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Mouflon's parasitofauna (*Ovis ammon musimon*, Pallas, 1811) from various natural and anthropised biotopes from the Republic of Moldova

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Abstract
Therefore, the study of the mouflon's parasitofauna of different ages from various biotopes of the Republic of Moldova highlighted a high and diversified level of their infestation with various parasitic agents. At the mouflons from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Teleneşti district, the following parasitic structure was highlighted: Trematoda Class - 3 species (D. lanceolatum, Fasciola hepatica, Paramphistomum cervi); Secernentae Class - 6 species (Strongyloides papillosus, Cooperia punctata, Capillaria bovis, Ostertagia ostertagi, Toxocara vitulorum, Trichostrongylus axei); Cestoda Class - one species: (Moniezia benedeni) and Conoidosida Class with 6 species: (E. asymmetrica, E. austriaca, E. ponderosa, E. capreoli, E. bovis, E. ovinoidalis). At the mouflons maintained in the Zoo from Chişinău town, was highlighted the following parasitic structure: Trematoda Class - 2 species (D. lanceolatum, Fasciola hepatica); Secernentae Class - 5 species (Strongyloides papillosus, Cooperia punctata, Capillaria bovis, Toxocara vitulorum, Trichostrongylus axei) and Conoidosida Class with 5 species: (E. asymmetrica, E. austriaca, E. ponderosa, E. capreoli, E. ovinoidalis). At the mouflons from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Teleneşti district, was highlighted a higher structure and level of infestation compared to that of mouflons maintained in the Zoo from Chisinau town, this being caused by the performance of irregular deworming works or even their lack at those from the Republican Reproduction Center, Forest Enclosure "Mândreşti", Teleneşti district.
Keywords: *mouflon's parasitofauna, various biotopes, different ages.*

Introduction

The European mouflon, also called the wild sheep, is the ancestor of the domestic sheep. It has the body's length of 1.1-1.3 m, the tail of 3–6 cm and the withers's height of 60–80 cm. The weight can reach 50 kg. It can make jumps of 2 meters high and 3-4 m long. The general appearance of the mouflon is similar to that of the ram. Especially the male has ringed, strong and twisted horns. They grow in spiral with the age of the animal, reaching at the 90 cm. Females sometimes wear horns, smaller, up to 10–12 cm. The hoof is the same as that of the domestic sheep. In Romania, the mouflon lived in the past, disappearing in an indefinite period. In the twentieth century it was reintroduced, after several attempts of acclimatization, first in Dobrogea, the center of Muntenia, Argeş county, Alba county. It developed well, although not as fast as would have liked those who took care of their introduction. It has settled mainly in the forests of low hills and hillocks, as well as in the plain forests with relatively high humidity, unlike the original area, rocky and quite arid. However, it also avoids areas with too much water and swims only in very exceptional cases [2, 7, 9].

At the mouflon the sense is very well developed, as well as the sharpness and surprising agility for those who are deceived by its seemingly cumbersome appearance. Grazing and watering take place in the evening and in the night.

Copulation takes place in October-November, and after about 22 weeks, the wild sheep is retiring alone, in hidden places and gives birth to one or, less often, two lambs. After a few days she returns with the lamb to the herd.

Mouflon's food is vegetal, consisting of the forest grasses, shoots, leaves, etc. Sometimes it enters and in the crops, if these are too close to its area. The natural enemies of the mouflon include stray dogs, the lynx, the wolf, the bear, and for lambs are the eagle, the fox, the jackal and the wild cat [1, 3, 5, 6, 8].

For mouflon the main threats are: hunting and poaching (for meat), road accidents, diseases transmitted by domestic animals, as well as habitat loss. In general, mouflons appear to be extremely tolerant to human disorders.

The weather conditions from the Republic of Moldova are very favorable for the mouflon, where it feels very good. So, eight years ago, with the purpose of accommodation and reproduction, mouflons were brought to the hunting tourist complex "Golden Pheasant" from Căușeni district. Here, on 20 ha of forest, the wild sheep have adapted wonderfully and have begun to multiply. But a problem arose - were born more males, so a few years ago it was decided to organize mouflon hunting. The number of foreigners wishing to hunt was increasing year by year. It should be mentioned that only one mouflon was shot down by a local hunter, because it is an expensive pleasure. Instead, it is a good support for the household for the maintenance of the herd. Currently, there are 15 heads at the complex, of which only two are female. It should be mentioned that only one person participates at the mouflon hunt, which is in a tower, not far from the animal feed gutter. But you should not be disappointed by this way of hunting, because the animals are very attentive and sensitive, so the hunter must show a lot of patience so as don't scare them when they approach the gutter [2, 4, 5, 7,10].

Materials and methods

The parasitological researches were carried out in the laboratory of Parasitology and Helminthology of the Institute of Zoology on 214 biological samples collected from the mouflon 2015-2017, from the Republican Reproduction Center, Forest Enclosure "Mândrești", and at the mouflons maintained in the Zoo from Chișinău town. In order to achieve the proposed objectives, were used the coproovoscopic methods (*Fulleborn, Darling*), the coprolarvoscopic methods (*Popov, Baermann*) and the successive washing method . The intensity of the invasion with nematodes was established in 5 g of faeces, and the oocysts of *Eimeria spp*., eggs of *Fasciola hepatica*, eggs of *Dicrocoelium lanceolatum*, etc. in 10 visual microscopic fields (10x40).

Systematic determination of parasite species was performed according to European fauna [6]. The parasitological evaluation is based on the determination of the extensivity of the *EI* invasion (%) and the Intensity of the invasion (specimens / animal) at the investigated animals. The obtained results were statistically processed in the Excel program.

Results and discussions

Like the domestic sheep, the mouflon is more prone to infestation with species of parasites from the Trematoda class (*Dicrocoelium lanceolatum, Fasciola hepatica*).

