Summary

Keywords: *Giardia spp.*, *Cryptosporidium spp.*, *Eimeria spp.*, Prevalence, Seasonal Dynamics, Mcmaster, Miniflotac, Immunofluorescence, Composite samples

Doctoral thesis entitled "Prevalence and risk of zoonotic parasitic intestinal protozoa in young cattle in the region of Moldavia, Romania" was developed within the Doctoral School of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" University as part of project "Improvement and development of human resources for research and innovation in the Doctoral School, HRD / CPP107 / DMI1.5 / S / 77222", co-financed from the European Social Fund through the Ministry of Labor, Family, and Social Protection or authority Managing Operational Programme Human Resources Development (MASOPHRD) within the Sectoral Operational Programme for Human Resources Development 2007 -2013.

The aim of the research was to conduct a comprehensive study about the protozoan parasites in calves from Moldavia. The objectives were satisfied: to fulfill the purpose defined were:

Sampling from cattle farms

Comparative evaluation methods in terms of parasitological examination of feces for protozoa parasite identification: direct smear examination (with or without staining)

Willis technique

Ziehl-Neelsen technique

Giemsa stain

Immunofluorescence technique

McMaster technique

Flotac technique

MiniFlotac technique

To develop new working protocols for achieving cost reduction and increased efficiency;
For improve our knowledge and continuous improvement of them we acomplish an internship in the laboratory of parasitology from the Faculty of Veterinary Medicine, Ghent University;
Making map work and application methods in Romania and Belgium

Following the headline targets drawn from our studies the following result with a degree of novelty as results of studies undertaken:

– Improve sampling methods to eliminate the risk of contamination of samples and decrease material costs and working time

– Completion and modification of protocols for analysis

– Develop and implement new methods of diagnosis.

The paper was write during four years, between 1.10.2010 – 30.09.2014 and is in accordance with the legal provisions in two main parts: the first part is represented by The current state of knowledge which includes 40 pages and represents 30% of the content of the paper and the second part, Personal contributions with 131 pages which represents 70%.

The research of this thesis has been recovered by the publication as first author of three scholarly articles in journals in international databases, the final results presented in the last two chapters are the subject of an article "in press", entitled: "The Effect of different detection Methods and pooling of samples on the detection of protozoan Infections in Housed calves'.

The content of the first part, the Current state of knowledge, is divided into two main chapters that briefly reporte the information from national and international literature on the subject of this paper work: the relation between the function an causality of the morphological particularities, physiological, pathophysiological, immune, feeding and housing, of young calves and the evolution of protozoa in calves

Monogastric digestive system of the calf at birth undergo modifications, changes and developments to the partitioning until becomes ruminant with pregastric compartments (rumen, reticulum, omasum and abomasum). The production mechanisms of diarrhea are presented as the main pathophysiological process that produces significant economic losses in addition to respiratory disease. A summary of the processes involved in specific and nonspecific immunity in defense against parasites is exposed. Placenta and immunoglobulins from colostrum have an important function in the development of future immune sistem of the organism Different ways

of maintenance, feeding and growth of young cattle that have influence in the transmission, evolution and prevention parasitosis are presented at the end of the first chapter.

