Endoparasites of rabbits (Oryctolagus cuniculus domesticus) in Southern Ukraine

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Introduction

One of the main tasks of ecological parasitology at the present stage is to establish patterns of spread of invasive diseases of animals in the environment through a thorough epidemiological examination, as well as to determine the main directions and factors of spread of parasites in Ukraine. Among the many pathogens, endoparasites play a major role in a number of diseases in rabbits with increased morbidity and mortality. In the body of wild and domestic rabbits several species of parasites that form a parasitocenosis can be localized. The latter have a pathogenic effect on organs and tissues, leading to reduced weight gain, premature slaughter and even mortality.

The prevalence of endoparasitoses was studied in weaned rabbits 60 days of age, 120 days of age at fattening, 180 days of age – mating age and adults of 320 days of age to determine the extensiveness and intensity of the dominant invasion. A total of 720 head was studied, 180 animals from each age group. Three species of nematodes were recorded in the rabbits: Trichonematidae retortaformis (Zeder, 1800), Nematodirus leporis (Ransom, 1907) and Passalurus ambiguus (Rudolphi, 1819); the cestode Taenia pisiformis (Bloch, 1780); three species of Eimeria: Eimeria stiedae (Lindemann, 1865), which parasitizes in the bile ducts of the liver and gallbladder; E. magna (Porul, 1925) and E. media (Kessel, 1929) – in the epithelial cells of the intestine. The prevalence of the infestation depends on the age of the animals. According to the data obtained, coccidiosis was one of the main parasitic diseases of the rabbits. Dominant invasions of Eimeria in the intestines of 60-day-old rabbits were found in the studied animals, their extensiveness reached 19.4%, while the spread of hepatic Eimeria was registered in 13.3% of the examined rabbits. The total infestation of weaned rabbits with helminths was 22.2%, in rabbits for fattening – 26.7%, and in rabbits of mating age and adults – 20.0% and 20.6%, respectively. Global climate change will change the distribution and dynamics of soil-borne helminthiases, but host immunity may also affect host-parasite interactions. Subsequent studies will be aimed at elucidating the effect of mono and mixed invasions on the body of rabbits. Updated data on helminthiasis will expand the screening strategy to maintain rabbit health and reduce economic losses.

Keywords: Trichonematidae retortaformis; Nematodirus leporis; Passalurus ambiguus; Taenia pisiformis; Eimeria spp.

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One of the main tasks of ecological parasitology at the present stage is to establish patterns of spread of invasive diseases of animals in the environment through a thorough epidemiological examination, as well as to determine the main directions and factors of spread of parasites in Ukraine. Among the many pathogens, endoparasites play a major role in a number of diseases in rabbits with increased morbidity and mortality. In the body of wild and domestic rabbits several species of parasites that form a parasitocenosis can be localized. The latter have a pathogenic effect on organs and tissues, leading to reduced weight gain, premature slaughter and even mortality.

The prevalence of endoparasitoses was studied in weaned rabbits 60 days of age, 120 days of age at fattening, 180 days of age – mating age and adults of 320 days of age to determine the extensiveness and intensity of the dominant invasion. A total of 720 head was studied, 180 animals from each age group. Three species of nematodes were recorded in the rabbits: Trichonematidae retortaformis (Zeder, 1800), Nematodirus leporis (Ransom, 1907) and Passalurus ambiguus (Rudolphi, 1819); the cestode Taenia pisiformis (Bloch, 1780); three species of Eimeria: Eimeria stiedae (Lindemann, 1865), which parasitizes in the bile ducts of the liver and gallbladder; E. magna (Porul, 1925) and E. media (Kessel, 1929) – in the epithelial cells of the intestine. The prevalence of the infestation depends on the age of the animals. According to the data obtained, coccidiosis was one of the main parasitic diseases of the rabbits. Dominant invasions of Eimeria in the intestines of 60-day-old rabbits were found in the studied animals, their extensiveness reached 19.4%, while the spread of hepatic Eimeria was registered in 13.3% of the examined rabbits. The total infestation of weaned rabbits with helminths was 22.2%, in rabbits for fattening – 26.7%, and in rabbits of mating age and adults – 20.0% and 20.6%, respectively. Global climate change will change the distribution and dynamics of soil-borne helminthiases, but host immunity may also affect host-parasite interactions. Subsequent studies will be aimed at elucidating the effect of mono and mixed invasions on the body of rabbits. Updated data on helminthiasis will expand the screening strategy to maintain rabbit health and reduce economic losses.

