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Abstract

Key words: *digestive parasites, serouses, horses, etiology, epidemiology, lesions, control*

Horses may be affected by more than 150 species of internal parasites. A horse can be infected by one or more parasites at a time, but the nature and the severity of the damage will vary with the type and degree of infestation (Güiris *et al.*, 2010). Digestive parasites can alter behavior, fertility, fitness, youth development, resistance to other pathogens or behavior performance for which they are breed animals (Cernea, 2008a; Cernea *et al.*, 2008c).

Romanian horses have always been appreciated for their outstanding qualities (energetic capacity, traction resistance, better use of food). Remarkable progress has been made in the last two decades regarding the information on parasites, on the pathogenetic and clinico-anatomical characters of parasitoses, on the means and methods of diagnosis, and thus, a change and acceptance of news criteria for classification of some of them is implied (Șuteu, 1994).

In our country, etiology and evolution of digestive parasitism and serous cavity have been less systematically studied, usually on small areas, requiring the completing of data with detailed analysis. In this context, we focused our research on the etiomorphopathogenetic and epidemiologic aspects aimed at controlling these parasitoses. To achieve this goal, several points of examination were set, the subject horses were from Suceava, Botoșani, Iași, Neamț, Bacău Counties and Bistrița-Năsăud, an additional region of Moldova.

In this context this paper has as general objectives the following:

- the analysis of the digestive and serous cavity parasitic population that colonizes these horses' organs from various areas of Moldova, in order to establish a complete etiologic diagnosis;
- obtaining data on the epidemiological situation of these parasites, respectively their extensivity against various intrinsic or extrinsic factors, knowledge of infestation degree and intensity of parasitic infection;
- comparative study of digestive and serous cavity parasitoses evolution in the two farming systems found in Moldova, respectively the semi-intensive and the household system;
- evaluation of parasitized organ morpho-lesional implications by necropsic, macroscopic and microscopic investigations, for a more precise assessment of the parasites pathogenicity;



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- contributions to the knowledge of antiparasitic treatments effectiveness by coproparasitological tests, respectively FECRT (faecal egg count reduction test) and control measures applied to a large number of the studied horses.

The paper was divided in two parts:

Part I (Chapters I, II, III, IV) includes bibliographic data and studies on morphological and functional particularities of the horses' digestive system and serous, the etiologial, epidemiological and morphopahetogenetic characteristics of main parasitoses of these organs, methods and diagnosis opportunities of parasitoses described, and some aspects of their prevention and combating. It has 62 pages, representing 33.15% and contains 14 figures and 3 tables.

Part II has over 125 pages (66.85%), contains 203 figures and 23 tables and is divided into 4 chapters, as follows:

Chapter V includes research on **Etiological and epidemiological diagnosis of intestinal and serous parasitoses in horses** as a first step for the study of parasitic entities that colonize these organs.

Parasite population analysis aimed at the identification of species, genus and families of protozoa and helminths localized in the digestive tract and serous cavities of horses from different areas of Moldova. 125 live animals from both extensive and semi-intensive growth systems were parasitologically examined, and 64 animals were by using a necropsically approach, all coming from the extensive system.

Working methods are represented by faecal qualitative and quantitative examination methods that revealed mostly microscopic parasitic elements, and the morphological examinations which mainly contributed to the identification of the adult parasites. The latter could also be harvested and identified by the administration of antihelminthic substances.

Research and analyzes on the digestive system and serous cavity in horses led to the diagnosis of the following parasitoses: eimeriosis, giardiasis, fasciolosis, dicroceliosis, cestodosis, habronemosis, parascaridosis, strongyloidosis, strongylosis, cyathostomosis, oxiurosis, setariosis and gasterophilosis.

Protozoa were represented as species of the genera *Eimeria* and *Giardia*. These were identified by the presence of oocysts or cysts in faeces. From the genera *Eimeria* were identified all three species known as equine parasites *E. solipedum*, *E. leuckarti* and *E. uniungulata*, based on morphological characters of the oocysts. The extensivity of infestation with *Eimeria* was found in 41.62% of the studied equine population. The dominant species was *E. solipedum*, followed by *E.*



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leuckarti, and *E. uniungulata* has rarely been seen. Highest prevalence was found among the horses coming from the semi-intensive system belonging to Rădăuți Stud Farm and Sports Club from Blăgești Com., Bacău County. A high incidence of infestation with *Eimeria* was observed in horses bred in the extensive systems in some villages from Suceava, Botoșani and Bistrița-Năsăud Counties.