As a result of the investigation of 36 biological samples from the mouflon youth (Ovis musimon Pallas 1811), from the Zoo, Chisinau town, structure was highlighted the following parasitic: Trematoda Class - 2 species: *Dicrocoelium lanceolatum* with EI- 6.2%, II-0.6 ex. and *Fasciola hepatica* with EI- 4.6%, II-1.3 ex .; Secernentae Class - 5 species: *Strongyloides papillosus* with EI- 18.6% and II-2.2 ex., *Cooperia punctata* with EI- 2.3% and II-0.4 ex., *Capillaria bovis* with EI- 6.2% and II-1.3 ex., *Toxocara vitulorum* with EI- 4.4% and II-0.6 ex., *Trichostrongylus axei* with EI- 2.6% and II-0.5 ex. and Conoidosida Class with 5 species: *Eimeria asymmetrica* with EI- 12.4% and II-1.6 ex., *E. austriaca* with EI- 4.2% and II-1.1 ex., *E. ponderosa* with EI- 16.5% and II-1.3 ex., *E. capreoli* with EI- 8.7% and II-1.8 ex. and *E. ovinoidalis* with EI- 18.4% and II-3.4 (table 1).

Out of the total of 36 samples examined from the mouflon youth - 8 samples (22.2%) were with monoinvasions, and 28 samples (77.8%) - mixed invasions. The samples with monoinvasions were consisting of: *Strongyloides papillosus* – 3 samples (37.5%); *E. ponderosa* - 3 samples

(37.5%); *Dicrocoelium lanceolatum* – one sample (12.5%) and *Fasciola hepatica* – one sample (12.5).

Out of the total samples with mixed invasions harvested from the young mouflon 28 (77.8%), more frequently were identified polyparasite associations consisted of 2 species - 8 samples (28.6%): *Strongyloides papillosus* + *E. ponderosa* - 3 samples (37.5%); *D. lanceolatum* + *E. ovinoidalis* - 3 samples (37.5%); *S. papillosus* + *E. ponderosa* - one sample (12.5%) and *D. lanceolatum* + *E. ovinoidalis* - one sample (12.5%).

Polyparasitic associations, at the mouflon youth from the Republican Reproduction Center, Ocolul Silvic "Mândreşti" Teleneşti district, consisted of 3 species were determined in 7 samples (25.0%) and consisting of: *Strongyloides papillosus* + *D. lanceolatum* + *E. ovinoidalis* - 3 samples (42.8%); *S. papillosus* + *Fasciola hepatica* + *E. ovinoidalis* - 2 samples (28.6%); *S. papillosus* + *Capillaria bovis* + *E. ovinoidalis* - one sample (14.3%); *S. papillosus* + *Toxocara vitulorum* + *Eimeria asymmetrica* – one sample (14.3%).

Polyparasitic associations consisted of 4 species were determined in 6 samples (21.4%) and consisting of: *S.papillosus* + *D. lanceolatum* + *Toxocara vitulorum* + *E. ovinoidalis* - 2 samples (33.3%); *S.papillosus* + *Dicrocoelium lanceolatum* + *E. ovinoidalis* + *E. capreoli* - 2 samples (33.3%); *S.papillosus* + *Fasciola hepatica* + *Toxocara vitulorum* + *E. ovinoidalis* - one sample (16.6%); *S.papillosus* + *D. lanceolatum* + *Capillaria bovis* + *E. capreoli* - one sample (16.6%).

Polyparasite associations consisted of 5 species were determined in 4 samples (14.3%) and consisting of: *S.papillosus* + *D. lanceolatum* + *Fasciola hepatica* + *E. ponderosa* + *E. ovinoidalis* - 1 sample (25.0%) *S.papillosus* + *Trichostrongylus axei* + *D. lanceolatum* + *E. ovinoidalis* + *E. ponderosa* - one sample (25.0%); *S.papillosus* + *Dicrocoelium lanceolatum* + *Fasciola hepatica* + *Capillaria bovis* + *E. ovinoidalis* - one sample (25.0%) and *S.papillosus* + *D. lanceolatum* + *Toxocara vitulorum* + *Trichostrongylus axei* + *E. ovinoidalis* - one sample (25.0 %).

Polyparasitic associations consisted of 6 species of parasites were determined in 3 samples (10.7%) and consisting of: *S.papillosus* + *D. lanceolatum* + *Fasciola hepatica* + *Toxocara vitulorum* + *E. ovinoidalis* + *E. ponderosa* - one sample (33.3 %) and *S.papillosus* + *D. lanceolatum* + *F. hepatica* + *Trichostrongylus axei* + *E. ponderosa* + *E. ovinoidalis* - one sample (33.3%) and *S.papillosus* + *D. lanceolatum* + *Fasciola hepatica* + *Capillaria bovis* + *E. ovinoidalis* + *E. ponderosa* - one sample (33.3%).

As a result of the investigation of 47 biological samples from adult mouflons, from the Zoo, Chisinau town, was highlighted the following parasitic structure: Trematoda Class - 2 species: *Dicrocoelium lanceolatum* with EI-12.5%, II-1.8 ex. and *Fasciola hepatica* with EI-8.4%, II-1.2 ex., Secernentae Class - 5 species: *Strongyloides papillosus* with EI-23.4% and II-2.4 ex., *Cooperia punctata* with EI-8.6% and II-1.1 ex., *Capillaria bovis* with EI-13.1% and II-1.8 ex., *Toxocara vitulorum* with EI-6.7% and II-1.2 ex., *Trichostrongylus axei* with EI-8.4% and II-1.3 ex. and Conoidosida Class with 5 species: *Eimeria asymmetrica* with EI-8.4% and II-2.2 ex., *E. austriaca* with EI-7.7% and II-0.5 ex., *E. ponderosa* with EI- 12.3% and II-1.4 ex.; *E. capreoli* with EI-5.2% and II-0.6 ex. and *E. ovinoidalis* with EI-12.1% and II-2.2 (table 1).

