SUMMARY

of the doctoral thesis entitled:

CONTRIBUTIONS TO THE STUDY OF ENDOPARASITOFAUNA IN WATER BUFFALOES FROM THE FAGARAS AREA

PhD-student: **PALTIN Andreia Cristina**

Scientific coordinator: *Professor, PhD* MITREA Ioan Liviu

KEYWORDS: digestive parasitophauna; etiology; epidemiology; Buffaloes; Tara Fagarasului; Romania.

Water buffalo (*Bubalus bubalis*) represent a species of special economic interest, due the important benefits of their products. Thus, buffalo milk, which represents 12% of the world milk production, being second in the world after cow's milk, has a high nutritional value, being used for obtaining the Mozzarella cheese, but also of other products; also, the meat has a special quality, due to its low fat and cholesterol content.

The breeding of buffaloes represents a traditional activity with a long tradition in Romania. A high interest in the growth of buffaloes is showed mainly in the center and western Romania, where the buffaloes are raised on farms and households. The Land of Făgăraş is an area where a large number of buffaloes are raised.

Maintaining a proper health status is one of the essential conditions in breeding of any animal species and for obtaining large productions. Parasitic diseases in buffaloes are of particular interest; they can have an impact on both animal health and its productivity. In this context, the studies on main parasitoses and/or parasitic infestations in buffaloes from the Făgăraş area, which are the subject of this thesis, are of particular interest.

Therefore, the general and specific objectives of this doctoral thesis aimed to study the endo-parasitofauna in buffaloes in the Făgăraş area (Center Romania), respectively to investigate the endo-parasitic community and epidemiology of endoparasitosis in buffaloes, by using specific parasitological (classical and modern) investigations in various age categories, including analysis of the dynamics of parasitic infestations and the main associated risk factors.

The doctoral thesis, structured according to the Recommendations of the Doctoral School, has two parts: the first which is a bibliographic study, comprising 3 chapters, and the second - personal research, with 5 chapters. The first part of the thesis represents 1/3 (30 p.) and the second part 2/3 (76 pages), according to the

recommendations. The selected bibliography comprises 130 relevant titles and the thesis annexes include seven tables and 51 figures (graphs and photos).

In **the first part** of the thesis, the Bibliographic Study, current data about the main parasitic diseases of buffaloes are presented according to the taxonomy of the etiologic agent. Therefore of the protozooses, main gastrointestinal protozoan diseases are described, namely the most important that affects mainly young animals, such as eimeriosis, cryptosporidiosis and giardiosis. Thereafter, there are described main aspects of the helminthoses, including: trematodoses, such as fasciolosis, paramphistomosis and dicroceliosis, cestodoses, and nematodoses, with repiratory strongylidosis (dictyocaulosis), then gastrointestinal strongylidosis and neoascaridosis. The presentation framework of these parasitic diseases considered the current data on the etiology, with taxonomy and morpho-biology data, epidemiology, and main characteristics and aspects of the pathology and clinical features.

The second part of the doctoral thesis includes the results of own research, carried out to achieve the thesis objectives. This section begins with the presentation of the general framework for organizing and conducting research, including: the morpho-biological characteristics of the genus *Bubalus*, but also current data on buffalo populations in Romania and in the world, the characterization of the study area, followed by the presentation of the working methods and methodology. Then, follows the studies undertaken, corresponding to the research and thesis research plan, with the results obtained described in the next three chapters, so that in the final chapter there are general conclusions and recommendations.

The Chapter V, on **the Epidemiology of endoparasitic infestations in buffaloes in Tara Făgăraşului,** describes research undertaken on the structure of endoparasitofauna and the epidemiological features of diagnosed parasitic infectiones, study on 105 animals from *the Research and Production Farm for the Breeding of Buffaloes, Şercaia*, county Braşov. Animals were grouped into 4age categories, (i) calves: <6 months; (ii) young aged from 6 months to 2 years; (iii) heifers (aged from 2 to 3 years); (iv) milking females (age> 3 years). The results of the copro-parasitological examinations performed showed that more 55.2% (95%CI:45.22–64.96) of the animals tested were positive; 43.8% had mixed infections (25.7% with two species and 18.10% with three parasitic species), and 11.4% had mono-specific infections. By age categories, the average prevalence values highlighted statistically significant differences ($p < 0.05$), as follows: in calves, 42.9% of animals were positive; In the category of 6 months-2 years, 50.0% of animals were positive; of the heifers, 73.3% were positive; also, of the milking females, 50.0% were positive at the parasitological examination.

Regarding the parasite community, a diverse endo-parasitophauna was found, including helminths - gastrointestinal strongyles (41.9%), respiratory strongyles - *Dictyocaulus* spp. (9.5%), trematodes - *Paramphistomum cervi* (24.8%), *Fasciola hepatica*

(21.0%), *Dicrocoelium lanceatum* (3.8%), cestodes - *Moniezia* spp. (1.9%) and protozoa - *Eimeria* spp. (16.2%), *Giardia duodenalis* (4.8%), *Cryptosporidium parvum* (3.8%).