The parasitism with *Giardia intestinalis* was found to be commonly associated with that of *Eimeria*. *Giardia* cysts were identified in 20 horses from Rădăuți Stud Farm (66.66%) and in all of the 7 animals originated from Blăgești Sports Club (100%). In other groups the prevalence was low.

Trematodes were also identified by coproscopic examinations; the parasitic elements are represented by *Fasciola hepatica* and *Dicrocoelium lanceolatum* eggs. Although they have been diagnosed with a reduced rate of incidence, these parasitoses had an unusual epidemiological situation, being encountered in horses bred in the semi-intensive system, where the contact probability with intermediate hosts and infested elements is reduced. Thus, they were found in horses from Blăgești Sports Club and Rădăuți Stud Farm, two of the animals from the first group being infected with both trematodes.

Equine parasitic cestodes had *Anoplocephala perfoliata* as representative species. Cestodosis was diagnosed in horses from the extensive system, revealing close contact with intermediate hosts due to grazing. Extensivity of infestation in these horses was 31.5 %. Eggs were identified with a prevalence rate of 16.66 % by coproscopic analysis, while by necropsic analysis, the extensivity of anoplocephale infestation was 34.37 %. Values of prevalence in horses coming from extensive system were: Botoșani - 44.44 % (4 of 9 animals examined), and Bacău - 40 % (8 of 20 animals examined), followed by Bistrița-Năsăud - 28.57 % (4 of 14 animals examined) and Suceava - 26.31 % (5 of 19 animals), the lowest prevalence was recorded in horses from Iași, respectively 18.18 % (8 of 44 animals examined). The degree of infestation revealed by necropsic analysis ranged from 3 to hundreds, even thousands, of copies, in massive infestations.

The identified **nematodes** belonged to the following genus and families: *Habronema* Genus, *Strongylidae* Fam., *Oxyuridae* Fam., *Strongyloides* Genus and *Setaria* Genus.

***Habronema* spp. infestation** was identified as a *Habronema muscae* species, on morphological characters of adults harvested from faeces after deworming. Habronemosis was the digestive nematodosis with the lowest range percentage, diagnosed only in horses owned by households from Iași County, reaching a prevalence of 10% in this group, and 3.77% for all horses coming from extensive system.



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***Strongylidaes* infestation** was diagnosed in horses of all the examined groups, being found in a similar percentage in both systems of growth. Etiologic diagnosis of these parasitic morbid conditions were achieved both by coproparasitological analysis and harvested adults after deworming, or directly in gastrointestinal mucosa after opening the large intestine and its necropsic examination.

Following microscopic examination of adult morphological characters, two species of large strongyles were identified: *Strongylus vulgaris* and *Strongylus edentatus*. Adult strongyles infestation observed in the large intestine of a necropsied animal showed differences depending on the species. *Strongylus vulgaris* species was present from a number of few tens, to a few hundreds, in some animals, while *Strongylus edentatus* was present in low numbers, from 5 to 12 parasites in one animal. *Strongylus vulgaris* was found in a higher percentage, being diagnosed in 54 necropsied animals, and in faecal samples from four dewormed animals coming from the extensive system (78.37%). Prevalence of *Strongylus edentatus* species was reduced, being identified in 5 necropsied animals (6.75%).

Along with the large strongyles, infestation with nematodes belonging to the *Cyathostominae* Subfam. had a prevalence of 100 %, being diagnosed by using the same methods. After examining a population of 705 adults, cyathostomes were identified in a number of 25 species belonging to the following seven types: *Cyathostomum*, *Coronocyclus*, *Cylicocyclus*, *Cylicostephanus*, *Skrjabinodentus*, *Tridentoinfundibulum* and *Petrovinema*. Regarding the number of species from a genus, the prevailed genera were represented by the *Cylicocyclus*, with 9 species, followed by the *Cylicostephanus* with 5 species, and *Coronocyclus* with 4 species. *Cyathostomum* genus was represented by 3 species, *Petrovinema* genus by two species and *Skrjabinodentus* genus and *Tridentoinfundibulum* genus by one species each. Regarding the dominance of individuals from a genus, we obtained the following percentage from all identified cyathostomes: *Cyathostomum* G. - 13.76 %; *Coronocyclus* G. - 11.11 %; *Cylicocyclus* G. - 41.35 %; *Cylicostephanus* G. - 28.97 %; *Skrjabinodentus* G. - 0.35 %; *Tridentoinfundibulum* G. - 0.36 %; *Petrovinema* G. - 4.1%.

From the identified cyathostomes, the presence of four species was distinguished, cited in literature as having the African continent as a starting point spreading area: *Cyathostomum montgomeryi*, *Cylicocyclus adersi*, *Cylicocyclus gyalcephaloides* and *Skrjabinodentus longiconus*.

