

**BANAT UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY
MEDICINE TIMIȘOARA**

FACULTY OF VETERINARY MEDICINE



**CONTRIBUTIONS IN THE KNOWLEDGE OF BIOLOGY,
EPIDEMIOLOGY, DIAGNOSIS AND CONTROL OF
CRYPTOSPORIDIOSIS IN WESTERN ROMANIA**

PHD THESIS

Scientific coordinator
Prof. dr. DĂRĂBUȘ GHEORGHE

PhD Student,
IMRE KÁLMÁN

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**PhD Thesis
for PhD title in veterinary medicine**

Scientific coordinator,
Prof. dr. DĂRĂBUȘ GHEORGHE

PhD student,
Dr. med. vet. IMRE KÁLMÁN
Parasitology and Parasitological Diseases Department

2010

The thesis contains:

Summary (romanian, english)
Abbreviations
Introduction
General part 68 pages;
Special part 128 pages;
Tables: 49;
Figures full color: 91;
Bibliography: 570 de bibliographical sources.

SUMMARY
OF PH.D. THESIS NAMED

**CONTRIBUTIONS IN THE KNOWLEDGE OF BIOLOGY, EPIDEMIOLOGY,
DIAGNOSIS AND CONTROL OF CRYPTOSPORIDIOSIS IN WESTERN ROMANIA**

The paper is structured in two parts: first part – *Bibliographic study* and the second part – *Own researches*.

The first part contains a short introduction and two chapters:
CAP.1 Etiology and biology of genus *Cryptosporidium*;
CAP.2 Epidemiological aspects in cryptosporidiosis

In the **first chapter** the information from literature regarding the etiology and biology of genus *Cryptosporidium* are actualized. Historical and taxonomical dates and the naming of the 19 valid *Cryptosporidium* species in mammals, birds, reptiles and fish are presented. Other information presented in this chapter is about the parasite-host co evolution and host adaptation of *Cryptosporidium*, implications for taxonomy and criteria used for naming the *Cryptosporidium* species. Informations regarding the life cycle of *Cryptosporidium* in mammals, reptiles, fish and birds and the resistance of the genus at physical and chemical agents are presented, respectively.

In the **second chapter** epidemiological dates regarding the sources and routes of infection, the modalities and mechanisms of transmission and possibilities of dissemination of *Cryptosporidium* in animals and humans are presented. The factors that induce the receptivity and information about molecular epidemiology are also described. This last subchapter contains dates regarding the recent evolution of epidemiological molecular methods in cryptosporidiosis and the distribution of species, genotypes and subtypes of *Cryptosporidium* genus in farm animals and humans.

The second part contains 8 chapters in which refers regarding the first part, are made:

CHAP. 3 Determination of prevalence using ELISA method, mapping of cryptosporidiosis in animals from Western Romania, establishing the associations with other enteropathogens;

CHAP. 4 Epidemiological investigations using ELISA on *Cryptosporidium* parasitism at human in Western Romania;

CHAP. 5 Morphometrical studies on direct smears with *Cryptosporidium* oocysts obtained from heifers calves;

CHAP. 6 Immunohistochemical detection of spontaneous *Cryptosporidium* spp. infection at calves;

CHAP. 7 Characterization of some *Cryptosporidium* species at animals and humans using molecular biology techniques;

CHAP. 8 Evaluation of some diagnosis methods in cryptosporidiosis

CHAP. 9 Testing of therapeutic and/or prophylactic efficacy of some nutritional supplements and drugs in natural and experimental infection with *Cryptosporidium* at calves;

CHAP. 10 General conclusions and recommendations.

In **chapter 3** are presented the results of epidemiological investigations, using ELISA technique, of the prevalence of cryptosporidiosis in association with other enteropathogens in some Counties from Western Romania, in different species. This chapter is composed from four subchapters:

- Determination of prevalence of bovine cryptosporidiosis in association with other enteropathogens using ELISA method in Western Romania;
- Determination of prevalence of swine cryptosporidiosis in association with other enteropathogen agents using ELISA in some Counties from Western Romania;
- Epidemiological study using ELISA on the parasitism with *Cryptosporidium* spp. in association with other enteropathogens at lambs;
- Determination of prevalence of cryptosporidiosis using ELISA method at other animals species;

❖ In the first subchapter informations about the prevalence of **bovine** cryptosporidiosis in association with other enteropathogens (rotavirus, coronavirus, *E. coli* F5) in Arad, Bihor, Caraș-Severin and Timiș Counties are presented.