Out of the total of 47 samples examined from adult mouflons, only 15 samples (32.0%) were with monoinvasions, and 32 samples (68.0%) - mixed invasions. The samples with monoinvasions were consisted of*: Strongyloides papillosus* - 5 samples (33.3%); *Capillaria bovis* – 3 samples (20.0%); *E. ponderosa* - 2 samples (13.3%); *E. ovinoidalis* - 2 samples (13.3%); *Toxocara vitulorum* - one sample (6.6%); *Dicrocoelium lanceolatum* - one sample (6.6%) and *Fasciola hepatica* - one sample (6.6%).

Out of the total of samples infested with mixed invasions 32 (68.0%), more frequently, were identified polyparasite associations, consisted of 2 species - 11 samples (34.4%): *S. papillosus* + *D. lanceolatum* - 3 samples (27, 3%); S. *papillosus* + *E. ovinoidalis* - 3 samples (27.3%); *D. lanceolatum* + *E. capreoli* - 2 samples (18.2%); *Capillaria bovis* + *Fasciola hepatica* - one sample (9.1%); *S. papillosus* + *E. ponderosa* - one sample (9.1%) and *F. hepatica* + *E. ovinoidalis* – one sample (9.1%).

Polyparasitic associations, from adult mouflons, from the Zoo, Chisinau town, consisted of 3 species were determined in 8 samples (25.0%) and consisting of: *S. papillosus* + *F. hepatica* + *E. ovinoidalis* - 3 samples (37.5%); *S. papillosus* + *D. lanceolatum* + *E. ovinoidalis* - 2 samples (25.0%); *S. papillosus* + *E. ponderosa* + *E. ovinoidalis* - 2 samples (25.0%) and S. *papillosus* + *F. hepatica* + *D. lanceolatum* – one sample (11.1%).

Polyparasitic associations consisted of 4 species were determined in 7 samples (21.9%) and consisting of: *S.papillosus* + *F. hepatica* + *Toxocara vitulorum* + *E. ponderosa* - 2 samples (28.6%); *S.papillosus* + *D. lanceolatum* + *E. ponderosa* + *E. capreoli* - one sample (14.3%); *S.papillosus* + *D. lanceolatum* + *Toxocara vitulorum* + *E. ponderosa* - one sample (14.3%); *F. hepatica* + *D. lanceolatum* + *Capillaria bovis* + *E. ponderosa* - one sample (14.3%); *S.papillosus* + *Trichostrongylus axei* + *D. lanceolatum* + *E. ponderosa* - one sample (16.6%) and one sample (16.6%) with *D. lanceolatum* + *Trichostrongylus axei* + *Toxocara vitulorum* + *E. ovinoidalis*.

Polyparasite associations consisted of 5 species were determined in 4 samples (12.5%) and consisting of*: S. papillosus* + *D. lanceolatum* + *F. hepatica* + *E. ponderosa* + *E. ovinoidalis* - 1sample (25.0%) ; *S. papillosus* + *D. lanceolatum* + *Trichostrongylus axei* + *Capillaria bovis* + *E. ponderosa* - one sample (25.0%); *S. papillosus* + *D. lanceolatum* + *F. hepatica* + *E. ponderosa* + *E. ovinoidalis* - one sample (25.0%) and *S. papillosus* + *D. lanceolatum* + *Trichostrongylus axei* + *F. hepatica* + *E. ovinoidalis* - one sample (25.0%).

Polyparasic associations consisted of 6 species of parasites were determined in 2 samples (6.2%) and consisting of *S. papillosus* + *D. lanceolatum* + *Fasciola hepatica* + *Capillaria bovis* + *E. ovinoidalis* + *E. ponderosa* - one sample (50.0 %) and *S. papillosus* + *D. lanceolatum* + *F. hepatica* + *Trichostrongylus axei* + *E. ponderosa* + *E. ovinoidalis* - one sample (50.0%).

As a result of the study of 62 biological samples from the mouflon youth (Ovis musimon Pallas 1811), from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Teleneşti district, the following parasitic structure was highlighted: Trematoda Class - 3 species: *D. lanceolatum* with EI- 21.2%, II-2.8 ex., *Fasciola hepatica with* EI-12.7%, II-1.3 ex., and *Paramphistomum cervi* with EI-4.2% and II-1.2 ex .; Secernentae Class - 6 species *Strongyloides papillosus* with EI- 100.0% and II-12.2ex., *Cooperia punctata* with EI- 17.2% and II-1.8 ex., *Capillaria bovis* with EI- 14.9% and II -2.3 ex., *Ostertagia ostertagi* with EI-18.6% and II-2.2 ex. „ *Toxocara vitulorum* with EI-11.2% and II-1.4 ex., *Trichostrongylus axei* with EI-6 , 7% and II-1.0 ex .; Cestoda Class - one species: *Moniezia benedeni* - with EI- 21.3% and II-1.0 ex. and Conoidosida Class with 6 species: E. asymmetrica with EI-54.4% and II-4.2 ex., *E. austriaca* with EI-22.4% and II-2.1 ex., *E. ponderosa* with EI - 57.2% and II-3.9 ex .; *E. capreoli* with EI-32.4% and II-3.2 ex. and *E. bovis* with EI-11.4% and II-1.6 ex .; and *E. ovinoidalis* with EI-56.7% and II-12.2 (tab.1).