Keywords: Trichonematidae retortaformis; Nematodirus leporis; Passalurus ambiguus; Taenia pisiformis; Eimeria spp.
and can be used to develop science-based measures to combat and prevent invasion diseases.

The aim of the study was to determine the extensity and intensity of endoparasites of rabbits in farms in Southern Ukraine.

Materials and methods

The experiments performed on animals did not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 “On protection of animals from cruel treatment”) and “General ethical principles of animal experiments”, adopted by the First National Congress of Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes, Strasbourg, 1985) (Simmonds, 2018; Kalabne & Buadel, 2019). The research program was reviewed and approved by the Bioethics Commission of the National Research Center “Institute of Experimental and Clinical Veterinary Medicine” in the current order.

The research was conducted on specialized rabbit farms of BBPPROM LLC, p. Shemetove of Berezovsky district (165 rabbits were inspected), LLC Odessa-MIAKRO with p. Nerusubis of Bilyav district (172 rabbits were inspected) and PSP “Druzhba” of Izmail district (115 rabbits were inspected) in which the type of feeding was mixed (hay, antiparasitic drugs. To detect eggs of helminths — in the laboratory of parasitology (75 rabbits were inspected) in which the type of feeding was mixed (hay, grain and root crops were additionally added to the granulated fodder).

The diagnosis was established taking into account laboratory studies and postmortem autopsy data, carried out in the laboratory of parasitology of the Odessa Experimental Station of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” and the slaughterhouse during planned and forced slaughter of rabbits.

The prevalence of endoparasitoses was studied in weaned rabbits 60 days of age, 120 days of age at fattening, 180 days of age – mating age and adults of 320 days of age to determine the extensiveness and intensity of the dominant invasion. A total of 720 head was studied, 180 animals from each age group. Prior to the examination, the rabbits did not receive antiparasitic drugs. To detect eggs of helminths Passalurus ambiguus, Nematoidea leporis and Trichonodraea retortiformis, faecal samples of rabbits were treated by standard parasitological methods of Fulleborn and Darling according to GOST 25383-82ST CMEA 2547-80. We used a biological light microscope “Biomed +” XSM-20 (“BioMed”, China) that serves as a guide for counting helminth eggs. After counting the helminth eggs, their number was multiplied by 15 and a number obtained that was taken as the number of eggs in 1 g of feces of the test animal.

The intensity of helminth infection in animals was considered to be the average number of parasite eggs in 1 g of each animal’s feces according to the results of three studies.

Immediately after slaughter, the carcasses were carefully examined for protozoa and helminths. The small intestine of each rabbit was separated from the mesentery, cut longitudinally and immersed in phosphate-buffered saline (pH 7.2) for 10 minutes. The mucosa was scraped with a scalpel, after which both the intestinal contents and the mucosal scraping were washed with tap water through a sieve and examined both with the naked eye and under a microscope (8 × 10) (Soubly, 1982; Bowman, 2020). Identification of parasites was performed on morphological grounds (Knopp et al., 2009; Dolbin et al., 2012; Jiménez et al., 2016).

The intensity of eimeriosis invasion was determined by counting the number of Eimeria oocysts in the microscopic specimen at low magnification (8 × 10). The oocysts in the faeces were counted according to a standardized combined method for determining their number in 1 g of faeces. Eimeria species identification was determined using biological characteristics such as oocyst size and morphology (curvature, presence or absence of oocyst residues, visible / invisible micropyle, sporulation time) (Bowman, 2020). The level of spontaneous coccidiosis in rabbits was determined visually after slaughter and at autopsy by the number of specific blisters on the internal organs.

