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Improving agricultural production of domestic rabbits in Serbia by follow-up study of their parasitic infections

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Summary

A study was made on the prevalence of some parasitic infections appearing in domestic rabbits obtained from individual breeders in Serbia in order to improve the agricultural production of these animals. Aside from economic reasons (meat production and reproduction) rabbits are bred for the research purposes, and races are kept as household pets. For these reasons, among others, it is important to gain knowledge of medical culprits including causes of parasitic diseases that compromise their health, well-being and cause economic losses. This parasitological research was conducted in the period from 2010 to 2015 in 8 epidemiological regions of Serbia, on 433 rabbits as representative samples of different races (154 individuals up to 1 years of age and 279 individuals older than 5). Out of the total number of examined rabbits parasitic infections were established in 82.68% of animals. We detected 3 species of endoparasites (*Eimeria* spp., *Trichostrongylus* spp., and *Passalurus ambiguus*) and 3 species of ectoparasites (Scabies from genera *Sarcoptes*, *Psoroptes* and *Notoedres*). In "kits" (small rabbits) coccidiosis was the most prevalent disease (50.65%), while in older animals trichostrongilidosis was common (39.07%). The most represented scabies infection was with the species *Psoroptes cuniculi* (12.01%). Aiming at better control on the health of rabbits, there is a growing need for continual monitoring of parasitic infections including appropriate diagnosis, application efficient therapeutic protocols and control measures.

Key words: Domestic rabbits, Individual breeders, Parasitic infection, Serbia

Introduction

Domestic rabbit (*Oryctolagus cuniculus var. domesticus*) originates from wild predecessor, which was domesticated in our past, cultivated to meet the needs for meat and fur. Certain research was initiated in ancient Rome, while the first relevant data were obtained in the middle ages. Since then, breeding selection of domestic rabbits has gone through numerous changes related to its appearance, behavior and fertility (Hemmer, 1990).

As opposed to other countries of Europe, in Serbia rabbit breeding is not appreciated as cattle breeding, this is why the state does not provide financial aid for those engaged in rabbit breeding. Although this branch of farming is less demanding and cheaper, there is only a few rabbit breeding locations in Serbia (regions of Vojvodina and central Serbia) engaged professionally with the aim of producing meat, wool and fur. Most of the breeding farms sell their product to veterinarian institutions involved in vaccine productions. The most prevalent races are Pannonia white rabbit and Hajkom breed, and German giant rabbit, the most massive of all (Urošević *et al*., 2000).

Aiming for better health of rabbits, it is necessary to provide proper hygiene, adequate nourishment and health control. This involves prevention, spread and control of bacterial, viral and parasitic etiologic disease (Okerman, 1994).

The analysis of available research data established the abundance scientific articles dealing with parasitic fauna of wild rabbits, compared to domesticate ones (Ilić *et al*., 2014). Parasitic infections of domestic rabbits were studied in Poland (Sadzikowski *et al*., 2008; Szkucik *et al*., 2014), Germany (Haupt, 1975; Düwel, 1980; Burger *et al*., 2006), France (Audebert *et al*., 2003), Spain (Gomez-Bautista *et al*., 1987; Tenora *et al*., 2002), Czech Republic (Pakandl and Hlásková, 2007; Pakandl *et al*., 2008; Pakandl, 2009), Italy (Papeschi *et al*., 2013), Egypt (Ashmawy *et al*., 2010; Sultan *et al*., 2015), India (Darzi *et al*., 2007; Mitra *et al*., 2014; Galdahar *et al*., 2015), Iraq (Altamemy, 2014), China (Cai *et al*., 2012), US (Alicata, 1932; Yeatts, 1994) and New Zeeland (Rinaldi *et al*., 2007; Sivajothi *et al*., 2014).

Today individual breeders dominate in Serbia, which motivated us to present the research of multiyear study, related to continual follow-up of parasitic infections in domestic rabbits including health issues and control.