• The study named *prevalence of cryptosporidiosis in association with other enteropathogens at calves in Arad County* was carried out between July 2007 – January 2009, on 130 calves aged between 3 days and 1 year, from nine localities of Arad County. The calves were from five dairy farms (localities: Utviniș, Fântânele, Zimandu Nou, Curtici și Sebiș) and from four micro farms (localities: Nădlac, Pâncota, Vărădia de Mureș și Chișinău Criș) from different areas of Arad County. The collected samples were examined using ELISA technique in the Immunodiagnostic ELISA laboratory at Parasitology and Parasitological Diseases Department of the Faculty of Veterinary Medicine, Timișoara. The BIO-X EASY-DIGEST (BIO K 151-Bio-X Diagnostics, Belgium), which is an antigenic „in vitro” diagnosis kit for bovine faeces and uses the principles of ELISA double-sandwich was used.

On the whole, the study of fecal samples, using ELISA, regarding four enteropathogens involved in calved diarrhea in Arad County showed that:

- *Cryptosporidium* spp. was identified in 74 samples out of 130 analyzed (54 cases as unique pathogen agent and 20 cases in association with other enteropathogens);
- coronaviruses were found in 20 samples out of 130 analyzed (two cases as unique pathogen agent and 18 cases in association);
- rotaviruses were found in 19 samples out of 130 analyzed (eight cases as unique pathogen agent and 11 cases in association);
- *E. coli* F5 enteropathogen was not identified.

The most receptive age category for infection with *Cryptosporidium* as unique pathogen agent was between 5 and 7 days and 8 and 14 days respectively.

This study revealed that in bovines aged between 3 days and one year, the **cryptosporidiosis**, evaluated using ELISA method in national premiere, developed in all 9 units investigated, having a prevalence of **56,9%** (41,5% as unique pathogen agent and 15,4% in association with other enteropathogens).

In the same age category **coronaviriosis** had a prevalence of **12,3%** (2% as unique pathogen agent and 10,3% in association with other enteropathogens). The **rotaviriosis** prevalence in young calves examined was **14,6%** (6,1% as unique pathogen agent and 8,5% in association with other enteropathogens). The infection with *E. coli* F5 enteropathogen was not notified.

It was observed that coronaviruses involved in the etiology of diarrhea, at age category 5 days-5 months, has an associate evolution with *Cryptosporidium*. Significant differences regarding the distribution of rota- and coronaviruses in age categories investigated were not observed.

• Another study from this subchapter was *the prevalence of cryptosporidiosis in association with other enteropathogens, at calves, in Bihor County*. 110 calves, aged between one day and 60 days, from seven localities of Bihor County, were investigated from April 2007- January 2009. The samples were examined using ELISA kit BIO-X EASY-DIGEST (BIO K 151-Bio-X Diagnostics, Belgium).

On the whole, the ELISA study regarding the weight of pathogen agents involved in neonatal diarrhea in calves in Bihor County revealed that:

- *Cryptosporidium* spp. was identified in 34 samples out of 110 analyzed (23 cases as unique pathogen agent and 11 cases in association with other enteropathogens);
- coronaviruses were identified in the faeces of eight calves out of 110 examined (five cases as unique pathogen agent and three cases in association);
- rotaviruses were found in 23 samples out of 110 examined (15 cases as unique pathogen agent and eight cases in association with other enteropathogens);

The most receptive age for infection with *Cryptosporidium*, as unique pathogen agent, was age category 8-14 days (30,4%), followed by age category 5-7 days and 15-21 days in equal proportions (25%). In young calves, in the first two months of life, **cryptosporidiosis** evaluated in all seven breeding units investigated in Bihor County, with a prevalence of **31,9%**.

In the same age category coronavirus had a prevalence of 7,2% (4,5% as unique pathogen agent and 2,7% in association with other pathogens).

The prevalence of rotavirus in the young calves examined was 20,9% (13,6% as unique pathogen agent and 7,3% in association with other pathogens).

Infection with *E. coli* F5 was not identified.

Cryptosporidium infection as unique pathogen agent varied between 0% and 30%.

The extensively and intensively of infection in calves from Bihor County was higher in age category 8-14 days. There were not semnalated significant differences regarding the distribution of rota- and coronaviruses in age categories investigated.

The frequency of rotaviruses was higher in diarrheic faeces with watery aspect and *C. parvum* was diagnosed in creamy diarrheic faeces.

- The study named *prevalence of cryptosporidiosis in association with other enteropathogens at calves in Caraş-Severin County* presents the results of ELISA investigations in 70 calves aged between two days and four months from four localities of this County. The same ELISA kit (BIO-X EASY-DIGEST - BIO K 151 Bio-X Diagnostics, Belgium) was used.