Prevalence of the studied cyathostome types from equine population was as follows: *Cyathostomum* G. - 52.4%; *Coronocyclus* G. - 51.2%; *Cylicocyclus* G. - 79.43%; *Cylicostephanus*



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G. - 81.57%; *Skrjabinodentus G.* - 5.2%; *Tridentoinfundibulum G.* - 12.12%; *Petrovinema G.* - 25.23%. This prevalence varied by region of origin, breeding system and lot of horses studied.

By coproscopic examination, *Strongylidae*s infestation prevalence reached 100%, parasitic elements represented by eggs, at different stages of development, and strongyles type larvae being emphasized. Based on morphological aspects, eggs from many genres of nematode could be identified: eggs of the *Cyathostominae* Subfam., *Triodontophorus*, *Strongylus* and *Oesophagodontus* Genus. Prevalence of *Strongylidae*s infestation based on faecal examination differ with genus: *Strongylus G.* - 90.69%; *Triodontophorus G.* - 37.87%; *Oesophagodontus G.* - 24.95%; *Cyathostominae* Subfam. - 85.8%.

From a quantitative perspective, the intensity of strongyles invasion determined by the number of eggs in the gramme of faeces (E.P.G.) was between 100 and 2200 E.P.G. The value was determined by using the MacMaster and Stoll methods. There were no significant differences between the values obtained by using any of the two methods, or between the samples collected during three moments of the day. Also, no significant differences were found in the E.P.G. values from the three days of the study.

In horses from the households we obtained E.P.G. values between 150 and 2200, with an average around 800 E.P.G., which shows generally moderate infestation to slightly increased. No significant differences were observed regarding age or sex factors, the values being close. *Strongylidae*s infestation of horses from semi-intensive system had values of E.P.G. between 50 and 800, with an average around 500 E.P.G. We can, therefore, speak of medium to low infestation. The influence of intrinsic or extrinsic factors such as sex, age, race or growth mode were insignificant, all categories of horses being similarly affected by these parasites.

***Parascaris equorum* infestation** was diagnosed by coproscopic and necropsic analysis, with a prevalence of 40.62%. Increased infestation cases were rare, the number of adults observed in necropsied animals ranging from 3 to 67 copies.

Eggs were coproscopically identified with a percentage of 19.83% extensivity. Extensivity of *Parascaris equorum* infestation also presented differences from one lot to another. For the coproscopic examined lots, extensivity varied as follows: horses from extensive system, Suceava County - 23%, horses from extensive system, Iași County - 38%; Rediu Farm, Iași - 83.33% Dumbrava Stallion Deposit - 16.66%.

***Oxyuris equi* infestation** was identified by necropsy in a similar percentage to ascarids infestation (37.5%). Number of *Oxyuris equi* adults from colon lumen was between 4 and 65



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individuals/animal. Extensivity was found to be low by coproscopic methods, "Scotch-test" respectively, and infestation was diagnosed in only 8% of the cases, compared to the necropsic analyses. We noted a high incidence of oxiurosis infection in horses from Bacău and Iași Counties, over 50% compared to other counties, where percentage was below 25%

***Strongyloides westeri* infestation.** *Strongyloides westeri* parasites were evidenced only by coproscopic analyses, identifying the characteristic eggs. The parasitosis had a low prevalence of 9.5% in live population of the examined horses. Three groups of horses were infested with prevalence values as follows: horses from Rădăuți Stud Farm- 16.66%; horses from the extensive system Suceava County - 7.69%; horses from the extensive system, Iași County - 17.24%.

***Setaria spp.* infestation** had two species as etiological agents - *Setaria equina* and *Setaria digitata*. Parasites had been found in the peritoneal cavity in a variable number, included from 3 to 16 individuals/animal, with an average of 9 individuals. Regarding the necropsic examined horses, setariosis had a extensivity of 34.37%. *Setaria equina* was diagnosed in a percentage of 74%, and *Setaria digitata*, in a percentage of 26%.

Widespread areas of the parasites were found in Iași and Bacău Counties, probably due to a more extensive development of vectors transmitters of infested elements in these areas. It was also noted that *Setaria equina* was especially prevalent in areas with high incidence and *Setaria digitata* in low incidence areas, and could thus be said that there is a different area for each species, depending on the environment factors and conditions aimed at developing agents' transmitters.

***Gasterophilus spp.* infestation** could be evaluated by necropsy and after observing larvae in faeces, after deworming. By stereomicroscopic examination of larvae one species was identified, namely *Gasterophilus intestinalis*. The number of larvae found in an animal ranged from a very low infestation, 3-4 larvae, up to 200 larvae infestations.