The datasets were expressed as mean (x) ± standard error of the mean (SE). Mathematical analysis of the study results was conducted in Statistica 13.3 IT Application (StatSoft Inc., USA). Differences between average values were considered statistically significant at P < 0.05 (ANOVA).

Results

Eimeria infestation of 60-day-old rabbits was 47.2%, 120-day-old rabbits – 18.9%, 180-day-old rabbits – 5.6% and 320-day-old rabbits – 1.7%, represented by three species: E. stiedae – parasitizes in the bile ducts of the liver and gallbladder, E. magna and E. media – in the epithelial cells of the intestine (Table 1).

Table 1

| Pathogen                      | 60 days of age | 120 days of age | 180 days of age | 320 days of age |
|-------------------------------|----------------|----------------|----------------|----------------|
|                               | inflected /   | intensity,     | inflected /   | intensity,     |
|                               | prevalence,%  | thousand        | prevalence,%  | thousand        |
|                               | in 1 g of    | oocysts in 1 g  | in 1 g of    | oocysts in 1 g  |
|                               | of feces     | of feces       | of feces     | of feces       |
| Eimeria stiedae (Lindermann, 1865) | 24 / 13.3    | 3.21 ± 0.92    | 18 / 10.0    | 7.28 ± 4.49    |
| E. magna (Perad, 1925)         | 35 / 19.4     | 8.56 ± 3.24    | 11 / 6.1     | 6.51 ± 2.21    |
| E. media (Kessel, 1929)         | 26 / 14.4     | 6.31 ± 2.12    | 5 / 2.8      | 4.32 ± 3.44    |

In rabbits aged 60 days, the intestinal form of eimeriosis was more often registered with an extent of 33.9%, while the hepatic form was registered in only 13.3% of rabbits. As the age of the rabbits increased, the extensiveness of invasion and intensity of the eimeriosis invasion decreased. In 120-day-old rabbits, there was a 2.5-fold reduction in eimeriosis lesions, but the hepatic form of E. stiedae was predominant, with a maximum invasion intensity of 7.28 ± 4.49 thousand oocysts in 1 g of feces. With a decrease in the invasion of rabbits 180 days of age with E. magna (5 times), there were changes in the intensity of the invasion, which was manifested by a decrease of 2.3 times and the detection of carriers of E. stiedae and E. media with indicators from 2.81 ± 3.49 to 4.36 ± 1.79 thousand oocysts in 1 g of feces. In 320-day-old rabbits E. media was not registered, while E. stiedae and E. media were also carriers. Thus, with the age of rabbits, the degree of damage by eimeriosis decreases, which proves the formation of non-sterile immunity and reduces the infection of animals in the case of reinvasion and superinvasion.
thickenings measuring 15 x 20 mm were visually visible in some areas of the hungry intestine.

In the hepatic form of eimeriosis, small speckled hemorrhages were observed in some parts of the liver, and whitish nodules of various sizes were observed on the surface of the organ (Fig. 2).

Fig. 1. Large bowel overflow of gases from *E. magna*

Fig. 2. Parasitic granulomas in rabbit liver with *E. stiedae*

The liver was enlarged, dark cherry colour, the capsule was moist, tense, unevenly hilly. The bile ducts were wide and well visible. In 120-day-old rabbits with a high level of *E. stiedae*, the gallbladder was enlarged 2-3 times due to excessive overflow of thick dark green bile (Fig. 3).

