Epidemiological study on the evolution of endoparasitosis in buffaloes in nortwestern Romania

(SUMMARY OF Ph.D. THESIS)

Ph.D. student **Bărburaș Diana Ancuța**

Scientific coordinator **Prof. univ. dr. Vasile Cozma**

Full member of Academy of Agricultural and Forestry Sciences Bucharest

ABSTRACT

Despite the decline in the buffalo population in the last 40 years, Romania ranks second in terms of the buffalo population in Europe, after Italy (BORGHESE, 2005; BORGHESE, 2013). Buffalo populations are found especially in Transylvania (north-west), where they were first recorded in the seventeenth century (KOS, 1975). Buffaloes are traditionally raised in Romania especially for milk and meat production and to a lesser extent for agricultural work. Buffalo milk is of interest for investment and research in many countries, due to its high nutrient content (AMARJIT and TOSHIHIKO, 2003). It ranks second in the world, after cow's milk, thus constituting over 12% of world milk production (CNIEL, 2002). Given the health implications and economic potential of buffaloes, investigations into parasitic infestations are of considerable relevance (RINALDI et al., 2007; VENEZIANO et al., 2007).

Parasitic diseases, due to their evolutionary peculiarities, with weak clinical manifestations, but with increasing spread in livestock. Some cause significant economic loss due to the zoonotic character.

Parasites cause local and general disorders, mortality, which leads to significant economic losses among livestock. Parasitic infestations are a major problem in buffalo herds, with effect on both morbidity and mortality in the affected livestock and the risk of transmitting parasites to humans. Thus, parasitic diseases play an important role in affecting the health of animals and prevent the development of animals. Local climatic conditions, animal husbandry practices and grazing largely determine the incidence and severity of various parasitic diseases in a given area (ALAM et al., 2016).

The prevalence of endoparasites in the household system is higher because there is a permanent source of contamination (RADOSTITS et al., 1994). Raising buffaloes on farms could be a method of prophylaxis that could lead to a decrease in the number of contaminated animals, and special attention should also be assigned to treatment.

A parasitological control in the herds of animals with a role in reducing the frequency and intensity of parasitic infestations is necessary in order to maintain a healthy and high productivity herd.

The doctoral thesis entitled " Studies regarding parasitic infections in buffaloes in northwestern Romania" includes several studies on the prevalence of parasitosis in buffaloes in northwestern Romania.

The originality elements of this paper are provided by the epidemiological study of parasitosis present in buffaloes raised in the household and farm system in northwestern Romania, as well as the correlations and statistical differences of the results obtained in serological, coproparasitological examinations and of molecular biology.

The thesis is structured in two parts: the first part entitled "Current state of knowledge", contains bibliographic data found in literature and other published works and the second part entitled "Own research" describes my research work and all the results obtained during my doctoral study.

The first part of the thesis, entitled "The current state of knowledge", extends over 7 chapters and contains general data from the literature on parasites studied in this thesis. For the elaboration of these chapters, documentation studies were carried out, using various sources: scientific articles, books, doctoral theses, specialized treatises, journals, as well as a series of recent web articles. Each chapter of the first part presents information on the impact of these parasites on buffaloes health.

Chapter I of this thesis contains general aspects regarding endoparasitosis in buffaloes.

Chapter II is divided into 3 subchapters, which cover etiology (taxonomic classification, morphology and biological cycle) pathogenesis, clinical signs and diagnostic methods for *Giardia duodenalis*.

Chapter III is divided into 3 subchapters, which cover etiology (taxonomic classification, morphology and biological cycle) pathogenesis, clinical signs and diagnostic methods for *Cryptosporidium* spp.

Chapter IV is divided into 3 subchapters, which cover etiology (taxonomic classification, morphology and biological cycle) pathogenesis, clinical signs and diagnostic methods for *Eimeria* spp.

Chapter V is divided into 3 subchapters, which describe aspects of morphology, life cycle and diagnostic methods for *Toxoplasma gondii*.

Chapter VI is divided into 3 subchapters, which cover aspects of morphology, life cycle and diagnostic methods for *Neospora caninum*.

Chapter VII is divided into 3 subchapters, which cover etiology (taxonomic classification, morphology and biological cycle) pathogenesis, clinical signs and diagnostic methods for *Toxocara vitulorum*.

The second part of the thesis, entitled "Own research", is composed of 4 chapters in which the results of the research are presented and discussed.