On the whole, the ELISA study regarding the weight of pathogen agents involved in neonatal diarrhea in calves in Caraş-Severin County revealed that:

- *Cryptosporidium* spp. was identified in 20 samples out of 70 investigated (5 cases as unique pathogen agent and three cases in association with other enteropathogens);
- rotaviruses were found in eight samples out of 70 analyzed (five cases as unique pathogen agent and three cases in association with other enteropathogens);

The most receptive age for infections with *Cryptosporidium* spp. as unique pathogen agent was between 8 and 14 days (46,6%) and 2-7 days (40%) respectively.

From the investigations carried out at calves in the first four months of life, from four farms in Caraş-Severin County, it was concluded that the major cause of diarrhea was the infection with *Cryptosporidium* spp.. In the four investigated farms with industrial breeding, **cryptosporidiosis** had a prevalence of **28,6%**.

Beside the infection with *Cryptosporidium*, rotaviruses are also a cause of morbidity, single or in associate infections. The infection with *Cryptosporidium* spp. as unique pathogen agent varied between 5,5% and 50%. The extensively and intensively of infection in calves from Caraş-Severin County was higher in 8-14 days age category.

The prevalence of rotavirus in young calves examined was 11,4% (7,1% as unique pathogen agent and 4,3% in association with other enteropathogens).

No infections with *E. coli* F5 and coronaviruses were identified.

- The determination of prevalence of *cryptosporidiosis in association with other enteropathogens in calves from Timiş County* was carried out from July 2007 until November 2008 on 110 calves aged between one day and seven months of life, from different areas of Timiş County. The diagnosis method used was ELISA, the BIO K 151 kit (Bio-X Diagnostics, Belgium).

On the whole the study of faeces using ELISA in Timiş County revealed that:

- *Cryptosporidium* spp. was identified in 47 samples out of 110 analyzed (27 cases as unique pathogen agent and 11 cases in association with other enteropathogens);
- coronaviruses were identified in 10 samples out of 110 investigated in all cases in mixed infections;

- rotaviruses were found in 17 samples out of 110 examined (six cases as unique pathogen agent and 11 cases in association with other three enteropathogens);
- *E. coli* F5 enteropathogen was found in two samples (one is monoinfection and one in association with other three enteropathogens).

The most receptive age for infection with *Cryptosporidium*, as unique pathogen agent, was between eight and 14 days (33,3%) followed by age category 22-30 days (29,1%). The results obtained showed that in young calves, in the first six months of life, the **cryptosporidiosis** developed in all five units from Timiș County having a prevalence of **42,7%**. In the same age category **coronavirosis** had a prevalence of **9,5%** and a evolution in association with other enteropathogens. The prevalence of **rotavirosis** in young calves examined was **15,4%** (5,4% as unique pathogen agent and 10% in association with other enteropathogens). The prevalence of **bovine colibacillosis**, determined by *E. coli* F5 enteropathogen, was **3,6%** (0,9% as unique pathogen agent and 2,7% in association with rotaviruses, coronaviruses and *Cryptosporidium* spp.).

The extensively and intensively of infection with *Cryptosporidium* spp. in cattle's from Timiș County is higher in 8-14 days age category.

- The first subchapter is finished with *epidemiological screening of the evolution of cryptosporidiosis in association with other enteropathogen agents in Western Romania*.

The ELISA study of faecies for the weight of enteropathogen agents, involved in neonatal diarrhea, in Western Romania relieved that:

- *Cryptosporidium* spp. was identified in 175 samples out of 420 analyzed (121 cases as unique pathogen agent and 54 cases in association with other enteropathogens);
- coronaviruses were found in 38 samples out of 420 analyzed (seven cases as unique pathogen agent and 31 cases associated);
- rotaviruses were found in 67 samples out of 420 examined (seven cases as unique pathogen agent and 33 cases in association with other enteropathogens);
- *E. coli* F5 enteropathogen was identified in four samples out of 420 examined (one case as unique pathogen agent and three cases in association).

Epidemiological investigations carried out in young calves, in the first six months of life, from four west Romanian Counties revealed a prevalence of **41,6% for cryptosporidiosis** (28,8% as unique pathogen agent and 12,8% in association with other enteropathogens).

In the same age category **coronavirosis** had a prevalence of **9%** (1,6% as unique pathogen agent and 7,4% in association with other enteropathogens).

The prevalence of rotavirosis in young calves examined was 15,9% (8% as unique pathogen agent and 7,9% in association with other enteropathogens).

The prevalence of bovine colibacillosis, produced by *E. coli* F5 enteropathogen was 0,9% (0,23% as unique pathogen agent and 0,67% in association with other enteropathogens).

From all the samples was investigated 32,6% were negative for all four enteropathogens tested. The extensivity and intensivity of *Cryptosporidium* spp. infection in bovines from Timiș County was higher at age category 8-14 days.