Out of the total of examined samples (62) from mouflon youth - 17 samples (27.4%) were with monoinvasions and 45 samples (72.6%) - mixed invasions. The samples with monoinvasions were consisted of: *Strongyloides papillosus* - 5 samples (29.4%); *E.ovinoidalis* - 4 samples (23.5%); *E. ponderosa* - 4 samples (23.5%); *D. lanceolatum* - 2 samples (11.7%); *Toxocara vitulorum* - one sample (5.9%) and *Fasciola hepatica* - one sample (5.9%).

Out of the total of samples infested with mixed invasions 45 (72.6%), more frequently were identified polyparasite associations consisting of 2 species - 16 samples (35.6%): *Strongyloides papillosus* + *E. ovinoidalis* - 5 samples (33.4 %); *Strongyloides papillosus* + *E. ponderosa* - 4 samples (25.0%); *Strongyloides papillosus* + *E. capreoli* - 3 samples (18.7%); *D. lanceolatum* + *E. ovinoidalis* - 3 samples (18.7%) and *D. lanceolatum* + *E. ponderosa* - one sample (6.2%).

Polyparasite associations, at the mouflon from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Telenești district, consisted of 3 species were determined in 12 samples (26.6%) and consisting of: *Strongyloides papillosus* + *D. lanceolatum* + *E. ovinoidalis* - 3 samples (25.0%); *S. papillosus* + *D. lanceolatum* + *E. ponderosa* - 2 samples (16.6%); *S. papillosus* + *E. asymmetrica* + *E. ovinoidalis* - 2 samples (16.6%); *S. papillosus* + *Fasciola hepatica* + *E. asymmetrica* - one sample (8.3%); *S. papillosus* + *E. asymmetrica* + *E. ponderosa* - one sample (8.3%); *S. papillosus* + *E. asymmetrica* + *E. ovinoidalis* - one sample (8.3%) and S. *papillosus* + *E. ovinoidalis* + *Moniezia benedeni* - one sample (8.3%).

Polyparasitic associations consisted of 4 species were determined in 9 samples (20.0%) and consisting of: *S.papillosus* + *E. asymmetrica* + *E. ovinoidalis* + *E. ponderosa* - 3 samples (33.3%); *S.papillosus* + *D. lanceolatum* + *E. asymmetrica* + *E. ovinoidalis* - 2 samples (22.2%); *S. papillosus* + *Dicrocoelium lanceolatum* + *Moniezia benedeni* + *E. asymmetrica* - one sample (11.1%); *S. papillosus* + *D. lanceolatum* + *Ostertagia ostertagi* + *E. asymmetric* - one sample (11.1%); *S. papillosus* + *Moniezia benedeni* + *D. lanceolatum* + *E. capreoli* - one sample (11.1%) and one sample (11.1%) with *F. hepatica* + *Toxocara vitulorum* + *E. asymmetrica* + *E. ponderosa.*

Polyparasitism consisted of 5 species was determined in 5 samples (11.1%) and consisting of: *S. papillosus* + *D. lanceolatum* + *E. asymmetrica* + *E. ponderosa* + *E. ovinoidalis* - 2 samples (40.0%); *S. papillosus* + *F. hepatica* + *E. capreoli* + *E. ovinoidalis* + *E. ponderosa* - one sample (20.0%); *S. papillosus* + *D. lanceolatum* + *Moniezia benedeni* + *E. asymmetrica* + *E. ponderosa* - one sample (20.0%) and *S. papillosus* + *Capillaria bovis* + *Moniezia benedeni* + *Toxocara vitulorum* + *E. ovinoidalis* - one sample (20, 0%).

Polyparasitic associations consisted of 6 species of parasites were determined in 3 samples (6.7%) and consisting *of S. papillosus* + *D. lanceolatum* + *F. hepatica* + *E. asymmetrica* + *E. ovinoidalis* + *E. ponderosa* - one sample (33 ,3%); *S. papillosus* + *D. lanceolatum* + *Toxocara vitulorum* + *Trichostrongylus axei* + *Eimeria bovis* + *E. asymmetrica* - one sample (33.3%) and *S.papillosus* + *D. lanceolatum* + *Capillaria bovis* + *Toxocara vitulorum* + *E. ponderosa* + *E. ovinoidalis* - one sample (33.3%).

Table 1

Parasitofauna at mouflons of different ages from various biotopes of the Republic of Moldova

Parasitic invasion	**Zoo, Chişinău town**				The Republican Reproduction Center, Forest Enclosure "Mândreşti" Telenești district,			
	Mouflon youth		**Adult mouflons**		**Mouflon youth**		**Adult mouflons**	
	EI (%)	II (ex.)	EI (%)	II (ex.)	EI (%)	II (ex.)	EI (%)	II (ex.)

TREMATODA CLASS								
Dicrocoelium lanceolatum	6,2	0,6	12,5	1,8	21,2	2,8	42,6	3,4
Fasciola hepatica	4,6	1,3	8,4	1,2	12,7	1,3	28,1	2,8
Paramphistomum cervi	-	-	-	-	4,2	1,2	12,4	1,4
SECERNENTEA CLASS								
Strongyloides papillosus	18,6	2,2	23,4	2,4	100,0	12,2	100,0	12,7
Cooperia punctata	2,3	0,4	8,6	1,1	17,2	1,8	29,8	2,6
Capillaria bovis	6,2	1,3	13,1	1,8	14,9	2,3	27,4	3,1
Ostertagia ostertagi	-	-	-	-	18,6	2,2	34,6	4,2
Toxocara vitulorum	4,4	0,6	6,7	1,2	11,2	1,4	23,3	2,3
Trichostrongylus axei	2,6	0,5	8,4	1,3	6,7	1,0	13,6	1,4
CESTODA CLASS								
Moniezia benedeni	-	-	-	-	21,3	1,0	46,6	1,2
CONOIDOSIDA CLASS								
Eimeria asymmetrica	12,4	1,6	8,4	2,2	54,4	4,2	42,4	3,8
Eimeria austriaca	4,2	1,1	7,7	0,5	22,4	2,1	18,3	1,5
Eimeria ponderosa	16,5	1,3	12,3	1,4	57,2	3,9	38,4	3,2
Eimeria capreoli	8,7	1,8	5,2	0,6	32,4	3,2	24,6	1,8
Eimeria bovis	-	-	-	-	11,4	1,6	6,4	0,7
Eimeria ovinoidalis	18,4	3,4	12,1	2,2	56,7	12,2	46,3	15,3
Total examined	**46**		**74**		**82**		**44**	