Materials and Methods

The research was conducted in the period from 2010 to 2015, covering 8 epidemiological regions of Serbia. Parasitological research was performed in 433 rabbits (representative sample) of different races (Pannonia white rabbit, Hajkom, German giant rabbit, Belgian

giant, New Zealand white rabbit, Chinchilla), and all originated from individual breeders, and the body mass was between 4.65 ± 0.85 kg. Rabbits were kept in a cage system with litter (straw or shavings). Animals were divided into two groups: younger individuals (154) - to 1 years of age and older ones (279) - older than 5 years. One group was bred for meat production. The second group was raised for breeding and sale as household pets.

Examined material (faeces and skin scarification) was sampled two times a year during March-April and October-November. Faecal samples were analyzed by qualitative techniques for separating and detecting lungworm larvae (method by Vajda) and concentrating eggs (Flotation method). The rabbit infection by lung nematodes (*Protostrongylidae*) was investigated by testing fresh faeces using the Vajda method. Approximately 3-4 g of faecal samples were examined by a conventional flotation method (according to Melhorn *et al*., 1993). The flotation solution was prepared by mixing 800 ml distilled water, 210 g NaCl (Carl Roth GmbH + Co KG, Karlsruhe, Germany) and 220 g $ZnCl_2$ (Sciencelab.com, Inc., Houston, Texas, USA) and adjusting the specific gravity 1.2-1.3 with a density hydrometer. Each sample was homogenized thoroughly on a vortexer in 50 ml preparation tubes (with sealing cap) with approx. 15 ml of the sodium chloride/zinc chloride solution. The suspension was sieved through a strainer into a 12 ml centrifuge tube, filled and centrifuged for 8-10 min at 300 g. Afterwards the tube was filled with flotation solution to form a convex meniscus at the top. Ten min later a coverslip was placed carefully in contact with the meniscus, lifted off and placed on a glass slide for microscopic examination. The cover glass was analyzed at ×100 magnification in a meandering pattern. Suspicious structures were confirmed at a higher magnification. Parasite stages found (eggs and/or oocysts) were recorded and classified according to genus, including coccidia oocysts (*Eimeria*) by Duszynski and Couch (2013) and nematode eggs (*Trichostrongylus*, *Passalurus*) by Skryabin *et al*. (1954) and Hugot *et al*. (1983).

Dermatological analysis was performed by the "klatch" of the preparation and by the method of cooking (Klayman and Schillhorn van Veen, 1981).

Regarding the fact that egg shedding of some species of helminths that infest rabbits is affected by circadian rhythm, the faeces of the suspicious animals were sampled in the afternoon and evening hours when the parasitic element is expected to be shed.

The statistical analysis was done in Graph Pad Prism software. For determining a statistically significant difference between age groups of domestic rabbits that tested positive for certain parasites we used the Hi-square (χ^2) test.

Results

The parasitic infections were diagnosed in 82.68% of the total number of examined rabbits (358/433), with the prevalence of 87.01% (134/154) in young animals and 80.27% (224/279) in older ones. We detected 3 species of endoparasites (*Eimeria* spp., *Trichostrongylus* spp., and *Passalurus ambiguus*) (Figs. 1A-C) and 3 species of ectoparasites (mange from the genera *Sarcoptes*, *Psoroptes* - Fig. 2A and *Notoedres* - Fig. 3A). In young rabbits the most prevalent was coccidiosis with frequency of - 50.65% (78/154), while in older ones, we detected *Trichostrongylus* spp - 39.07% (109/279). In mange investigation we diagnosed the highest prevalence of infection with the species *Psoroptes cuniculi* - 12.01% (52/279). In older individual rabbits it is statistically important ($P<0.001$) that all three species of ectoparasites and endoparasites were found higher, compared with younger individuals, except for *Trichostrongylus* spp. with no statistical significance. Also, in a significant number of older rabbits ($P<0.05$) no difference was detected between young and old individuals (Table 1).