Age category 15-21 days was most receptive for infections with rotaviruses.

In evolution of coronavirosis was higher at age category 15-21 days.

❖ In the second subchapter are presented the results of epidemiological investigations regarding the prevalence of cryptosporidiosis in association with other enteropathogens at swines, determined by ELISA method, in Arad and Timiș Counties.

- In **Timiș County** the study was carried out in July - December 2008, on an effective of 104 piglets aged between one day and 47 days, from eight intensive farms. For ELISA investigations the kit BIO K 151 kit (Bio-X Diagnostics, Belgium) was used.

On the whole the ELISA investigations regarding the weight of enteropathogen agents involved in diarrhea in piglets in Timiș County revealed that:

- *Cryptosporidium* spp. was identified an a number of 32 samples from 104 analyzed (27 cases as unique pathogen agent and 5 cases in association);
- coronaviruses were found in one sample in association with *Cryptosporidium*;
- rotaviruses were found in 16 samples out of 104 examined (12 cases as unique pathogen agent and 4 cases associated);

- *E. coli* F5 enteropathogen was not identified.

The most receptive age for *Cryptosporidium* infection, as unique pathogen agent, was between 29 and 47 days. This investigations demonstrate that in piglets aged between 0 and 47 days, **cryptosporidiosis**, evaluated by ELISA in national premier, developed in all eight breeding units investigated in Timiș County with a prevalence of **31%** (26,2% as unique pathogen agent and 4,8% in association with other enteropathogens).

In the same age category, **coronaviriosis** had a prevalence of **0,9%** and was associated with *Cryptosporidium* in one case.

The prevalence of **rotaviriosis** in piglets examined was **15,5%** (11,5% as unique pathogen agent and 3,9% in association with cryptosporidiosis).

The infection with *E. coli* F5 enteropathogen was not observed.

- From Arad County, 29 samples from swines with diarrhea, aged between 110 and 133 days, from three breeding farms were examined using ELISA.

On the whole, from 29 faecies samples examined from piglets aged between 110 and 133 days, was semnalated a prevalence of **17,2%** from **cryptosporidiosis**. From all samples analyzed 82,8% were negative for all four enteropathogens tested (rotavirus, coronavirus, *E. coli* F5).

- An *epidemiological screening* regarding the evolution of *Cryptosporidium* in association with other enteropathogens, at swines, in the **two western Counties of Romania** investigated, revealed that the infection with *Cryptosporidium* spp. was the most prevalent (27,7%).

The prevalence of swine **rotaviriosis** was **12%**.

Swine **coronaviriosis** was diagnosed in **0,7%** of samples and the colibacillosis determined by *E. coli* F5 enteropathogen was not observed.

The extensivity and intensivity of infection at swines from Timiș and Arad Counties was higher at 29-47 days age category.

❖ Subchapter three of the first chapter of own researches comprise dates regarding the prevalence of cryptosporidiosis in association with other enteropathogens in **lambs**, determined by ELISA on 66 lambs in the first three weeks of life, from five localities of four Counties of Western Romania (Arad, Bihor, Satu-Mare, Timiș).

The investigations revealed a prevalence of **cryptosporidiosis** in lambs in a percentage of **9,09%**. The prevalence of **rotaviriosis** in lambs was **7,57%**. The prevalence of *E. coli* F5 infection in the lambs investigated was **1,51%**.

No infections with coronaviruses on associations between enteropathogens were found.

❖ In the last subchapter dates regarding the presence of *Cryptosporidium* in other species, using ELISA technique are presented.

63 **dogs** aged between 0 and 10 years, from different areas (urban and rural) of Arad, Bihor, Caraș-Severin, Hunedoara and Timiș Counties were investigated. Dogs faecies were individually collected and were evaluated using ELISA kit BIO K 346 (Bio-X Diagnostics, Belgium). For certain diagnosis every sample was stained with Ziehl-Neelsen method modified by Henricksen. No infection with *Cryptosporidium* in dogs using ELISA and Ziehl-Neelsen method modified by Henricksen, was observed, in the 63 faecal samples prelevated from dogs.

The same results was obtained in the investigations carried out on 184 chickens, broiler breed, in the first seven weeks of life, from four localities of Hunedoara, Satu Mare and Timiș Counties. The birds were raised in intensive system (localities: Satu-Mare, Giarmata, Deva) and extensive system (Conacul Iosif).

In **chapter 4** epidemiological findings obtained using ELISA method on the parasitism with *Cryptosporidium* spp. in **humans** from Western Romania, were mentioned.