As a result of the study of 44 biological samples from adult mouflons (Ovis musimon Pallas 1811), from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Telenesti district, was highlighted the following parasitic structure: Trematoda Class - 3 species*: Dicrocoelium lanceolatum* with EI– 42,66%, II-3,4 ex., *Fasciola hepatica* with EI- 28,1%, II-2,8 ex., and *Paramphistomum cervi* with EI- 12,4% and II-1,4 ex .; Secernentae Class - 6 species: *Strongyloides papillosus* with EI- 100.0% and II-12.7 ex., *Cooperia punctata* with EI- 29.8% and II-2.6 ex., *Capillaria bovis* with EI- 27.4% and II-3.1 ex., *Ostertagia ostertagi* with EI- 34.6% and II-4.2 ex. „ *Toxocara vitulorum* with EI- 23.3% and II-2.3 ex., *Trichostrongylus axei* with EI- 13.6% and II-1.4 ex.; Cestoda Class - one species: *Moniezia benedeni* - with EI- 46.6% and II-4.2 ex. and Conoidosida Class with 6 species: *Eimeria asymmetrica* with EI-4 2.4% and II-3.8 ex., *E. austriaca* with EI- 18.3% and II-1.5 ex., *E. ponderosa* with EI- 38.4% and II-3.2 ex .; *E. capreoli* with EI- 24.6% and II-1.8 ex., *E. bovis* with EI-6.4% and II-0.7 ex.; and *E. ovinoidalis* with EI- 46.3% and II-15.3 (table 1).

Out of the total of examined samples (44) from the mouflon - 11 samples (25.0%) were with monoinvasions, and 33 samples (75.0%) - mixed invasions. The samples with monoinvasions consisted of: *Strongyloides papillosus* – 3 samples (27.2%); *Eimeria asymmetrica* - 2 samples (18.2%); *E. ovinoidalis* - 2 samples (18.2%); *Toxocara vitulorum* - one sample (9.1%); *Dicrocoelium lanceolatum* - one sample (9.1%) *Fasciola hepatica* - one sample (9.1%) and *Capillaria bovis* - one sample (9.1%).

Out of the total of samples infested with mixed invasions 33 (75.0%), more frequently were identified polyparasite associations consisting of 2 species - 12 samples (36.4%): *Strongyloides papillosus* + *Moniezia benedeni* - 4 samples (33.4%) *Ostertagia ostertagi* + *E. ovinoidalis* - 3 samples (25.0%); *Moniezia benedeni* + *E. capreoli* - 2 samples (16.7%); S. *papillosus* + *Capillaria bovis* - one sample (8.3%); *S. papillosus* + *E. ponderosa* - one sample (8.3%) and *D. lanceolatum* + *E. ovinoidalis* - one sample (8.3%).

Polyparasitic associations, at the mouflon from the Republican Reproduction Center, Forest Enclosure "Mândreşti", Telenești district, consisting of 3 species were determined in 9 samples (27.3%) and consisting of: *Strongyloides papillosus* + *Moniezia benedeni* + *E. ovinoidalis* - 2 samples (22.2%); *S. papillosus* + *D. lanceolatum* + *E. ovinoidalis* - 2 samples (22.2%); *S. papillosus* + *Paramphistomum cervi* + *E. ovinoidalis* - one sample (11.1%); *S. papillosus* + *Fasciola hepatica* + *Ostertagia ostertagi* - one sample (11.1%); *Ostertagia ostertagi* + *Moniezia benedeni* + *E. ovinoidalis* - one sample (11.1%); *S. papillosus* + *Moniezia benedeni* + *E. ovinoidalis* – one sample (11.1%) and *S. papillosus* + *E. ovinoidalis* + *Ostertagia ostertagi* - one sample (11.1%).

Polyparasite associations consisted of 4 species were determined in 6 samples (18.2%) and consisting of: *S. papillosus* + *Moniezia benedeni* + *Toxocara vitulorum* + *E. ponderosa* - one sample (16.6%); S. *papillosus* + *Dicrocoelium lanceolatum* + *E. ovinoidalis* + *E. capreoli* - one sample (16.6%); *S. papillosus* + *Cooperia punctata* + *Toxocara vitulorum* + *Moniezia benedeni* - one sample (16.6%); *Moniezia benedeni* + *D. lanceolatum* + *Capillaria bovis* + *E. capreoli* - one sample (16.6%); S. *papillosus* + *Moniezia benedeni* + *Dicrocoelium lanceolatum* + *E. ovinoidalis* - one sample (16.6%) and one sample (16.6%) with *Dicrocoelium lanceolatum* + *Ostertagia ostertagi* + *Moniezia benedeni* + *E. ponderosa.*

Polyparasitic associations consisted of 5 species were determined in 4 samples (12.1%) and consisting of: *S. papillosus* + *D. lanceolatum* + *Moniezia benedeni* + *E. ponderosa* + *E. ovinoidalis* - one sample (25.0%); *S. papillosus* + *D. lanceolatum* + *Ostertagia ostertagi* + *E. ovinoidalis* + *E. ponderosa* - one sample (25.0%); S. *papillosus* + *Dicrocoelium lanceolatum* + *Fasciola hepatica* + *Capillaria bovis* + *E. ovinoidalis* - one sample (25.0%) and *S. papillosus* + *D. lanceolatum* + *Moniezia benedeni* + *Ostertagia ostertagi* + *E. ovinoidalis* - one sample (25.0 %).