In Chapter II, Methods for identifying intestinal protozoa in young cattle, of the first part was done a bibliographic study on standard methods used in the diagnosis of parasitic protozoa. Methods were both qualitative and quantitative. The most accessible and popular method used for detection of vegetative forms of flagella or cilia; of coccidia is direct smear, with or without colorants or clarifying solutions. The protocol has been described as Cosoroabă et al., 2002. Willis technique described by Cosoroabă et al., 2002 and Mircean V. et al.., 2011.is suitable for identification of light eggs, wich float in hypersaline solution. For diagnosis of *Cryptosporidium spp.* are described as literature both Ziehl Neelsen modified by Henricksen (Iacob O. 2000) and Giemsa stain method (Cosoroabă et al., 2002). Immunofluorescence technique allows identification of *Giardia spp.* cysts and Cryptosporidium spp by examining with immunofluorescence microscopy. To acomplish our studies we used the kit Cryptosporidium / Giardia detection kit Merifluor firm. The technique is very easy to achieve and the identifying of the cysts is done by examining with objective 200x or 400x. The positive samples in the microscopic field are observed as formations with specific morphology and dimensions stained in fluorescent green on a red-orange background. Microscopic examination can be done by quantitative methods: McMaster, Flotac and MiniFlotac for *Eimeria spp.*. McMaster method is the most used method in veterinary worldwide parasitology, was developed to facilitate the diagnosis of parasitosis in sheep from New Zealand and is based on the principle of flotation parasitic elements. To facilitate drug efficacy studies innovative methods were developed, wich combine flotation with centrifugation (Flotac), flotation and translation or methods less time consuming and laboratory equipment isrequired -MiniFlotac and FillFlotac device. For these two methods the top of the solution to be examined is translated, which allows for easy examination without the presence of debris (remaining at the bottom of the chamber for examination) the sensitivity is increased in comparison with othe conventional methods, and thus is suitable to be used for achieving the efficiency of drugs and to conduct prevalence studies of different species of parasites.

The second part of this thesis, PERSONAL CONTRIBUTIONS, consists of six chapters followed by general conclusions:

Chapter III - PURPOSE AND OBJECTIVES motivates the theme of this thesis, the importance of the study and use of modern methods of identification of parasitic protozoa in cattle and the improvements of the clasical techniques.

Chapters IV and V presents the locations were studies has been done, sampling points and the pathogens studied. In the laboratory of Parasitology from Veterinary Medicine Faculty Iaşi were conducted the most of the research from Romania. The fluorescence examination was performedusing fluorescence microscope from the Laboratory of Immunology and Virology of the Department of Veterinary and Food Safety Health Iasi. The samples included in the studies, carried out over the four years, were from entire part of Romania but especially from Moldavia. Different maintenance methods, harvest time and different methods used for examination have led to varied results also validated by international literature. The study in between October 2010-March 2013, analyzed 354 samples Willis method and obtained a prevalence of coccidiosis in calves of about 20%, the most common species: *Eimeria bovis* and *Eimeria zuernii*. (Tronciu et al, 2014).

In the second stage of the study in Romania 160 samples were collected and analyzed by the methods: McMaster (modified) MiniFlotac and IFA (modified). Performing in parallel the McMaster technique (modified) and MiniFlotac technique allowed a comparative study between these methods, validating the use of composite samples and determine the prevalence of coccidiosis, giardiasis and cryptosporidiosis in calves from farms studied. In farms with dairy cows, the calves are housed in individual bunks, located primarily outside the shelter. On family farms with extensive maintenance type, calves are housed in common pens inside the shelters. On beef cows farms calves may stay until weaning, with mothers. Both type of farm, extensive or intensive, with cows for meat or dairy, maintenance mode and feeding calves influence the evolution of parasitic protozoa. These results were presented in the articles entitled: The distribution of the parasitic complex of Eimeria, Giardia and Cryptosporidium in housed calves

from Moldavia, Preliminary research on the parasitic complex of Eimeria, Giardia and Cryptosporidium in young cattle from Northern Moldavia, and The prevalence of Eimeria species in dairy farms from Romania. Modified immunofluorescence technique allows establishing the EPG for *Giardia spp* and *Cryprosporidium spp.*

During January 2013-May 2013 period of travel has been completed, within the project "Improvement and Development of Human Resources for Research and Innovation by Doctoral School" Contract: HRD - CPP107-DMI1 / 5 / S / 77222, in Belgium, at the Faculty of Veterinary Medicine, University Ghent. Studies were performed in research team led by Professor Edwin Claerebout and under the supervision of Dr. PhD. Bruno Levecke. Here we consolidated knowledge of modified McMaster methods, MiniFlotac, immunofluorescence, composite samples. The coprologic material was recolted from 22 farms located in the western part of Belgium, in the Flanders region. Farms are focused on raising beef cows, predominant breed is Belgian blue and white, the cows are housed in pens situated in closed or opened shelter, in number of 8-10 individuals for each pen. Calves has unlimited access to colostrum and milk until weaning.