Fig. 3. Rabbit gallbladder is full of bile with a high level of *E. stiedae*

In 180-day-old rabbits, the extent of pathogen damage was the lowest, but the intensity of the invasion increased. Due to reinvasion, the intensity of *P. ambiguus* increased 4.0 times, *T. retortaeformis* increased 3.3 times and *T. pisiformis* increased 2.8 times compared to 60-day-old rabbits. During the dissection of rabbits, small spindle-shaped white helminths *P. ambiguus* were found in the cavity of the colon. The intensity was high and was 167.24 ± 2.47 specimen/animal (Fig. 5). Also in rabbits of this age group a high degree of intensity of *T. pisiformis* was registered – 67.14 ± 2.22 cysticerci. The largest number of cysticercosis vesicles was on the mesentery and omentum, and at low intensity of cysticercosis invasion, the vesicles were localized only on the serous membrane of the rectum (Fig. 6). In 320-day-old rabbits, along with live cysticerci, the presence of degenerated *T. pisiformis* as a result of different life expectancy of parasites and re-infection of animals was recorded. It should be noted that 42% of cysticerci were dark brown, i.e. degenerate, which indicated their non-viability (Fig. 7).
Fig. 5. *Passalurus ambiguus* in the cavity of the colon

Fig. 6. *Taenia pisiformis* on the mesentery

Fig. 7. Degenerated *Taenia pisiformis* on the mesentery

Adult rabbits also showed a 7.1-fold increase in the intensity of *T. retortaeformis* lesions, leading to intestinal enlargement and bloating (Fig. 8).

Discussion

Parasitism is one of the antagonistic trophic relations in which an organism uses another species (host) as a temporary or constant place for living and source of nutrients. It completely depends on the host, negatively affects the host’s condition, and can cause the host’s death (Boyko et al., 2016). According to numerous studies, parasitoses contribute to development of somatic diseases, complications of chronic diseases, and affect the immune system. Characteristic of most helminths is the chronic course of the disease, related to the long term presence of the pathogen in the organism and possibility of repeating the infestation (Karilova et al., 2020, 2021). At the same time, clinical features are manifested poorly, and pathological-anatomical changes are accompanied by productive processes which can practically change the functioning of part of the organ. A significant role in the pathogenesis of helminthoses is considered to be played by their mechanical impact conditioned by fixation of the parasites (using suckers, spikes, mouth capsules, cutting plates) and traumatization of the tissues or the host’s organ, and is also related to the migration of larvae in the organism. During migration, nematode larvae synthesize enzymes which activate their penetration to their places of localization. At the same time, helminth larvae provide opportunities for penetration of infectious agents (Thompson et al., 1994).

Fig. 8. *Trichostrongylus retortaeformis* in the chyme of the intestinal mass

Researchers believe that intestinal parasitosis is primarily a sanitary problem, and its prevention should be based on constant sanitary and parasitological monitoring (Atehmengo & Nnagbo, 2014; Takeuchi-Storm et al., 2019). One of the conditions that can cause susceptibility of the host organism and transmission of parasites is the occurrence of concomitant infections. Parasites persist directly inside the body and often these interactions modulate the host’s immune response (Cattadori et al., 2007; Foster & Elsheikha, 2012).

Domestic rabbits belonging to the species *Oryctolagus cuniculus* are social animals and are descended from European animals (Owuor et al., 2019). It is well known that domestic rabbits are intended for meat consumption and are a valuable source of a number of nutrients, but may contain many different endoparasites, including *Passalurus ambiguus* (Abdel-Gaber et al., 2019), *Trichostrongylus retortaeformis* (Cattadori et al., 2019), *Nematodirus leporis* (Tizzani et al., 2020), *Cysticercus pisiformis* (Chen et al., 2021) and *Eimeria* spp. (Ladron de Guevara et al., 2019).

Gastrointestinal disorders in rabbits with coccidiosis are characterized by diarrhea, which can lead to high mortality (Shola et al., 2019). At the same time, intensive rabbit breeding has led to an increase in the transmission of parasites with a direct life cycle (*P. ambiguus* and *Eimeria* spp.) (Rinaldi et al., 2007; Kornaś et al., 2015). *Passalurus ambiguus* and *Eimeria* spp. are two common intestinal parasites in rabbits that can, among other symptoms, cause diarrhea and, in severe cases, death (Sioutas et al., 2021).