Polyparasitic associations consisted of 6 species of parasites were determined in 2 samples (6.0%) and consisting of *S. papillosus* + *D. lanceolatum* + *Fasciola hepatica* + *Moniezia benedeni* + *E. ovinoidalis* + *E. ponderosa* - one sample (50.0 %) and S.*papillosus* + *D. lanceolatum* + *F. hepatica* + *Ostertagia ostertagi* + *E. ponderosa* + *E. ovinoidalis* - one sample (50.0%).

Conclusions

At the mouflons from the Republican Reproduction Center, Forest Enclosure "Mândreşti" Telenești district, was highlighted a higher structure and level of infestation compared to that of mouflons maintained in the Zoo from Chisinau town, this being caused by the performance of irregular deworming works or even their lack at those from the Republican Reproduction Center, Forest Enclosure "Mândreşti", Telenești district.

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Parasitic fauna at the hare (*Lepus Europaeus Pallas*, 1778) from the "Codrii" natural reservation, Republic of Moldova

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Abstract
The study of the parasitic fauna at the hare (Lepus europaeus Pallas, 1778) from the "Codrii" natural reservation, Republic of Moldova revealed species of parasites that fall taxonomically in 3 classes (Trematoda, Secernentea and Conoidasida), 9 families (Fasciolidae, Dicrocoeliidae, Trichuridae, Strongyloidida, Trichostrongylidae, Oxyuridae, Trichuridae, Molineidae, Eimeriidae) and 9 genera (Fasciola, Dicrocoelium, Trichuris, Strongyloides, Trichostrongylus, Passalurimus, N and Passalurimus), and Parasitological research has shown an increased level of infestation at hares with various parasitic agents: Tematoda class 2 species (Fasciola hepatica with EI of 7.5% cases and II of 4,6 ex., Dicrocoelium lanceolatum with EI – 11.3% of cases, II-2.4 ex.); Class Secernentea 8 species (Trichocephalus leporis with EI – 16.1% cases, II -7.1 ex., Strongyloides papillosusi with EI – 69.2% cases, II- 14.7 ex., Trichostrongylus retortaeformis with EI – 5.3% cases, II -2.4 ex., Passalurus ambiguus with EI – 34.6% cases, II-5.2 ex., Trcihostrongylus probolurus with EI – 17.1% cases, II- 4,3ex., Trichuris leporis with EI – 7.1% cases, II-3,5ex., Graphidium strigosum with EI – 2.3% cases, II -1,4 ex., Nematodirus abnormalis with EI – 3.7% cases, II- 5.5 ex.) and Conoidasida Class with 6 species: Eimeria leporis with EI – 51.4% cases and II- 18.5 oocysts. Eimeria magna with EI – 31.3% of cases, II- 12.4 oocysts, Eimeria stiedae with EI – 57.1% cases, II- 18.7 oocysts, Eimeria perforans with EI – 12.9% of cases, II- 17.6 oochişti; Eimeria exigua with EI – 48.1% of cases, II- 15.3 oocysts; Eimeria intestinalis with EI – 14.3% of cases, II – 17.2 oocysts. It has been established that hares from the "Codrii" Natural Reserve are infested in the form of monoinvasions in 28,1% of cases, and in the form of polyinvasions - in 71,9% of cases.
Keywords: *parasitic fauna, hare, "Codrii" natural reservation*

Introduction

Changes in climatic factors, along with socio-anthropic changes, which have been reported in recent years, cause changes in the structure of wildlife communities as a whole and those of economic interest. In the last two decades, agrocenoses have undergone structural changes expressed through the parcelling of land with a greater diversity of agricultural crops, the disappearance of monocultures, the appearance of ponds, etc [3, 6].

Along with global warming, droughts are quite common during the summer and early fall. These factors have a contradictory influence in the increase of the number of fur game, especially of the most important game species in the open lands - the agrocenoses in our country, of the hare. Other factors are parasitic diseases, poaching, the excessive presence of predators - foxes, stray and wild dogs and cats [1].

Neutralizing these negative factors and implementing the necessary recommendations will give the opportunity to organize the hunting household at an effective hunting level. We must mention that the management of hunting households in Central and Eastern European countries (Czech Republic, Slovakia, Hungary, Romania) has shown that acclimatization of hares imported from other regions does not give spontaneous results, so it is necessary to maintain and optimize local population density by making recommendations for the protection of various parasitic and infectious diseases, stimulating the reproductive process and rational exploitation of the species. The most visible changes in the reporting period are as a result of assessments of the number of main game species and hunting results [5, 6].

The hare (*Lepus europaeus Pallas*, 1778) or the common rabbit is a mammal of the leporidae family. This species inhabits open and semi-open lands in the temperate zone of Europe and parts of Asia. The number of hares is declining due to the intensification of agriculture. The

hare is not related to the domestic rabbit (Oryctolagus cuniculus domesticus) and does not breed with it [5, 6, 7].

This species is one of the largest from the lagomorphs. The length of the body together with the head varies from 60 to 75 cm and the length of the tail from 7.2 to 11 cm. The weight is usually between 3 and 5 kg. The color of the fur is brownish-yellow on the back; reddish on shoulders, feet and neck; white on the lower body and black on the tail and on the tips of the ears [6, 10].

The hare is a mostly nocturnal animal and spends a third of its time feeding. During the day, the hare hides in the shelter in a hollow in the ground. The hares can run at up to 70 km /h and, in the event of a predator attack, they rely on their superiority in speed [6, 8, 14].