A total of 730 individual samples were analyzed between October 2010 to May 2014 through qualitative and quantitative methods.

Chapter V presents the incriminated etiologic agents, describing the etiology, diagnostic methods, treatment and risks of zoonotic or economic for:

Giardia spp.,

Cryptosporidium spp.

Eimeria spp.

Giardia spp. present the highest prevalence in calves up to six months aged and the zoonotic subtypes (A and B) are found in 20% of positive cases in calves. Studies show very high levels of protozoa spread in calves, sometimes, infestation can be at 100% rate. Biological cycle allows excretion of infested items after 72 hours from infestation. Trophozoites have pear shape, with dimensions of 12-15 µm long and 5-9 µm wide. Cyst are oval-shaped, approximately 5 to 7-10 mm diameter and presents four nuclei. The wall has a thickness of 0.3-0.5 µm and is composed of a network of filaments of 7 to 20 nm. Fixing trophozoites is via adhesive disc. High

pH makes *Giardia spp* trophozoites. to develop blisters, which are not normally observed in trophozoite. The cyst is oval shaped and contains two or four nuclei, basal body and axonema are located much closer to the nuclei and fragments of adhesive disc. Pathogenesis in *Giardia spp.* should be regarded as a multifactorial process including both parasite characteristics, and immune response. Destruction of the brush border microvilli, and decrease the efficiency of enzymes, in particular lipases, certain proteases, and lactase also maltose is given by the toxins of the trophozoites. Diffuse destruction of microvilli leads to loss water, electrolytes and nutrients absorption in the small intestine. The combined effect of this reduction and enzyme deficiency results malabsorption, diarrhea, and decreased body weight score. Decreased lipase activity, and increased production of mucus can explaine the steatorrhea described in hosts infected with *Giardia spp.* (Lujan et al., 2011). The main clinical manifestation is diarrhea that does not respond to antibiotic treatment or nor at the coccidiostats. Elimination of pasty feces with mucous appearance reveals giardiasis, especially when diarrhea occurs in young animals.

Laboratory diagnosis is made by identification of trophozoites or cysts in feces, cysts can be colored, often with iodine. IFA kits is using antibodies anti cystic protein contain by the cyst walls. The most sensitive diagnostic method is PCR that was originally used to typing and identification of different species of *Giardia spp* .. Theoretically, the detection limit of the PCR is a cyst, which allows increasing significantly diagnostic sensitivity. (Geurden T., 2007). Production and role of anti - *Giardia spp.* immunocompetent mice infected with *Giardia muris* produce antibodies against *Giardia muris* trophozoites. It is an evidence that antibodies play an important role in immunity. It was shown that with the introduction of *Giardia lamblia* trophozoites in the duodenal lumen of rats' specific IgA apear. Fenbendazole is a greater efficiency in the oral treatment of giardiasis at a dose of 15 mg per kg for 3 consecutive days. If treatment with Fenbendazole no change is observed fecal consistency, and the disappearance of clinical symptoms. Also is interrupted and intermittent removal of the cyst. However, *Giardia duodenalis* infestation is less severe as compared with coccidiosis, which may cause fatal form of the disease. The persistence of the disease in calves infected groups can take up to 6 months, suggesting that *Giardia duodenalis* has a potentially large impact on cattle farms. (Appelbee AJ

et al 2003, Santina M. et al., 2012) Differences in body growth in calves treated for *Giardia spp.* eere about 3 kg within 4 weeks. (Geurden T. et al., 2010)

Albendazole, along fenbendazole can be used to treat calves. Contributing to the removal of trophozoites, the fenbendazole help the microvilii of brush border to recover at 7 days after treatment. Even if using an effective treatment, reinfection are common if the source of contamination persist in the environment or collectively. Colostrum and milk with anti-Giardia antibody, protectes young calves, and thus there is the potential for the production of a vaccine. (ME Olson et al., 2004)