The prevalence of different parasitic species has also shown differences according to age categories:

- in calves protozoan infections were predominantly recorded, most with *Eimeria* spp. (28.6%), followed by *Cryptosporidium* spp. (19.0%) and *Giardia* spp. (9.5%);
- in young animals (6 months - 2 years): the highest prevalence was registered for digestive strongyles (35.0%) and *Dictyocaulus* spp. (25.0%); relatively close prevalence values were registered for infections with *Eimeria* spp. (20.0%), *G. duodenalis* (15.0%) and *F. hepatica* (15.0%); lower values were recorded for *Moniezia* spp. (10.0%) and *Paramphistomum* (5.0%);
- in heifers (2 - 3 years), the highest prevalence values were registered for digestive Strongyles (66.7%), *Paramphitomum* (46.7%) and *Fasciola* (26.7%), followed by *Eimeria* spp. (16.7%), *Dictyocaulus* spp. (10.0%) and *Dicrocoelium* (3.3%);
- For milking cows, 50.0% were positive for digestive strongyles, 32.4% for *Paramphistomum* and *Fasciola*, followed by *Dicrocoelium* spp. (8.8%), *Dictyocaulus* spp. (5.9%) and *Eimeria* spp. (5.9%).

Chapter VI, **Characterization of endoparasitofauna in buffaloe calves in Tara Făgăraşului**, aimed to identify the digestive parasite community in calves and the associated risk factors in the studyfarm. In the study were included a total of 63 calves, aging between 3 and 20 weeks, grouped into three categories. The results of the copro-parasitological examinations revealed that 77.8% of the analyzed samples were positive, the majority-53.9% as single parasite infections. The endoparasitic profile included the following species*: Eimeria* spp. [66.7%; OPG = 2569)], *Toxocara vitulorum* [prev.15.9%; epg=3507)], *G.duodenalis* (14.3%), *Strongyloides papillosus* (6.3%). Parasitic associations (polyparasitism) were identified in 23.8% of tested animals, with two parasitic species [*Eimeria+Toxocara*; *Eimeria+Giardia*; *Eimeria+Strongyloides*; *Giardia + Strongyloides*)] or three parasitic species (*Eimeria + Toxocara + Strongyloides*).

Particular aspects were recorded according to the age group, as follows:
- *Eimeria* spp. infections were identified in all age groups, its prevalence varied from 47% to 80.0%; - *Toxocara vitulorum* was identified in calves under 13 weeks of age, with prevalence ranging from 12.55% to 36.8%; - cysts of *G. duodenalis* were identified in claves over 6 weeks of age, with the rate prevalence from 16.7% to 25%; - positive samples for *Strongyloides* had a prevalence between 4.2% and 15.0%.

The study described in Chapter VII, on Molecular epidemiology of cryptosporidiosis in buffaloes, in the Făgăraş area, was performed on calves (n=20) of up to 6 weeks old, an age that favors *Cryptosporidium* infections. Both classical parasitological diagnostic investigations (examination of faeces in direct fecal smear), as well as molecular identification (PCR) and genetic characterization of *Cryptosporidium* spp. isolates were

carried out in order to asses its zoonotic risk. Thus, through the direct smear technique, for 20% of the tested samples *Cryptosporidium* oocysts were observed.

Following the amplification of the *Cryptosporidium* spp. SSU-rRNA gene (DNA extracted from calves feces), by *nested PCR,* in 30% of the analyzed samples amplicons of approximately ~850 bp size, specific to the *Cryptosporidium* genus, were identified. The molecular analysis of the SSU-rRNA gene (18S) amplified, respectively theanalysis of the RFLP-restriction fragment length polymorphism, the species with zoonotic potential, *C. parvum*, was identified, the differentiation carried out with the help of the endonuclease *VspI* highlighting bands at the regions of 629 and 104 bp, specific for *C. parvum*. Thus, the present study demonstrated that buffaloes are natural reservoir hosts for *Cryptosporidium* spp. and potential zoonotic risk.

Subsequently of the carried-out studies'results, the thesis ends with a Chapter of General conclusions and recommendations. Thus, altogether the studies'results demonstrate that in Făgăraș region there are conditions which are favorable for evolution and spread of these parasitoses in buffalo herds, underlining once again the importance of periodic parasitological screening studies for etiological diagnosis as a basis for applying a sustainable program of parasitological control, with specific measures in all age groups. Also, the results of the studies highlight that the presence of digestive parasites can have a relevant impact on the health of animals, with implications in digestive pathology, especially in young animals, but also emphasize that these animals can be a source of risk for human health for the detected pathogens with zoonotic potential.

In conclusion, the studies undertaken in the realization of this doctoral thesis, which aimed at the study of the endoparasitofauna in buffaloes from the Făgăraș study region, respectively the investigation of the epidemiology and the evolution of gastrointestinal parasitic infestations in buffaloes in the conditions of central Romania, contribute to the development of scientific knowledge in the field of parasitic infestations in buffaloes in our country, but also bring aspects of practical value, for the benefit of farmers, owners and veterinarians, for better knowledge and updating of information on the importance and control of parasitic diseases in water-buffaloes.