The study was carried out between January 2007 and August 2008 on 421 samples from children and adults, who lived in rural and urban areas of Arad, Caraș-Severin and Timiș Counties. Faecies samples were prelevated from every person and examined using ELISA kit BIO-X EASY-DIGEST - BIO K 070 (Bio-X Diagnostics, Belgium) which is an antigenic „in vitro” diagnostic kit for *Cryptosporidium* oocysts.

The results obtained from 421 samples examination from children and adults living in urban or rural areas of Timiș, Arad and Caraș-Severin Counties revealed a prevalence of **4,03%** for **cryptosporidiosis**.

The prevalence of human cryptosporidiosis registered on Counties was: 7,14% in Arad County, 11,1% in Caraș-Severin and 3,08% in Timiș County respectively.

In the age category one month-80 years, cryptosporidiosis was coprological diagnosed in: group 0-1 year (4 cases); group 2-15 years (7 cases); group 20-30 years (6 cases).

From 17 cases diagnosed with cryptosporidiosis, seven had as primary etiology HIV infection.

The **5-th chapter** presents informations about morphometrical measurements of *Cryptosporidium* oocysts from direct faecies smear in calves.

The oocysts identification was carried out in five dairy farms and one micro farm from June 2007 until April 2008. These breeding units were located in five localities of Arad, Bihor and Timiș Counties. Diarrheic samples from 29 samples with cryptosporidiosis were prelevated directly from rectum. The oocysts evidentiatio n was made using the microscopical evaluation of direct smears and the identification, from each smear, of 10 oocyst. The identified oocysts were appreciated regarding the shape, length, width and the shape index using Motic microscope with video extension.

In direct smears the *Cryptosporidium* oocysts had characteristic shape and are distinguished from other parasitary forms with the same dimensions and close shape or other pathogen agents.

The appearance of oocysts in direct smear is an identification criteria and oocysts typical pink refringence. Also, the black point from intern mass and suture lines can be identified in many oocysts. Based on the morphological criteria, in 28 from 29 samples *Cryptosporidium parvum* was identified. In one sample *C. andersoni* was identified based on the measurements.

In **chapter 6** are revealed some observations on anatomopathological modification in natural infection with *Cryptosporidium* spp., and immunohistochemical identification in calves.

Four calves were included in the study; all of them were 7 days, died from diarrhea syndrome and were diagnosed wit *Cryptosporidium* spp. infections. In the first six hours after the death of the calves, parts of intestine were prelevated for histopathological examination. The samples were stained with hematoxilin-eozin (H.E.) technique. The histological sections were imunohistochemically stained with monoclonal antibodies for *C. parvum*, using the kit *Cryptosporidium parvum* (BDI 370): sc-57693 (Santa Cruz Biotechnology, INC) in conformity with manufacturer's instructions.

In microscopically examination of the infected ileum, the main morphological lesions were: blood vessels hyperemia and submucosal edema, epithelial denudation and ulcer, chistisation of some glands, massive invasion of polimorphonuclear leucocytes. At immunohistochemical identification the cryptosporidias appear as spherical or oval shaped, having different dimensions, depending on the section place, included in enterocytes.

This identification technique for cryptosporidial infection in calves is modern and exactly and reveal important anatomopathological informations about the pathogenity of cryptosporidias.

Chapter 7 named „Characterization of some *Cryptosporidium* species at animals and humans using molecular biology techniques” has three subchapters in which are presented informations about *Cryptosporidium* species and subtypes, in calves in their first month of life and in humans.

These subchapters are:

- Molecular identification using polymerase chain reaction (PCR) of *Cryptosporidium* spp. at young calves in Western Romania;
- Subtyping of *Cryptosporidium parvum* species from calves in Western Romania, using sequential analysis of gene gp60;
- Molecular identification (PCR) of *Cryptosporidium* species at humans in Western Romania;

❖ The first subchapter presents the molecular characterization (PCR) of *Cryptosporidium* spp. in **calves**, in the first month of life, from some farms in western part of the country.

The study was carried out in February –May 2008 on 76 calves aged between one day and 30 days. The unweaned calves, Holstein-Freez e breed, were from four dairy farms (20-50

calves/effectives), located in Arad, Bihor and Timiș Counties, with diarrhea and the presence of *Cryptosporidium* spp. The faecies were examined using Ziehl-Neelsen stain modified by Henricksen. Positive samples (22 from 76 examined) were analyzed using PCR-RFLP for the species determination. The gene characterized was SSU-rRNA (18S).

Using molecular analyze technique the SSU-rRNA (18S) from *Cryptosporidium* oocysts was characterized for calves in the first month of life from Western Romania and the parasite specie identified was *C. parvum*.

The purpose gene SSU-rRNA (18S) was easy to amplify with a dense thick and evident shine of migration bands, which indicate a good quality of the tested DNA.