Poly-parasitism of two causative species was diagnosed as 9 different mixed infections, of which the most prevalent in older individuals was the infection with species from the genera *Eimeria* and *Trichostrongylus* (12.90%), while younger ones were infected mostly with *Eimeria* spp. - *Sarcoptes cuniculi*

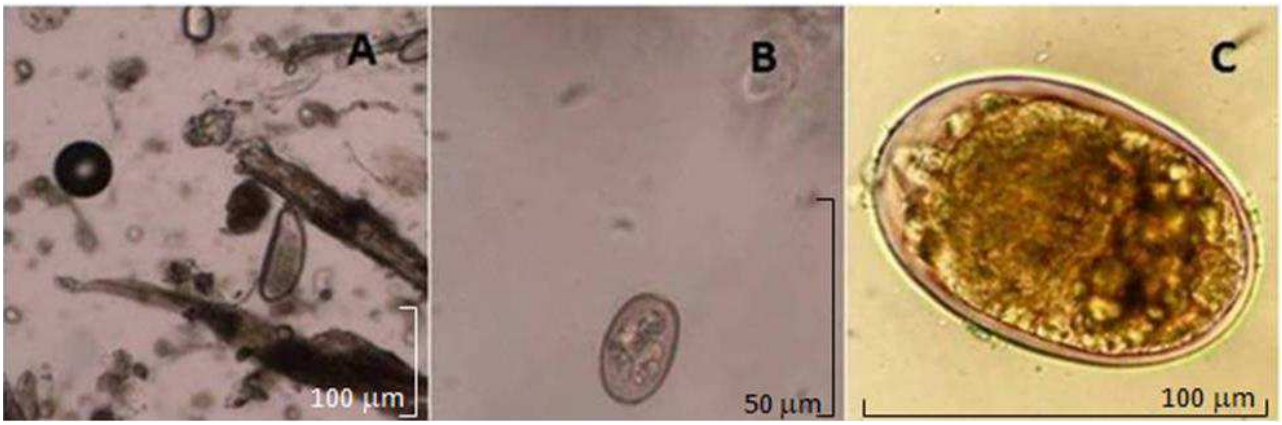

Fig. 1: Coprological examination of rabbits - Flotation method. (**A**) *Passalurus ambiguus* egg (×100), (**B**) Sporulated oocyst *Eimeria* spp. (×400), and (**C**) *Trichostrongylus* spp. egg (×400)

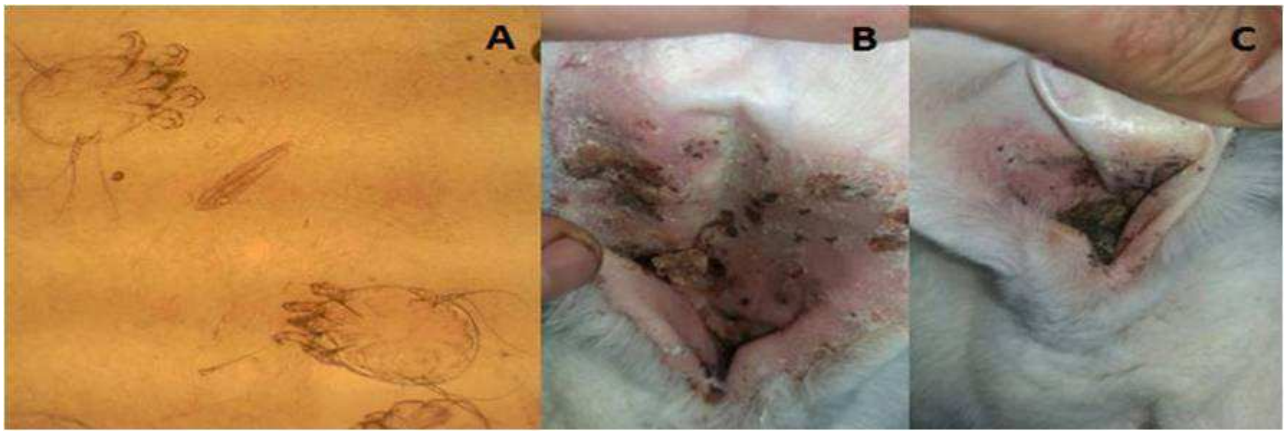

Fig. 2: Parasitologic and clinical findings of mange *Psoroptes cuniculi*. (**A**) Adult forms, (**B**) left ear, and (**C**) Right ear – clinical findings on rabbit (New Zealand white) ears