The first chapter (II.1) aimed to determine the prevalence of Toxoplasma gondii infection in buffaloes in northwestern Romania. The study was conducted by taking samples from 197 animals from the household system and 74 samples from the slaughterhouses.. The age of the animals ranged from 2 weeks to 300 months (25 years), with a mean of 84.4 ± 77.6 months (7 ± 6.5 years). In terms of age, samples were collected from 25 young buffaloes (0-6 months), young animals (6-30 months) and adult animals (> 30 months). Most of the animals included in the study were raised in the household system (166/197; 84.3%) and were female (172/197; 87.3%).

The frequency, prevalence and the 95% confidence interval (CI) were calculated for each detection method. The concordance between serological methods (ELISA and MAT) and between the indirect methods (ELISA, MAT) and the direct (biosample, PCR) was measured using the Cohen kappa (k) in PeiTools.

The total prevalence of anti-*T. gondii* antibodies in buffaloes in Romania was 12.7% (25/197). Depending on the method used, the seroprevalence was 6.6% (13/197) by ELISA technique and 8.1% (16/197) by MAT technique. Four samples (n = 4) were positive by both methods, while nine samples (n = 9) were positive by the ELISA technique only, and twelve animals (n = 12) were positive by the MAT technique only. Prevalence (by ELISA, MAT techniques) was not influenced by sex and growth system. It has been found that adults are more likely to have anti-*T. gondii* antibodies., detected either by ELISA or MAT technique, compared to young animals (p = 0.05) with 2.73 (95% CI CI: 0.74-10.0). The maximum dilution at which anti-T. gondii antibodies were detected using MAT were 1:768. Comparing the results obtained by the ELISA technique and by the MAT technique, there was an almost perfect concordance between the 2 methods (k=0.219). The total concordance was 93.3%, and the percentage of positive concordance between the 2 methods was 85.7%, for both methods, 4 samples were positive, the seroprevalence being 2% (4/197).

Following the bioprobing (n=74), all samples were negative. No specific T. gondii IgG was detected in the mouse serum by the MAT technique (n=148). The olfactory lobes and the median region of the mouse brain (n=148) were examined by light microscopy and no *T. gondii* cysts were identified. Also, following the PCR analysis, all samples (homogenized by the brain of mice) were negative. There was no relation between indirect methods and bioprobing.

All tissue samples collected from buffaloes (heart, liver, mesenteric lymph nodes, mediastinal lymph nodes and digested diaphragm) were subjected to PCR, nPCR and qPCR methods. By nPCR and qPCR methods, T. gondii DNA was detected in a mesenteric lymph node sample from a 252-month-old (21-year-old) buffalo by nPCR and in a diaphragm digestion sample from a 30 months (2.5 years) male by qPCR, the prevalence being 2.7% (2/74; 95% CI: 0.33-9.42). There was no relation between indirect methods and PCR detection, as nPCR and qPCR positive animals were seronegative to both methods (both MAT and ELISA).

Chapter II (II.2) aimed to determine the prevalence of Neospora caninum infection in buffaloes in northwestern Romania. The study was performed by taking samples (n=197) from buffaloes from the household system and from the slaughterhouse (n=74). 123 (n=123) samples were collected in field conditions, the rest of the samples were collected from the slaughterhouse (n=74). The buffaloes came from 5 counties in north-west of Romania, respectively: Sălaj, Cluj, Maramureş, Bihor and Bistriţa-Năsăud.

Epi Info TM 7 software (CDC, USA) was used for statistical analysis. The frequency, prevalence and the 95% confidence interval were calculated for the results obtained with ELISA, cPCR and nPCR.

Total prevalence of anti-*N. caninum* antibodies in buffaloes in Romania was 68.5% (135/197), by ELISA technique. According to the age category, seroprevalence was significantly higher (p=0.0009) in adults compared to young buffaloes. Regarding the sex of the animals studied, the prevalence was significantly higher (p=0.009) in females, while depending on the origin, the prevalence was significantly higher (p=0.00004) in the case of animals from households compared to the system industrial growth prevalence was significantly higher (p=0.00004).

By conventional PCR, all the samples examined (samples subjected to digestion like diaphragm, heart samples and mesenteric lymph nodes) were negative, and via nPCR, 8.1% (n=6) of the digested diaphragm samples were positive, from which three animals (50%) were serologically negative.