Due to an increased prevalence of both *Giardia spp* and *Cryptosporidium spp.* in cattle farms worldwide zoonotic transmission risk is very high. *Giardia spp.* shows host specificity with the two zoonotic subtypes found in dogs, cats, calves and wildlife. There is the possibility to encounter mixed infections with *Cryptosporidium spp* whose main host remains calves. Children represent a group at high risk; poor hygiene has been shown to be a crucial factor. *Cryptosporidium spp.* and *Giardia spp.* can be transmitted through water, drinking water sources are included, waters used for recreation as well as lakes and rivers. Diarrheal episodes may occur if contamination with *Giardia spp.*is produced. .From the point of view of public health isolates collected from ruminants is morphologically and immunologically similar to *Giardia duodenalis* in humans. The greatest zoonotical risk represin the subtype A and subtype B. Studies show that approximately 20% of calves are infected with subtype A. This leads us to evaluate the zoonotic risk, either by direct contact or through contamination of surface waters. (Andrew R. C. Thompson, 2000). Molecular biology studies (PCR) identified zoonotic genotypes of *Giardia spp.* in cattle farms. (Trout JM et al., 2006; O'Handley RM et al., 2000)

Criptosporidium spp. infections have been reported around the world. From the perspective of human health, cattle were often involved as a source of contamination by Cryptosporidium species. (G. Ortega et al., 2009). Calves can be infected by oro-fecal contamination but is possible by vector path (tools, caretakers, birds or arthropods). At an early age calves main source of contamination can be represented by dam. On dairy farms, where calves are reared in individual bunks the infestation level is smaller compared to beef farms, where technological system involves accommodation of the calves with dams. Three species of *Cryptosporidium*

have been identified in calves: *C. parvum* (59.4%), followed by the *C. bovis* (20.3%) and *C ryanae* (9.8%). Mixed infections *C. parvum / C. bovis* and *C. parvum / C ryanae* accounted for 9.8%. (Josephine Su Yin, 2012). *C. parvum* is responsible for about 85% of infections in unweaned calves and only 1% in those weaned. Schizogony starts by ingesting infective oocysts containing four sporozoites and a residual body. Once inside the host organism under the influence of gastrointestinal juices, the oocyst wall releases the sporozoites. Unlike other coccidia that invade enterocytes, they produce fusion and microvilli expansion resulting double membrane surrounding the parasite. (Hanna Borowski et al., 2008). The macrogametocit creates a macrogamet and a zygote, which is converted in the intestinal lumen, into oocyst, which is sporulated by fecal elimination and is containing four sporozoites. Oocysts are round or slightly oval, medium size is 5µm, with a double membrane, while the four sporozoites are banana-shaped bacillary or of about 1µm, marginal place in U shape (Mitrea IL, 2002)

Microvilli atrophy is caused by the destruction of enterocytes and the retraction of microvilli in an attempt to maintain the continuity of the epithelial barrier. Cryptic hyperplasia also appears as an effort to replace damaged epithelial cells. Thus absorption is altered due to the disappearance of mature enterocytes in the microvilli and their transporter function .the distruction of the cells from extreme absorptive microvilli, and increased secretion of chlorine in the crypts leads to increased secretion. At the same time the total area of absorption decreased. (Andrew S et al 2005)

Clinical symptoms are diarrhea and malabsorption. Diarrhea can be mild to severe watery or with mucoid yellow feces. Calves with severe disease may require longer periods up to several weeks to recover. It is believed that the pathogenicity is diminished in older calves because of immunological processes intervention. (Geurden T., 2007) Either directly or stained microscopic examination can be done. The most used methods are the methods Ziehl-Neelsen staining and staining with carbolfuchsin. The morphological differences between cryptosporidiosis species are indistinguishable in the microscopic field and other components may occur that lead to a misdiagnosis. Compared with microscopic examination, the IFA and PCR techniques offers high sensitivity even at low concentrations of oocysts. The only drug in Romania, registered for the treatment of cryptosporidiosis in calves, is halofuginone lactate (HALOCUR, Intervet). The

therapeutic dose varies depending on the weight and purpose. If is used for prophylactic treatment on calves weighing 35-45 kg the daily dose is 8 ml with application for 7 days, beginning with the first 24-48 hours after birth. The calves weighing between 45-60 kg are recommended to receive 12 ml HALOCUR. (I. Kalman, 2010; Andrew S et al. 2005)