In generally they are thought to be solitary, but can be seen in large and small groups. They don't seem to be territorial, living in vital areas in common of about 300 ha. They communicates through a wide range of visual cues. To show interest, they raises their ears, while their lowered ears warn the others to stay away. The hare can live up to 12 years.

Childbirth takes place in a hollow in the ground. The hare can have up to 3 calvings per year with a gestation period of 41-42 days. The bunnies weigh about 130 g at birth. The bunnies are covered in fur and are able to leave the nest at shortly time after birth, an adaptation to the absence of physical protection comparable to that offered by a den. The bunnies disperse during the day and gather in the evening near the place where they were born to be breastfed by their mother. After two weeks they can eat solid food and after four weeks they are already weaned [5, 6].

The hare is an important part of the hunting fauna. Starting with 2012, we have an increase in the number of hares in the autumn population from 62 thousand in 2012 to about 233 thousand in the fall of 2018, with an increase of the average annual number for the field hare population in the hunting fund of open lands of 20.5% [1, 7].

Most parasitological studies have focused on domestic animals, but it has recently been established that parasitic infestations are equally common and important in wildlife, which can serve as a potential reservoir for parasites. The hare hosts a wide range of parasites, which are a great interest to hunting fund managers and veterinarians, being considered important sources of zoonotic agents [4,8,13].

The study of the process of infestation of wild animals with various parasitic agents, the development of innovative measures to reduce and control them is an important, fundamental and, especially, applicative problem, because some species serve as definitive hosts in the development cycle and as their vectors being dangerous for both domestic animals and humans. Parasitosis is the most common disease in wildlife in game, resulting in substantial economic losses [2, 3, 7, 9, 11, 12].

The fauna of hunting interest is the component part of the national hunting heritage. Both the number and the total spectrum of main and complementary species determine the value of this fund. Therefore, the study of parasitic fauna at wild animals from hunting fauna has a special significance [9,14].

The purpose of the research is to study the diversity of parasitic fauna at the hare (*L. europaeus Pallas*), from the "Codrii" Nature Reserve from the Central Area of the Republic of Moldova.

Materials and methods

The parasitological researches were carried out in the laboratory of Parasitology and Helminthology of the Institute of Zoology on 214 biological samples collected from the hare during 2017-2019, from the forest ecosystem of the Nature Reserve "Codrii".

In order to achieve the proposed objectives, were used the coproovoscopic methods (Fulleborn, Darling), the coprolarvoscopic methods (Popov, Baermann) and the successive washing method . The intensity of the invasion with nematodes was established in 5 g of fetuses, and the oocysts of *Eimeria spp*., eggs of *Fasciola hepatica,* eggs of *Dicrocoelium lanceolatum*, etc. in 10 visual microscopic fields (10x40).

Systematic determination of parasite species was performed according to European fauna [6]. The parasitological evaluation is based on the determination of the extensivity of the *EI* invasion (%) and the Intensity of the invasion (specimens / animal) at the investigated animals. The obtained results were statistically processed in the Excel program.

Results and discussions

The parasitological research carried out on the study of parasitofauna at the hare from the forest ecosystem of the "Codrii" Nature Reserve, Republic of Moldova, shows that they are parasitized with various dangerous parasitic agents with various locations, systematically classified into 3 classes (*Trematoda, Secernentea, Conoidas*), 9 families (*Fasciolidae, Dicrocoeliidae, Trichuridae, Strongyloididae, Trichostrongylidae, Oxyuridae, Trichuridae, Molineidae, Eimeriidae*) and 9 genera (*Fasciola, Dicrocoelium, Trichuris, Strongyloides, Trichostrongidus E* and *Trichostrongidus*.

As a result of the parasitological research carried out, an increased level of infestation was established at hares with various parasitic agents: Tematoda class 2 species (*Fasciola hepatica* with *EI* of 7.5% cases and *II* of 4.6 ex., *Dicrocoelium lanceolatum* with *EI* – 11.3% of cases, *II*-2.4 ex.); Class Secernentea 8 species (*Trichocephalus leporis* with *EI* – 16.1% cases, *II* -7.1 ex., *Strongyloides papillosusi* with *EI* – 69.2% cases, *II*- 14.7 ex., *Trichostrongylus retortaeformis* with *EI* – 5.3% cases, *II* -2.4 ex., *Passalurus ambiguus* with *EI* – 34.6% cases, *II*-5.2 ex., *Trcihostrongylus probolurus* with *EI* – 17.1% cases, *II*- 4.3ex., *Trichuris leporis* with *EI* – 7.1% cases, *II*-3.5ex., *Graphidium strigosum* with *EI* – 2.3% cases, *II* -1.4 ex., *Nematodirus abnormalis* with *EI* – 3.7% cases, *II*- 5.5 ex.) and Conoidasida Class with 6 species: *Eimeria leporis* with *EI* – 51.4% cases and *II*- 18.5 oocysts. *Eimeria magna* with *EI* – 31.3% of cases, *II*- 12.4 oocysts, *Eimeria stiedae* with *EI* – 57.1% cases, *II*- 18.7 oocysts, *Eimeria perforans* with *EI* – 12.9% of cases, *II*- 17.6 oochişti; *Eimeria exigua* with *EI* – 48.1% of cases, *II*- 15.3 oocysts; *Eimeria intestinalis* with *EI* – 14.3% of cases, *II* – 17.2 oocysts (tab.1).

The parasitological examination performed on 214 coprological samples collected from hares from the forest ecosystem of the "Codrii" Nature Reserve showed that in 203 samples (94.8% of cases) are present parasitic agents.