The BLAST analysis resulted in five unique sequences, which were 97-100% identical to the Liverpool strain of *N. caninum* (Accession Umber LN714488). Our sequences were grouped into a single cluster, along with three other *N. caninum* isolates from Europe and Asia.

Chapter III (II.3) aimed to determine the prevalence of parasitosis by age category in buffaloes raised in the household and farm system in north-western Romania. The study was performed by taking fecal samples from 180 buffaloes. The 180 fecal samples came from 101 males, 28 young buffaloes and 51 adult buffaloes. Thus, 122 females and 58 males, aged between 2 weeks and 25 years. Most of the animals were raised in the household system (160/180), the rest of the animals were from a farm in Cluj county.

For all faecal samples, a coproparasitological examination was performed by the flotation technique (Willis), sedimentation and by the McMaster method.

At the coproparasitological examination, parasitic infections/infestations were identified in 57.22% (95% CI: 49.65-64.55) of the evaluated animals. The parasitic elements were represented by oocysts of *Eimeria* spp. (78/180; 43.33%; 95% CI: 35.98-50.91), *Buxtonella sulcata* cysts (3/180, 1.7%; 95% CI: 0.35-4.79), eggs of *Fasciola hepatica* (8/180, 4.44%; 95% CI: 1.94-8.57), eggs of *Paramphistomum cervi* (5/180, 2.8%; 95% CI: 0.91-6.36), oncospheres of *Moniezia* spp. (1/180 , 0.6%, CI: 95%: 0.01-3.06), *Toxocara vitulorum* eggs (21/180, 11.7%; 95% CI: 7.37-17.28), strongyle eggs (6/180, 3.33%; 95% CI : 1.23-7.11), eggs of *Strongyloides* spp. (9/180, 5%; 95% CI: 2.31-9.28) and eggs of *Capillaria* spp. (1/180, 0.6%, IC (95%: 0.01-3.06).

In both the buffaloes that came from the farm and those that came from the household system, the infection with *Eimeria* spp. had the greatest extent.

The number of oocysts/g feces (OPG) was: *Eimeria* spp.- OPG = 2176.55, N. vitulorum- OPG = 4348.19 and strongyle digestives- OPG = 126.33. There were no statistically significant differences in OPG value by age group and sex (p>0.05).

Monospecific infestations with *Eimeria* spp., *F. hepatica*, *P. cervi*, *Moniezia* spp., *T. vitulorum*, digestive strongyle and *Strongyloides* spp. Regarding polyspecific infestations, the most frequent parasitic association was found between *Eimeria* spp. and *T. vitulorum* (7.22%; 95% CI: 3.9-12.03). The association between *Eimeria* spp. and *Strongyloides* spp. was also observed in 6 (3.33%) buffaloes and in 2 (1.11%) buffaloes the association between *Eimeria* spp. and *F. hepatica*. Other parasitic associations identified were *Eimeria* spp. with digestive strongyles, *Eimeria* spp. with *B. sulcata*, *Eimeria* spp. with *Capillaria* spp. and *Eimeria* spp., *B. sulcata* and *T. vitulorum* in a single buffalo (0.56%).

Chapters IV (II.4) aimed to determine the prevalence of parasites in young buffaloes in the first three months of life in Romania, and to identify *Eimeria* species based on morphological characteristics and genotypes of *Cryptosporidium* and *Giardia*. The study was performed by taking 104 faecal samples from 38 young buffaloes aged 2-11 weeks. Fecal samples were collected at 14-day intervals, starting at 2-3 weeks of age and finishing at 10-11 weeks of age. The young buffaloes came from the household system, from 4 localities of Sălaj county, respectively: Românaşi, Păuşa, Poarta Sălajului and Chichişa.

The frequency, prevalence and the 95% confidence interval were calculated for each identified parasitic infection / infestation. Prior to statistical processing, data distribution was verified using the D'Agostino-Pearson normality test. Statistics were performed using the dedicated software EpiInfo v3.5.1 and MedCalc Statistical v19.0.4.

Following the coproparasitological examination, the following were identified: infection with *Criptosporidium* spp. (10.5%), *G. duodenalis* (2.6%) and *Eimeria* spp. (84.2%), respectively infestation with *N. vitulorum* (36.8%) and *S. papillosus* (15.8%).