Bovine eimeriosis (known as red diarrhea) is produced by various species of *Eimeria.* The most pathogenic are. *E.bovis* and *E.zuernii*. Contamination is achieved by oral route after ingesting oocysts with water or food. Biological cycle have two multiplying phases, sexual and asexual and coincide with oocysts entering the digestive tract, where under the action of trypsin and bile the oocyst were they are exchistate, eliminating sporozoites. Clinical coccidiosis is most often caused by *Eimeria bovis* and *Eimeria zuernii* the gametes of these two pathogenic species produce lesions in the large intestine, causing severe diarrhea or even death. (W.C.A. Cornelissen et al., 1995). Lesions are most destructive in the last 2 meters of ileum on day 19 post-infection. The microvilii were shortened, the peaks being ulcerate, lamina was bare and covered with fibrin.

Clinical symptoms appear after a 20 days long incubation o, with different stages. Animals with clinical forms of coccidiosis present delays of raise, and the threat to become economically unprofitable. Even subclinical coccidiosis, otherwise less obvious for farmers or veterinarians can affect gut physiology and therefore decrease growth and feed conversion of animals. It is accepted that subclinical eimeriosis is more damaging economically because of higher recurrence. (Daugschies A. et al., 2007). Clinical trials concluded that the use of toltrazuril for coccidiosis prevention is better than Diclazuril, sulfamidin or amprol. (HC Mundt et al., 2005; Mohamed M. Ghanem et al., 2008).

Chapter VI presents the methods used for research with the changes made in order to facilitate the identification ofintestinal protozoan parasites in young cattle. The technique used, McMaster was modified to achieve a higher sensitivity factor (50 EPG). Unlike the method described Cosoroabă (2002) and Mircean V (2010) the amount of feces used was 4 grams.

MiniFlotac technique was developed by researchers of the University Federico II of Naples by simplifying method Flotac, the method is based on flotation and translation the upper part of solution. The standard operating protocol provided by the manufacturer recommends the

use of hypersaline and sugar solution for identifying oocysts of intestinal protozoa in the feces of calves. Because the sugar solution has higher viscosity and when translating upper part of solution occur, with a higher incidence, air bubbles than in hypersaline solution, the amount of solution examined can be modified.

Immunofluorescence test for identifying Giardia and Cryptosporidiosis is based on using the kit sold by Merifluor Company,"*Cryptosporidium / Giardia kit"* (Meridian Diagnostics Inc., Cincinnati, Ohio) and by modify the method by Xiao and Herd (1993). By standardizing quantities used a qualitative test is obtained with sensitivity factor of 50 EPG.

Data about the prevalence of protozoa, presented in Chapter VII of the thesis, in the eastern part of Romania, were obtained by conducting two studies in different periods, 2010-2011 and 2013-2014, which were based on principles of identifying methods, both classic and modern. Prevalence of intestinal parasitic protozoa was obtained by analysis of three genera: *Eimeria spp., Giardia spp.* and *Cryptosporidium spp* with different methods.

Prevalence of coccidiosis in cattle farms is constant, both in Romania, Moldova and in Flanders, Belgium. By using Willis technique, the prevalence coccidiosis was about 20%, but this is a low sensitivity qualitative method. After analyzing the samples harvested from the 16 farms in Moldova through different methods we conclude that the McMaster method reveal a 36.87% prevalence and MiniFlotac 38.12%.