Fig. 3: Parasitological and clinical finding of mange *Notoedres cuniculi*. (**A**) Egg and adult form, (**B**) left ear, and (**C**) right ear - clinical findings on rabbit ears (Belgian gigant)

Table 1: Prevalence of parasitic infections of domestic rabbits of different age

Parasites species	Number of domestic rabbits (prevalence %)						χ^2	P
	Age category							
	Young animals (to 1 year old) (n=154)		Adult animals (over 5 years) (n=279)		Total (n=433)			
	No.	%	No.	%	No.	%		
Eimeria spp.	78	50.65	105	37.63	183	42.26	6.89	0.01***
Trichostrongylus spp.	66	42.85	109	39.07	175	40.41	0.59	0.44
Passalurus ambiguus	9	5.84	65	23.29	74	17.09	21.33	0.00***
Psoroptes equi var. cuniculi	0	0	52	18.63	52	12.01	32.62	0.00***
Notoedres cati var. cuniculi	0	0	23	8.24	23	5.31	13.41	0.00***
Sarcoptes scabiei var. cuniculi	18	11.69	0	0	18	4.15	34.03	0.00***
Without parasites	20	12.98	55	19.71	77	17.78	3.14	0.08*

* P<0.05, and *** P<0001

(11.04%). Poly-parasitism of the infectious was diagnosed only in older rabbits as 5 different mixed infections of which the prevalence was lower than 1.5%.

In older animals we found statistically significant prevalence (P<0.001) of mixed infections (*Eimeria* spp. - *P. cuniculi*; *Trichostrongylus* spp. - *P. ambiguus* and *Trichostrongylus* spp. - *P. cuniculi*) compared to younger individuals where a significantly higher appearance (P<0.001) of mixed infections with *Eimeria* spp. - *S. cuniculi* compared with older individuals was observed (Table 2). In addition, a statically significant prevalence (P<0.05) of mixed infections (*Eimeria* spp. - *P. ambiguus*; *Trichostrongylus* spp. - *Notoedres cuniculi* and *P. ambiguus* – *P. cuniculi*) was established (Table 2).

In 18.63% (52/279) of rabbits older than 5 years we diagnosed psoroptic mange, which was mainly presented as mixed infection with *Eimeria* spp., *Trichostrongylus* spp. and *P. ambiguus*. Pathological alterations were clinically manifested at the bottom of an ear lobe, which

Table 2: Prevalence of mixed parasitic infections of domestic rabbits of different age

Mixed infection	Number of domestic rabbits (prevalence %)						$\chi 2$	P
	Age category							
	Young animals (to 1 year old) (n=154)		Adult animals (over 5 years) (n=279)		Total (n=433)			
	No.	%	No.	%	No.	%		
Eimeria spp. *Trichostrongylus* spp.	15	9.74	36	12.90	51	11.78	0.96	0.33
Eimeria spp. *P. ambiguus*	0	0	10	3.58	10	2.31	5.65	0.02*
Eimeria spp. *Psoroptes cuniculi*	0	0	15	5.37	15	3.46	8.56	0.00***
Eimeria spp. *Notoedres cuniculi*	0	0	7	2.51	7	1.61	3.93	0.053
Eimeria spp. *Sarcoptes cuniculi*	17	11.04	0	0	17	3.92	32.06	0.00***
Trichostrongylus spp. *P. ambiguus*	0	0	12	4.30	12	2.77	6.81	0.00***
Trichostrongylus spp. *Psoroptes cuniculi*	0	0	14	5.02	14	3.23	7.98	0.00***
Trichostrongylus spp. *Notoedres cuniculi*	0	0	11	3.94	11	2.54	6.23	0.02*
P. ambiguus *Psoroptes cuniculi*	0	0	10	3.58	10	2.31	5.65	0.02*
Eimeria spp. *Trichostrongylus* spp. *P. ambiguus*	0	0	3	1.07	3	0.69	1.67	0.19
Eimeria spp. *Trichostrongylus* spp. *Psoroptes cuniculi*	0	0	3	1.07	3	0.69	1.67	0.19
Eimeria spp. *Trichostrongylus* spp. *Notoedres cuniculi*	0	0	1	0.36	1	0.23	0.55	0.45
Eimeria spp. *P. ambiguus* *Notoedres cuniculi*	0	0	1	0.36	1	0.23	0.55	0.45
Trichostrongylus spp. *P. ambiguus* *Psoroptes cuniculi*	0	0	1	0.36	1	0.23	0.55	0.45