The *Cryptosporidium* parasitism reveal the existence of a zoonotic specie, in calves in the first month of life, with a very high pathogen potential for humans, especially for the people who work in farms and for the veterinarians directly involved in the disease epidemiology.

These molecular biology techniques were used for the first time in Romania, to diagnose the cryptosporidiosis in calves.

❖ The second subchapter has dates regarding the subtyping of *Cryptosporidium parvum* specie, in calves from Western Romania, using the sequential analysis of gp60 gene. The study was carried out in February-May 2008 on 12 *C. parvum* isolates, from calves, Holstein Freeze breed, in their first month of life, living in two dairy farms from two localities of Bihor and Arad Counties. The characterized gene was gp60.

The sequentialising of PCR amplified product of gp60 gene revealed that 12 isolates of bovine *C. parvum* from Western Romania to belong to one familial subtype IIa.

In this familial subtype, the IIaA15G2R1 subtype was observed in 66,6% from all 12 samples sequential zed, being the dominant subtype.

The second subtype identified was IIaA16G1R1 in 33,3 percent of the samples.

The presence of familial subtype IIa in calves in the first month of life in Western Romania means that the parasitism has a high zoonotic potential. These molecular biology techniques of calves cryptosporidiosis were effectuated for the first time in Romania and are a major contribution in the knowledge of the disease in the world.

❖ The last subchapter presents informations about molecular identification (PCR) of human cryptosporidiosis in Western Romania. For oocysts identification the faecies from 107 persons were examined using Ziehl-Neelsen stain modified by Henricksen. The oocysts from positive samples (two from 107 examined) were molecular characterized based on SSU-rRNA (18S) gene using PCR-RFLP.

Using molecular analysis of SSU-rRNA (18S) gene of *Cryptosporidium* oocysts isolated from humans from Western Romania, the parasitism with *C. parvum* was identified.

C. parvum parasitism, in humans, demonstrates the existence of a zoonotic specie, with possibilities of infection for ruminants. These techniques of molecular biology in human cryptosporidiosis were performed for the first time in Romania.

Chapter 8 presents the evaluation of efficacy of five usual and immunological diagnosis methods in cryptosporidiosis.

Diarrheic and non diarrheic faecies prelevated from 35 calves were examined for *Cryptosporidium* infection using the following methods: direct smear examination, Ziehl-Neelsen stain modified by Henricksen, rapid test for *C. parvum* detection (Bio-K 155- Bio X Diagnostics, Belgium), direct immunofluorescence and ELISA double-sandwich test (Bio-K 070 - Bio X Diagnostics, Belgium). On the other part the purpose of this paper was to make to clinician or epidemiological choice more easier regarding the diagnosis method used, depending on the circumstances and purpose.

For efficacy characterization, the standard method for identification of *Cryptosporidium* spp. oocysts was Ziehl-Neelsen stain modified by Henricksen.

It was concluded that all the five methods used allow the identification of *Cryptosporidium* spp. oocysts.

The most sensitive methods were direct immunofluorescence and ELISA test (100%) and the less sensitive was direct smear method (73%). The rapid test had a moderate sensitivity (80%).

The best specificity was found at rapid test (95%) and the lowest was ELISA test (70%). The direct smear method and direct immunofluorescence had a specificity of 90%.

In Ziehl-Neelsen stain modified by Henricksen method and direct smear method experience, knowing the oocyst aspect and other similar elements are needed for parasite identification in the microscopic field.

Based on the results obtained, in clinical infections indicated by diarrheic aspects of faecies, we recommended the direct smear method for emergency cases and Ziehl-Neelsen stain modified by Henricksen for certain diagnosis.

In case of large amount of samples for diagnosis in a short time or for epidemiological survey ELISA test is recommended. This method can be used also for asymptomatic cases.

In **chapter 9** the therapeutically effects of some nutritional supplies and drugs in natural and experimental infection with *Cryptosporidium* spp. at calves are described.

This chapter has two subchapters:

- Testing of efficacy of Diakur^{® PLUS} (*Boehringer Ingelheim*) in natural criptosporidial infection at calves;

- Testing of prophylactic and therapeutic efficacy of Scourban^{® PLUS} (*Bomac*) in experimental criptosporidial infection at calves.

❖ In the first subchapter the efficacy of Diakur^{® PLUS} (*Boehringer Ingelheim*) in natural infection with *Cryptosporidium* spp. in calves was tested. For the research three groups of calves, Holstein-Freeze breed, from three localities of Arad and Timiș Counties were tested. From the three groups tested, two groups of calves with diarrhea were formed and treated orally for seven days. Group one (placebo group) had six calves treated with 100 ml water mixed with 2 liters of powder milk, two times/day. Group two (Diakur group) had nine calves treated with 100 g product Diakur^{® PLUS} (*Boehringer Ingelheim*) powder mixed in 2 liters milk. It was concluded that Diakur^{® PLUS} (*Boehringer Ingelheim*) had no efficacy against *Cryptosporidium* oocysts elimination.