Data obtained during October 2010 to March 2011 were used in carrying out the paper: "The prevalence of *Eimeria spp* in dairy farms from Romania" published in the Journal of Scientific Works of the Faculty of Veterinary Medicine, Iasi, 2014 Vol. 57 (1 -2): 151-154,

For the prevalence giardiasis, carried out from July 2013 to May 2014, we used calves aged between one and five months. Of the 140 individual samples collected from farms 63 calves were positive, which means a prevalence of 45%. Prevalence *Giardia spp.* was influenced by housing method, the calves from the farms with collective pens were positive at rate of 42.66% comparing with the calves from individual pens which had a higher rate of infestation: 47.69%.

Cryptosporidiosis is one of the diseases of young cattle clinically manifested by producing diarrhea and metabolic imbalances. Like *Giardia spp.* infestation varies between 1-60%. Up to 90% of farms are positive for *Cryptosporidium spp.* Differences causes are polyfactorial: diagnostic techniques, age, number of calves, farm management, disinfection. Of the 140 individual samples collected from a total of 12 calves were positive, which means a prevalence of 8.57% of positive samples, but especially because of age calves, the disease being most common in calves to 8- 14 days. Depending on housing of calves in individual pens and collective the results were: of the total of 75 samples from four farms with collective pens (5.33%) were positive. On the other side of the total of 65 samples from farms with bunks or individual pens 8 (12.03%) were positive.

Sampling points were visited between July 2013 and May 2014. The results show that in the winter months appear elevated levels of giardiasis (61.62%), the 37.2% coccidiosis is constant throughout the year, and Cryptosporidium infestation spp. show the lowest level (8.13%) of protozoa studied.

Preliminary data obtained during the conduct of the research were presented at the Review of Scientific Works Facultăţii of Veterinary Medicine, Timisoara Vol. XLVII (3), 120-124 - 2014 ISSN - 1221-5295, with "Preliminary research on the parasitic complex of *Eimeria, Giardia* and *Cryptosporidium* in young cattle from northern Moldavia "

The increased prevalence of intestinal parasitic protozoa in calves, confirmed both by the literature data and the data obtained from our research urged us to attempt improving existing methods (by increasing sensibiltate factor), but also an attempt decrease in costs. Article with data about the prevalence of parasitic protozoa in calves in Moldova was presented at the 53rd Scientific Symposium of the Faculty of Veterinary Medicine, entitled: "Towards a Global Health" published in Review of Scientific Works of the Faculty of Veterinary Medicine, Iaşi, 2014 Vol. 57 (1-2): 146-150, entitled "The distribution of the parasitic complex of *Eimeria, Giardia* and *Cryptosporidium* in calves housed from Moldavia"

To achieve flotation methods the only cost are water and salt consumption (excluding initial acquisition flotation chambers). Making composite samples is another way that diagnosis is made quickly and easily. These improvements favor both specialized staff from laboratories and farmers by decreasing costs and waiting time. Previous studies have shown that Flotac has the highest sensitivity 66.7% compared with 41.7% McMaster to detect eggs strongyls gastrointestinal composite samples. It requires completion of literature studies on the possibility of using newly developed methods (especially MiniFlotac®) in the diagnostic parasite in cattle. (Boscol et al., 2014)

Chapter VIII presents improvements to the methods of collection and analysis of fecal samples to identify intestinal protozoa in calves. The increased prevalence of intestinal parasitic protozoa in calves, confirmed both by the literature data and the data obtained from our research urged us to attempt of improving existing methods (by increasing the sensitivity factor), but also an attempt decrease in costs. To achieve flotation methods only cost water and salt consumption (excluding initial acquisition flotation chambers).Using composite samples are another way to make diagnose faster and easily. These improvements favor both laboratories specialized staff and farmers by decreasing costs and waiting time of the results. Previous studies have shown that Flotac has the higher sensitivity 66.7% compared with 41.7% McMaster for detecting gastrointestinal strongyls eggs in composite samples. It requires completion of literature datas on the possibility of using newly developed methods (especially MiniFlotac) in the parasitic diagnostic in cattle. (Antonio Boscol et al., 2014)