* P<0.05, and *** P<0.001

followed a spread, encompassing the whole lobe and outer ear duct. Sick animals tended to scratch their ears with their back legs, shake their heads and banded to the sides, left or to the right, according to which the ears were affected. The ear lobe was filled brownish contents resembling scales, and the skin beneath was red and covered with nod-like structures and filled with many different inflamed cells (Figs. 2B and C).

Microscopic evaluation of the scrapings revealed the presence of eggs and adult stages of mites (Fig. 2A). The pedicels of the mites were long and segmented. The tarsal suckers were observed on the pedicels of the first, second, and third pairs of legs in the male mite and on the first, second, and fourth pairs of legs in the adult female mite. Based on the morphology these mites were identified as *Psoroptes* spp. (Soulsby, 2006).

Sarcoptic mange was diagnosed in 11.69% (18/154) of rabbits up to 1 year of age mostly as mixed infection with coccidia. Microscopic evaluation of the morphology and physical characteristics of the mites can be used to differentiate sarcoptic mites from other mites that are found in rabbits. Sarcoptic mites have a thick body wall, with large spines on the dorsal surface (Radi, 2004). The mites have a round body, short legs, a long, un-jointed stalk with a sucker on front pairs of legs, vertical setae and a terminal anus (Radi, 2004). The dorsum has scales, cones, and bladelike setae. The female mites are white, covered with fine parallel striate, and measure 303 to 450

μm in length and 250 to 350 μm in width (Scott *et al*., 2001).

Notoedres cuniculi was established in 8.24% (23/279) of rabbits older than 5 years, with typical clinical alterations on ears (Figs. 3B and C). In the one third of examined rabbits from this age group we detected mixed infection with coccidiae - 2.51% (7/279), and in 0.36% (1/279) as a mixed infection with *Eimeria* spp. and *P. ambiguus*, *Eimeria* spp. and *Trichostrongylus* spp. respectively (Fig. 4).

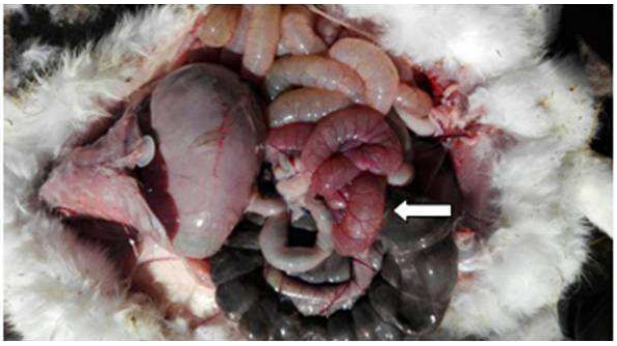

Fig. 4: Necropsy showed a haemorrhagic enteritis in the duodenum (arrow) caused by *Eimeria* spp.

Discussion

According to the last reports of the Statistical Office of the Republic of Serbia (Dissemination and Public Relations Division), the only official report is in 2012. Exactly 9966 individual owners in the Republic of Serbia were breeding rabbits, of which 9962 were private enterprises. The number of females for breeding was 47022. An individual owner is maintaining at least 50 females.