In the analyzed equine population, gasterophilosis was diagnosed with a prevalence rate of 43.21%. When horses come dewormed, larvae were observed at a rate of 34.86%, and in necropsied horses the percentage was of 51.56%. The wide spread of the disease was reported in Iași and Bacău Counties, especially in the extensive system.

Chapter VI contains the results of the **clinical diagnosis and pathology of the digestive parasitoses and peritoneal serous**. Following clinical examination, there was a general trend of an inapparent subclinical evolution of digestive parasitic diseases without obvious problems, despite high extensiveness of these disorders and mixed parasitic colonization of the digestive tract. In some

animals, with maintenance deficiencies and heavily infested, they could find general symptoms such as weakness, fatigue on exertion, irregularity, loss of hair gloss, ruffle, pale mucous membranes.

Specific symptoms were noted only in the oxiurosis infection, represented by perianal itching with scratching trend, depilations and hair ruffle from the tail, accompanied by thickening and scaling of the skin in these areas.

A number of 33 horses slaughtered in the Nicşan abattoir, Şerbăuţi Com., Suceava County, were examined morphopathologically; 20 horses slaughtered in the Nicbac Prod abattoir of Nicolae Bălcescu Com., Bacau County, and 11 specimens were examined in the Anatomy and Morphopathology labs of the Faculty of Veterinary Medicine Iaşi.

The necropsy was performed by using the organics systems methods and apparatus (Abricosov method), and the histopathological examination was performed following permanent histological preparations obtained by the method of inclusion in paraffin and stained by the HEA method.

By necropsy of digestive tract and cavitory serous parasitism in the following sections and organs was revealed: stomach, duodenum, jejunum, ileum, cecum, colon, rectum and peritoneal serous, all animals being diagnosed with multiple parasites simultaneously.

Macroscopic pathology panel was dominated by ulcers determined by digestive mucosa parasite attachment, congestions and catarrhal inflammation of the digestive mucosa. Without special pathological implications, but with an increased frequency, setariosis was diagnosed as a parasitic disease of the peritoneal cavity.

Histopathologically, in addition to non-specific reactions dominated by eosinophilic inflammation of the mucosa and submucosa along the digestive tract, parasitic granulomas containing strongyls and oxiuris larvae in chorion of intestinal mucosa and *Eimeria* oocysts were identified.

Chapter VII entitled "Observations on the control of digestive parasitoses in horses and effectiveness of some antiparasitic compounds" provides data from several lots of both equine growth systems, aimed at prevention and control issues.

The analyzed substances include ivermectin, fenbendazole and pyrantel (as pamoate) most of them used like antiparasitic substances in horses. In vitro FECRT test (faecal egg count reduction test) was conducted to assess treatment effectiveness in combating strongylidoses. Results obtained in two tested lots for ivermectin, showed high efficiency, 100% and 98.33%; 97.11% for



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fenbendazole (one tested lot); 98.21% for pyrantel (one tested lot), which shows no strongyls resistance phenomena on the lots of examined horses.

In addition to testing the efficacy of these three substances for strongylidosis, combating data were obtained about applied chemically control protocol of equine digestive parasitoses from farms and households of the studied areas. Chemical compounds used for this purpose are: avermectins (ivermectin, abamectin), benzimidazoles (fenbendazole, albendazole), tetrahydropyrimidines (pyrantel pamoate), piperazine derivatives (praziquantel). The administration of these substances is often made orally with commercial products in paste form or oral suspension.

A wide use of ivermectin in all lots of horses has been found, either alone or in alternation with benzimidazolic products or less, with tetrahydropyrimidines. The alternation of substances is made annually or seasonally, especially in small farms from semi-intensive system. In the extensive system, treatment is usually carried out at the request of the owners, generally twice a year and with the same substance, rarely alternating the used products. The treatment is administered once, in some cases, repeated every two weeks.

Regarding the resistance of equine digestive parasites to some antiparasitic compounds, there have been found by numerous national and international studies, mostly cases of benzimidazoles decreased efficiency in combating cyathostomosis. Recurrence of eggs in the faeces after treatment and nematode *Parascaris equorum* resistance to macrocyclic lactones has been shortened.

In order to extend the control possibilities and avoid chemoresistance installing, some studies on the efficacy of herbal extracts to combat strongylidosis both *in vivo* and *in vitro*, often with promising results, have been nationally performed.

Chapter VIII - "Conclusions" - includes a synthetic assessment of all findings presented in each chapter, highlighting the main issues aimed at by researches and their practical importance.