But Diakur^{® PLUS} (*Boehringer Ingelheim*) product can induce clinical improvement in cryptosporidiosis by reducing the number of diarrheic calves and improvement of general status of the animal.

Because of the lack of specific treatment, the therapy with a mixed powder including energize substances, minerals and antimicrobial substances is very important. This conclusion is based on the lowering of diarrheic calves weight and a improvement in general status at Diakur group.

❖ The second subchapter presents the results obtained in efficacy test for Scourban^{® PLUS} (*Bomac*) in experimental infection with *Cryptosporidium* spp. at calves.

The study was carried out on an effective of 12 new-born calves, Holstein- Freeze breed, randomly divided in three different groups. The first group (infected and untreated) was the control group. The calves from group II (infected and treated preventively) were treated preventively six hours before oocysts inoculation with 0,5 ml/kg Scourban^{® PLUS} (*Bomac*) and seven days after the inoculation

❖ The second subchapter presents the results obtained in efficacy test for Scourban^{® PLUS} (*Bomac*) in experimental infection with *Cryptosporidium* spp. at calves.

The study was carried out on an effective of 12 new born calves, Holstein-Freeze breed, randomicaly divided in three different groups. The first group (infected and untreated) was the control group. The calves from group II (infected and preventive treated) were treated preventively, six hours before oocysts inoculation twice/day (morning and evening). The third group was composed of infected and curatively treated calves in witch Scourban^{® PLUS} (*Bomac*) was administrated in doses of 1 ml/kg seven days after the oocysts appeared in the faecies. The calves were experimentally infected with 10⁶ *Cryptosporidium* oocysts in 20 ml distillate water. The criterias for treatment efficy where: oocysts elimination, prepatent period, patent period and diarrhea during treatment.

The results of the study revealed that preventive administration of Scourban^{® PLUS} (*Bomac*), in doses of 0,5 ml/kg, reduce the number of excreted *Cryptosporidium* oocysts and reduces the prepatent and patent period, compared with control group.

Anticryptosporidian effects of Scourban^{® PLUS} (*Bomac*) demonstrated in this study, suggest that this drug can be successfully used in the control of newborn cryptosporidiosis, in preventive and curative treatment.

In the **chapter 10** are presented the 25 general conclusions and recommendations that result from this thesis. They are:

- Present paper is the first epidemiological screening of *Cryptosporidium* spp in association with other enteropathogens using ELISA in Western Romania at calves, piglets and lambs.
- From all five species investigated (bovines, swines, ovines, candies and poultry) in our epidemiological study, from Western Romania (Arad, Bihor, Caraș-Severin, Hunedoara, Satu Mare and Timiș) natural infections with *Cryptosporidium* spp. were identified in: bovines, swines and ovines.
- The diagnosis of lamb cryptosporidiosis was the first identification in this part of the country.
- Epidemiological investigations carried out at calves from Western Romania revealed that *Cryptosporidium* spp. was the main enteropathogen from the four investigated (rotavirus, coronavirus, *E. coli* F5).
- The ELISA double-sandwich epidemiological screening in young calves in the first six months of life, from four Western Counties of Romania, revealed a prevalence of 41,6% for cryptosporidiosis, 9% for coronavirus, 15,9% for rotavirus and 0,9% for infections produced by *E. coli* F5 enterotoxigen. 32,6 percent of all analyzed faeces samples were negative for the enteropathogens tested.
- The prevalence of cryptosporidiosis in the investigated Counties was: 56,9% in Arad County, 31,9% in Bihor County, 28,6% in Caraș-Severin County, and 42,7% in Timiș County, respectively.
- Bovine rotavirus was semnalated in all four investigated Counties with a prevalence of 14,6% in Arad County, 20,9% in Bihor County, 11,4% in Caraș-Severin County and 15,% in Timiș County.
- Enteric coronavirus was found in three from four investigated Counties, with a following prevalence: 12,3% in Arad County, 7,2% in Bihor County, and 9,1% in Timiș County, respectively
- Infections with *E. coli* F5 were semnalated only in Timiș County with 3,6 percent positivity.
- In calves from four investigated Counties the extensivity and the intensity of *Cryptosporidium* infection was higher in 8-14 days age category. But, in age category 15-21 days the calves were more susceptible for infections with rota- and coronaviruses.
- Epidemiological survey for swines from two Western Romania Counties, revealed a prevalence of 27,7% for cryptosporidiosis, 12% for rotavirus and 0,7% for coronavirus. Swine colibacillosis determined by *E. coli* F5 was not observed.
- The extensivity and intensity of infection in swines from investigated Counties (Arad and Timiș) was higher at age category 29-47 days.