We determined presence of coccidiosis in 42.26% of examined rabbits (50.56%) and second in prevalence in older individual (37.63%). In rabbits the most frequent are mixed infections of intestinal coccidiae, which cause clinical coccidiosis in offspring, with anemia, diarrhea, dehydration, lagging in growth and development (Sadzikovski *et al*., 2008; Pakandl, 2009; Szkucik *et al*., 2014). Coccidiosis is controlled with prophylactic application of anti-coccidials, among which most frequently: salinomycin (20-25 ppm), robenidin (66 ppm), monensin (20 ppm), lasalocid (90.125 ppm) and synergistic mixture - clopidol 20% + methylbenzoquate 1.67% (216.7 ppm). Robenidin is very efficient against intestinal coccidiae, but of low efficiency against *E. stiedae* (Polozowski, 1993; Pakandl, 2009). High degree of efficiency in lowering of discharged coccidia *E. stiedae*, was achieved by toltrazuril. It is applied in drinking water at: 7-10 mg/kg 1 x/day, during 7 days, or 2.5-5.0 mg/kg 2 x/day during 5-6 days, and 5 this treatment is repeated for 5 days (Duszynski and Couch, 2013). Narasin and maduramicin (4.5 ppm) are toxic to rabbits (Polozowski, 1993).

Anticoccidials added to chicken feed are very convenient for kits if it is possible to apply them in drinking water (sulfonamides or toltrazuril) (Pakandl, 2009). Sulfonamides are mostly used for treatment: sulfamethazine (128 mg/kg in duration 1-3 of days), sulfadimetoxine (0.5-0.7 g/1 L of water or 75 mg/kg/daily during 7 days or 15 mg/kg p/o every 12 h during 10 days), sulfaquinoxaline (3.5 g/3.7 L in water during 3 weeks), sulfadimerazine (2 g/1 L of water), trimetoprim-sulfamethoxasole (30 mg/kg every 12 h during 10 days) and sulfadimetoxine-diaverdine (3:1/100 g in food, 3 days or 100 ml/1l of water during 8 days) (Duszynski and Couch, 2013). The remaining mentioned anticoccidials can be used only as prevention.

In this research trichostrongilidosis was diagnosed in 40.41% and were the most prevalent parasites in older (39.07%) and second most prevalent in young animals (42.85%). As diagnostics was based exclusively on coprological examination, identification was based solely on genera. Species that are localized in small intestine (*T. retortaeformis*, *T. affis* and *T. calcaratus*) cause sub-acute or chronical inflammation, while ones localized in stomach (*Graphidium strigosum* and *Obeliscoides cuniculi*) cause chronical inflammatory or fibrinous gastritis, with difficulties in secretions digestion (Audebert *et al*., 2002). Sometimes in wild rabbits *Trichostrongylus* spp. of ruminants may parasitize (Ilić *et al*., 2011a), demonstrating that these wild animals' species can be vectors for domestic rabbits (Saulai and Cabaret, 1998; Musongong *et al*., 2004; Ilić *et al*., 2011b; Ilić *et al*., 2014).

For the treatment of trichostrongilidosis of rabbits it is recommended to use medications from the group Benzimidazole: fenbendazole (10-20 mg/kg, p/o, repeat in 10-14 days), thiabendazole (100-200 mg/kg, p/o), albendazole (10 mg/kg) and ivermectin (0.2-0.4 mg/kg, p/o, s/c, repeat in 10-14 days) (Anderson, 2000).

Passalurosis was diagnosed with prevalence of 17.09%. This oxyurid is one of the most frequent gastro-intestinal nematodes persisting in domestic rabbits, in wild rabbits and in rodents all over the world (Pritt *et al*., 2012). Identification of these nematodes may be of great veterinarian and medical importance, especially between two related species: *P. ambiguus* and *P. nonanulatus*, whose morphological distinction aids in better understanding of taxonomic and phylogenetic (Sultan *et al*., 2015). The prevalence of this oxyuridae varies depending on age and season (Nosal *et al*., 2006; Ashmawy *et al*., 2010). In Egypt, *P. ambiguus* is one the most prevalent helminthes found in domestic rabbits, up to 40% of samples are infected with it and younger animals are more commonly infected than adults (Ashmawy *et al*., 2010).