Parasitological examination of the internal organs of domestic rabbits revealed damage by coccidia (78.8%), nematodes (*Obolelloides cuniculi, Graphidium strigosum, Trichostrongylus* spp., *Strongylodes* spp., *Passalurus ambiguus, Trichuris leporis* (16.4%) and cestodes *Cysticercus pisiformis* (4%)). The intestinal form of coccidiosis was dominant and reached 56.5%, while the extensiveness of the hepatic form of coccidiosis was significantly lower – 3.3% of the surveyed rabbits (Szkucik et al., 2017).
2014). In Finnish domestic rabbits, *Eimeria* oocysts were detected in 27% of samples, *Passalurus ambiguus* eggs were detected in 3% of samples, and *Trichuris leporis* eggs and cestode eggs, respectively, were detected in 1 sample (0.25%) (Mäkitaipale et al., 2017). Some studies show that the largest proportion of infected rabbits (95%–100.0%) are animals from rabbit farms (Harrad et al., 2019; Sioutas et al., 2021). Excessive stocking density of animals with extensive housing system, contributes to the rapid spread of the invasion. Significantly lower prevalence of coccidiosis (8.3–51.0%) is recorded in rabbits living in the wild (Foronda et al., 2005; Silva et al., 2015). In the province of Grosseto (Central Italy), necroscopic analysis of wild hares revealed the helminths *Trichostrongylus retortaeformis* (87.1%), *Passalurus ambiguus* (12.9%), and *Andrya* spp. (6.4%) in the intestines, *Protostrongylus canicularum* (8.3%) in the lungs and *Dicrocoelium dendriticum* (16.7%) in the liver. The prevalent intestinal helminths in breeding hares were *Passalurus ambiguus* (12.1%) and *Trichostrongylus retortaeformis* (3%). Coprological analysis showed the prevalence of coccidia at the level of 64.9% in wild hares and 45.5% in bread hares (Sergi et al., 2018). Chylinski et al. (2009) note that the intensity of *Trichostrongylus retortaeformis* increased and reached a maximum in young rabbits and decreased slightly in adults. The causative agent of *Trichostrongylus retortaeformis* was the second most numerous species of nematode with an extent of 42% among various breeds of domestic rabbits, while in wild rabbits it had a prevalence of 11.6% (Yagoob & Hossein, 2011).

Mignati et al. (2016) note that global climate change will change the distribution and dynamics of soil-borne helminthiases, but host immunity may also affect host-parasite interactions. Most of the problems that affect the physiological state of the domestic rabbit's gastrointestinal tract are caused by hereditary or housing factors, many of which can be addressed through basic veterinary procedures and proper diet (Harrenstein, 1999; Nowland et al., 2015). Thus, knowledge of the dependence of the extent and intensity of the invasion of diseases of the digestive organs of rabbits on their age and season is a well-founded indicator in the planning and implementation of treatment and prevention measures.

Conclusions

It has been proved that in the south of Ukraine, rabbit endoparasitoses are a fairly common invasive disease, the causative agents of which are *Trichostrongylus retortaeformis, Nematostrongylus leporis, Passalurus ambiguus, Taenia pisiformis and Eimeria stiedae*. *E. magna*. 60-day-old rabbits are most infected with the intestinal *Eimeria E. magna* and *E. media*, 120-day-old rabbits are most infected with the hepatic form of coccidiosis caused by *E. stiedae*, while in adult rabbits coccidiosis is recorded as a carriers. *Trichostrongylus retortaeformis* and *Taenia pisiformis* were mostly recorded in 120-day-old rabbits, while the extensiveness of *Passalurus ambiguus* and *Nematostrongylus leporis* was almost the same in all age groups.

Authors state no conflict of interest.

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