- In sheep farms and micro farms from four Western Romania Counties investigated, the lambs examined had a prevalence of 9,09% for cryptosporidiosis, 7,8% for rotavirus and 1,51% for *E. coli* F5. Infections with coronaviruses or associations between enteropathogens were not observed.
- In dogs aged between 0 and 10 years and broiler chickens in the first weeks of life, infections with *Cryptosporidium* spp. were not identified.
- The results of ELISA investigation of 421 samples from humans, from three Western Counties, revealed a prevalence of 4,03%.
- The prevalence of human cryptosporidiosis in investigated Counties were: 7,14% in Arad County, 11,1% in Caraş-Severin County and 3,08,% in Timiș County. From all 17 cases of human cryptosporidiosis, seven had HIV infection as primary etiology. This monitoring of human cryptosporidiosis is the first extensive study in Western Romania.
- Following the measurements of *Cryptosporidium* oocysts in calves, based on morphological study, *C. parvum* was identified in 96,9% percent. In on calve, six month age, *C. andersoni* was identified.
- The microscopically examination of the ileum infected with *Cryptosporidium* spp. the main anatomo-pathological lesions were: blood vessels hyperemia, submucous edema, epithelial denudations and ulcer, cystisation of some glands and massive polimorphonucleare leucocytes infiltrate. The immunohistochemical technique for *Cryptosporidium* spp. identification reveals that *Cryptosporidium* oocysts appear as spherical or oval shape forms, having different dimensions, based an the section place, included in brush edge of enterocytes.
- Using molecular analysis of SSU – rRNA (18S) gene from *Cryptosporidium* oocysts prelevated from calves in their first month of life, from Western Romania, the specie *Cryptosporidium parvum* was identified. This technique, used for the first time in Romania, demonstrated the existence of zoonotic specie with high pathogen effects in human.
- The sequentialising of PCR product, amplified at gp60 gene showed that the 12 isolates of bovine *C. parvum* from Western Romania belong to one family subtype IIa. In this familial subtype, the subtype IIaA15G2R1 was found in 66,6% of samples and the subtype IIaA16G1R1 in 33,3%. The applicability of this technique for molecular biology second generation has a major contribution in the knowledge and implementation of this molecular epidemiology techniques in our country.
- In SSU – rRNA (18S) gene analysis of *Cryptosporidium* oocysts isolated from human samples prelevated from Western Romanian hospitals, using PCR-RFLP analysis the parasitism with *C. parvum* zoonoti specie was identified.
- The following diagnosis methods: Ziehl-Neelsen stain modified by Henricksen, direct smear, direct immunofluorescence, rapid immunoenzymatic test on nitrocellulose substrate and ELISA double – sandwich method permit the identification of *Cryptosporidium* oocysts.
- Evaluation of five diagnosis methods demonstrates a high sensibility for direct immunofluorescence test and ELISA (100%), and a middle sensibility for rapid test (80%) and direct smear test (73%). The best specificity was found for rapid test (95%) and the lowest specificity for ELISA (70%). Direct smear and direct immunofluorescence methods had a specificity of 90%.
- Based on the results obtained, in clinical infections indicated by diarrheic aspect of faecies, we recommend the direct smear test for emergency cases and Ziehl-Neelsen modified by Henricksen stain for certain diagnosis. In case of high number of samples that needed to be examined in short time or for epidemiologic survey ELISA test is recommended.

- The results of efficacy test for Diakur^{® PLUS} (*Boehringer Ingelheim*) product in calves cryptosporidiosis revealed that the product has no efficacy on *Cryptosporidium* oocysts elimination but produces a clinical improvement in general status of the calves and reduces the faecies elimination.
- Based on the results obtained we recommend the using of Diakur^{® PLUS} (*Boehringer Ingelheim*) in symptomatic treatment of cryptosporidiosis.
- Preventive administration of Scourban^{PLUS}® (*Bomac*), in doses of 0,5 ml/kg, reduces the excretion of *Cryptosporidium* oocysts, prepatent and patent period compared with control group, which was infected and untreated. The using of this product in curative purpose in calves who excreted *Cryptosporidium* oocysts, slowly reduces the number of parasites compared with control group.
- The results obtained after Scourban^{PLUS}® (*Bomac*) administration, recommend this drug for prophylactic and curative treatment of cryptosporidiosis.

The present thesis is based on 570 **bibliographic** titles from 16 were web sights and 12 were original papers of the author, published from the thesis content.