In the passalurosis therapy the best effect was achieved with: piperazine adipate (200 mg/kg, p/o, repeated in 14 days) (Hillyer and Quensenberry, 1997), fenbendazole (20 mg/kg, p/o, repeated after 10-14 days), thiabendazole (100-200 mg/kg, p/o, one treatment) (Brown, 1993) or 110 mg/kg one treatment + 70 mg/kg for eight doses (Hillyer and Quensenberry, 1997), mebendazole (20-50 mg/kg one treatment), oxibendazole (15 mg/kg, repeated in 14 days). Ivermectin dose 0.4

mg/kg, showed complete lack of efficiency (Tsui and Patton, 1991).

From the point of view of public health sarcoptic mange is of special importance, keeping in mind that causative agent is directly transmitted to humans by hand shake and may well cause self-limiting dermatitis. In rabbits between 7-9 months of age *S. scabiei* was diagnosed with prevalence of 11, 69%. Such a finding is in accordance with results of some researchers from India who determined sarcoptosis in animals 3-10 months of age, which is very frequent and presents a major obstacle in commercial production in this country (Mitra *et al*., 2014). Infected animals are treated with single dose of ivermectin (350 µg/kg, s/c). It is an accepted practice to submerge the whole body of animal in 1/1000 solution of Cypermetrin and administer AD_3E injection (0, 5 ml) to each rabbit in the period of 7 days. Effects of this therapy are obvious even after 14 days, when the skin lesions begin to withdraw and gradually disappear (Altamemy, 2014).

In scientific research, psoroptosis (Ear pest) was diagnosed among older rabbits with the prevalence of 18.63%, a similar finding to that in Kenya. They found this species of mange in over 16% of examined rabbits (Aleri *et al*., 2012; Okumu, 2014). Cases of oto-acarinosis caused by *P. cuniculi* were recorded in rabbits in India (Sivajothi *et al*., 2014), Italy (Fichi *et al*., 2007), Turkey (Kurtdede *et al*., 2007), Romania (Mederle, 2010), Korea (Kyung-Yeon and Oh-Deog, 2010), and USA (Yeatts, 1994).

In the therapy of the mange, success was achieved with ivermectin at 200 µg/kg (1 dose s/c and 10 doses p/o), combined with antibiotic to control secondary bacterial infections (Sivajothi *et al*., 2014) or 400 µg/kg (2 doses s/c, in 15 days interval) in *N. cuniculi* (Galdhar *et al*., 2015).

Predisposing factors responsible for ear infections of parasitic etiology in rabbits may be high temperatures and increased relative humidity, inferior hygiene, feeding methods, way of handling animals and follow the principle of quarantine (Morton *et al*., 2005). As the consequence of global warming climate conditions in Serbia during the last decade are significantly altered. Typical continental climate with four seasons was altered by increasing number of days with high temperatures and abundant rain, thus creating tropical conditions favoring the appearance of psoroptosis (Aiello *et al*., 1998; Ulutas *et al*., 2005). It is assumed that this disease is an antropozoonoses. Keeping in mind that in India a case of infection in humans was recorded in a man engaged as a technician on a rabbit farm and directly exposed to sick animals (Swarnakar *et al*., 2014).

In individual rabbits breeding in Serbia in the period of 5 investigated years, the prevalence of parasitic infections was 82.68%. Coccidiosis was diagnosed in more than half (50.65%) of young rabbits, and in more than one-third (39.07%) of older individuals, trichostrogylidosis was found. For this reason health monitoring of these animals including epidemiological status of parasitic infections, proper time diagnosis of the agents, educating breeders about the importance and necessity of conducting a regular parasitological screening, in order to avoid indirect or direct damage in rabbit breeding while maintaining health measures is of exceptional importance.

Since Serbia is adjusting to European policies, it is to be expected that in the near future rabbit breeding will be recognized as a branch of animal husbandry, which will open foreign markets for our breeders and subsidies.

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