MiniFLOTAC is also a method based on the buoyancy of parasitic elements. This innovative method was developed by researchers at the University Federico II of Naples, Italy and simplification method derived from FLOTAC ©. Besides buoyancy the method use the translation of the upper part of the examined solution. Usually infestation intensity, determination is made by the individual analysis of the samples, but it is time and material consuming, being very expensive when a major study is performed. An alternative is the analysis of composition samples of different sizes. A common practice is that the farmer gives treatment to the calves against coccidiosis, but without any parasitic status check, either before or after

treatment. Causes of this practice could be the increased price of individual analise and waiting time. Using composite samples will allow dropping examination price and so being more available to farmers and veterinarians practitioners. Data were analyzed using Excel; statistical values were calculated 2 "Pearson coefficient" and "P value". In most cases the Pearson correlation index values ranged between 0.900607 and 0.999809. When we have a close relationship between the two variables, with positive direction and form strong positive linear association exists.

Particularly the comparison chart between the average individual values analyzed by McMaster and 10 composite sample resulting from the same samples, and the P value is 0.0000182 pearson factor is 0.90175303. Compared with the results obtained from the comparison between the average individual values analyzed by MiniFlotac and 10 composite sample resulting from the same samples where both P value is 0 and pearson coefficient is 0.981194479 follows that the sample preparation composite of 10 individual samples results that MiniFlotac is recommended to be used for composite samples. A different situation arises if two samples commpozite 10 individual samples for *Giardia spp.* Pearson coefficient and P value of 0.02235909 0.565754138 being a reasonable correlation is positive even if compared with P5 *Giardia spp.* Where Pearson coefficient is 0,900607 and P value is 0. Thus P5 is recommended for composite samples *Giardia spp.* in cattle analyzed by IFT. If cryptosporidiosis poolings both P5 (0.999809) and the P10 (0.999974508) have values close to the maximum in this case the result may indicate a very high correlation with a very close relationship between variables or indicate an error. Given that the prevalence of cryptosporidiosis was very little we can not say that it (P5 / P10) is a reliable method. It is recommended to test the sampling recovery from calves younger than one month to get a higher number of positive samples.

Conclusions:

1. Prevalence of eimeriosis in the 36 dairy and beef farms is 20.6% according to the results of the study between October 2010 and March 2011. The predominant species were *Eimeria bovis 12,99%*,and *Eimeria zuernii 3,38%* of the total samples

2. The average prevalence of coccidiosis in the 16 dairy farms in Moldova is 37%, according to results from the study conducted during July 2013 to May 2014.

3. The prevalence of giardiasis in the 16 dairy farms in Moldova is 45%. Depending on the maintenance of calves, in individual pens or common ones, the prevalence was 5.03% higher for individual maintenance (47.69%) than 42.66% in calves in common pens.

4. Prevalence of cryptosporidiosis on calves aged between one month and five months in farms where they collected samples was 8.57%. As in the case of giardiasis, calves in individual housing systems showed a higher prevalence (12.03%).

5. Eimeriosis is a constant diseases in farms across Romania with small seasonal fluctuations influenced also by the method of diagnosis used.

6. Giardiasis calves occurs in winter at a rate of 61,62% and in spring at a rate of 27,77%

7. Seasonal dynamics of cryptosporidiosis shows that the disease evolves mainly in spring (13, 88%).

8. MiniFlotac is an innovative method with higher sensitivity than McMaster method.

9. The method of composite sample works for both composite samples of 5 individual samples and composite samples consisting of 10 samples.

10. When infestations have lower levels is recommended to use diagnostic methods with high sensitivity (MiniFlotac, Flotac).

11. The method of composite sample 5 has a higher sensitivity than the method of composite samples 10 and MiniFlotac method is recommended because conclusive results are obtained compared to the McMaster method.

12. Identification of *Giardia spp.* by immunofluorescence method can be done also using composite samples method of 5 or 10.

13. Prevalence of cryptosporidiosis had the lowest values compared to eimeriosis and giardiasis.


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