish colour and was mixed with gas bubbles unlike that in other types of enteritis. Studied samples from calves with colibacillosis revealed thickening of duodenal, jejunal and ileal intestinal villi, with colonies of *E. coli* at some sites on epithelial cell surface as specific features. On the basis of our results, we agree with what other authors have affirmed so far: that alterations in the middle compartment of small intestine (jejunum) were prevailing, with highly intensive desquamative necrobiotic processes in epithelial cells (Angelov & Karadzhov, 1978; Evelyn, 2008).

The analysis of histological samples in cases of *Cryptosporidium parvum* infection demonstrated extensive atrophy and rounding of villi, responsible for their ball-like shape in the area of the ileum and proximal colon as specific microscopic lesions. Sometimes, the etiological agents (*Cryptosporidium parvum*) attached to villous surface could be observed, leading to desquamation of the epithelium and its shedding in the intestinal lumen. Similar cryptosporidiosis lesions have been reported by other researchers as well (Evelyn, 2008; Hur *et al*., 2013).

In our investigations on these two diseases, cryptic epithelium was affected by cryptosporidial infection, as ileal crypts showed many developmental forms of the protozoan *Cryptosporidium parvum*. Their presence could be used for confirmation of diagnosis made on the basis of results from preliminary antigenic and parasitological analyses (Lanz *et al*., 2008; Nguyen & Vu-Khac, 2011). Our results suggested that formed inflammatory infiltrations in the mucosa and submucosa in these enteric disorders could be important from differential diagnostic point of view. In *E. coli* infections, lymphocytes were mainly prevailing, unlike findings in cryptosporidiosis, where only neutrophils have been observed.

## CONCLUSIONS

The analysis of research literature and discussion of own results allowed concluding that the established macroscopic and microscopic lesions in the gastrointestinal tract of calves with neonatal diarrhoea (colibacillosis and cryptosporidiosis) could definitely have a diagnostic and differential diagnostic value in these problematic disorders. The primary macroscopic and microscopic lesions in cases of NCD are concentrated in the digestive system, mesenteric lymph nodes and some parenchymal organs: liver, kidneys, and heart in the septicaemic form of disease. In complicated NCD with involvement of multiple etiological agents, the course of disease is more dynamic, and observed lesions are more pronounced and characterised with variability of expression site.

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