Eight species of *Eimeria* were identified: *E. auburnensis* (8.2%), *E. bareillyi* (32.3%), *E. bovis* (1.7%), *E. canadensis* (3.9%), *E. cylindrica* (14.7%), *E. ellipsoidalis* (14.2%), *E. subspherica* (2.6%) and *E. zuernii* (22.4%), the prevalence being variable depending on the age of the young buffaloes. The most prevalent species of *Eimeria* in the age categories 2-3 weeks, respectively 6-7 weeks was *E. bareillyi*, in the age categories 4-5 weeks, respectively 8-9 weeks was *E. zuernii*, and in the category of age 10-11 weeks was *E. ellipsoidalis*.

Elimination of *Eimeria* spp. oocysts have been observed since the age of 2-3 weeks. The highest prevalence of 60.5% was registered in the age category of 4-5 weeks. The arithmetic mean of oocysts/g (OPG) was 417,155.6 in *Eimeria* spp., 26,509.9 in *N. vitulorum* and 444.12 in *S. papillosus*.

With Henriksen staining, 4 positive samples were identified, and by the nPCR method 3 positive samples, regarding the infection with *Crypotosporidium* spp, also the infection with *Giardia duodenalis* was identified in a single malac by the nPCR method.

Elimination of *Cryptosporidium* spp. oocysts have been recorded since the age of 2-3 weeks, the highest prevalence being found in the age category 4-5 weeks, but after this age no positive animals were identified.

Eggs of *N. vitulorum* were identified starting with the age of 2-3 weeks, with the highest prevalence in the age category of 8-9 weeks, showing statistical significance of the cohabitation of young buffaloes together with young buffaloes (p=0.0009754566) and adults (p=0.0050705694), and *S. papillosus* eggs were identified starting with the age of 4-5 weeks, the highest prevalence being registered in the age category of 10-11 weeks.

Following the genotyping to determine the species of *Cryptosporidium* spp. that evolves in young buffalos, the species *C. ryanae, C. bovis* and *C. xiaoi* were identified, also the genotype E of *Giardia duodenalis* was identified.

The final objective of this thesis was to complete the information about the epidemiology of parasitosis in buffaloes in the north-west of Romania.

REFERENCES

1. ALAM, M.R., ERFAN, R., SEN, A.B., DAS, S., RAHMAN, M.M., NATH, S.K., 2016, Prevalence of gastrointestinal helminthiasis in naturally infested buffalo in Sylhet district. Int. J. Adv. Multidiscip. Res. 3(8): 52-58.
2. AMARJIT, S., TOSHIHIKO, N., 2003, Role of buffalo in the socioeconomic development of rural Asia: current status and future prospectus. Anim Sci J 74, 443-455.
3. BORGHESE, A., 2005, Buffalo production and research, Food and Agriculture Organization of the United Nations, Rome. Buffalo Production and Research. FAO (ed), REU Technical Series 67:1-315.
4. BORGHESE, A., 2013, Buffalo livestock and products. CRA – Council for Research in Agriculture, 511 pp. ISBN 978-88-97081-27.
5. CNIEL (CENTRE NATIONAL INTERPROFESSIONNEL DE L'ECONOMIE LAITIÈRE), 2002, L'economie laitière en chiffres, 183 pp.
6. KOS, K., 1975, Contribuţii la cercetarea etnografică a creşterii bivolilor, Anuarul Muzeului Etnografic al Transilvaniei, VII, seria 1974-1975, 121-136.
7. RADOSTITS, O.M., BLOOD, D.C., GAY, C.C., 1994, Veterinary Medicine: A text book of disease of cattle, sheep, pigs, goats and horse. 8th ed. BaillereTindall Publication. London, 1223-1225, 1237-1238.
8. RINALDI, L, MUSELLA, V., CONDOLEO, R., SARALLI, G., VENEZIANO, V., BRUNI, G., CONDOLEO, R.U., CRINGOLI, G., 2007, Giardia and Cryptosporidium in water buffaloes (Bubalus bubalis). Parasitol Res 100, 1113-1118.
9. VENEZIANO, V., SANTANIELLO, M., CARBONE, S., PENNACCHIO, S., MORGOGLIONE, M,E., SCHIOPPI, M., CONDOLEO, R., CRINGOLI, G., 2007, Lice (*Haematopinus tuberculatus*) in water buffalo farms from central Italy. IJAS 6, 926-927.