Table 1

The diversity of parasitic fauna at the hare from the forest ecosystem of the "Codrii" Nature Reserve

Class	Family	Species	EI, %	II, ex.
Trema-toda	Fasciolidae	*Fasciola hepatica (Linnaeus, 1758)*	7.5	4.6
	Dicrocoeliidae	*Dicrocoelium lanceolatum (Rudolphi, 1819)*	11.3	2.4
	Trichuridae	*Trichocephalus leporis (**Frolich, 1789**)*	16.1	7.1

Secernentea	Strongyloididae	*Strongyloides papillosus (Wedl, 1856)*	69.2	14.7
	Trichostrongylidae	*Trichostrongylus retortaeformis (Zeder,1800)*	5.3	2.4
		Trcihostrongylus probolurus (Railliet,1896)	17.1	4.3
		Graphidium strigosum (Dujardin, 1845)	2.3	1.4
	Oxyuridae	*Passalurus ambiguus (Rudolphi, 1819)*	34.6	5.2
	Trichuridae	*Trichuris leporis (Frölich, 1789*	7.1	3.5
	Molineidae	*Nematodirus abnormalis (May, 1920*	3,7	5,4
Conoidasida	Eimeriidae	*Eimeria leporis* ***(Nieschulz, 1923)***	51.4	18.5
	Eimeriidae	*Eimeria magna (Pérard, 1925)*	31.3	12.4
	Eimeriidae	*Eimeria stiedae* ***(Lindemann, 1865)***	57.1	18.7
	Eimeriidae	*Eimeria perforans(Leuckart, 1879)*	12.9	17.6
	Eimeriidae	*Eimeria exigua (Yakimoff, 1934)*	48.1	15.3
	Eimeriidae	*Eimeria intestinalis (Cheissin, 1948)*	14.3	17.2

Parasitic invasions consisting of a single parasite species (monoinvasions) were present in 57 samples (28.1% cases), and parasitic associations consisting of several species of parasites (polyinvasions) being recorded in the remaining investigated samples -146 samples (71.9%).

From the total of polyparasitic samples, the following polyparasite associations were established: with 2 species of parasites - 60 samples (41.1%): *Strongyloides papillosus* + *Eimeria stiedae* –14 samples (23.3%); *Strongyloides papillosus* + *Eimeria leporis* - 11 samples (18.3%); *Strongyloides papillosus* + *Eimeria exigua* - 8 samples (13.3%); *Strongyloides papillosus* + *Trichocephalus leporis* - 7 samples (11.6% of cases); *Passalurus ambiguus* + *Eimeria leporis* - 7 samples (11.6%); *Strongyloides papillosus* + *Dicrocoelium lanceolatum* - 4 samples (6.7%); *Strongyloides papillosus* + *Fasciola hepatica* - 4 samples (6.7%); *Strongyloides papillosus* + *Trichostrongylus retortaeformis* - 3 samples (5.0%); *Trichostrongylus retortaeformis* + *Nematodirus abnormalis* - 2 samples (3.3%).

In 46 samples (31.5%) examined, had been established polyparasite associations consisting of 3 species of parasites *: Strongyloides papillosus* + *Eimeria leporis* + *Eimeria stiedae* - 13 samples (28.3%); *Passalurus ambiguus* + *Eimeria perforans* + *Eimeria intestinalis* - 9 probe (19.5%); *Strongyloides papillosus* + *Eimeria leporis* + *Dicrocoelium lanceolatum* - 8 samples (17.4%); *Trichocephalus leporis* + *Dicrocoelium lanceolatum* + *Eimeria exigua* - 7 samples (15.2%); *Strongyloides papillosus* + *Trcihostrongylus probolurus* + *Graphidium strigosum*- 5 probe (10.9%); *Nematodirus abnormalis* + *Trichuris leporis* + *Fasciola hepatica* - 4 samples (8.7%).

Polyparasitic associations consisting of 4 species of parasites were identified in 25 samples (17.1%) and consisting of: *Strongyloides papillosus* + *Eimeria magna* + *Fasciola hepatica* + *Eimeria stiedae* - 8 samples (2.8%); *Dicrocoelium lanceolatum* + *Fasciola hepatica* + *Trichocephalus leporis* + *Strongyloides papillosus* - 6 samples (1.8%); *Strongyloides papillosus* + *Trichuris leporis* + *Passalurus ambiguus* + *Eimeria stiedae* - 6 samples (2.3%); *Dicrocoelium lanceolatum* + *Strongyloides papillosus* + *Eimeria leporis* + *Nematodirus abnormalis* - 4 samples (2.8%).

As a result of the laboratory parasitological examination, it was possible to highlight in 11 samples (7.5%), parasitic associations consisting of 5 species of parasites: *Strongyloides papillosus* + *Trichuris leporis* + *Trichocephalus leporis* + *Fasciola hepatica* + *Eimeria stiedae* - 3 samples

(27.2%) ; *Strongyloides papillosus + Trichostrongylus retortaeformis + Eimeria stiedae + Passalurus ambiguous + Eimeria leporis* - 2 samples (18.2%); *Strongyloides papillosus + Dicrocoelium lanceolatum + Nematodirus abnormalis + Eimeria magna + Eimeria leporis* - 2 samples (18.2%); *Strongyloides papillosus + Trichuris leporis + Passalurus ambiguus + Eimeria stiedae + Trichocephalus leporis* -2 sample (18.2%); *Strongyloides papillosus + Eimeria magna + Dicrocoelium lanceolatum + Graphidium strigosum + Trichuris leporis* - one sample (9.1%); *Strongyloides papillosus + Eimeria leporis + Trichocephalus leporis + Trichuris leporis + Eimeria stiedae* - one sample (9.1%).

Polyparasitic associations consisting of 6 species of parasites were identified in 4 samples (2.8%) and consisting of: *Strongyloides papillosus + Trichostrongylus retortaeformis + Trichuris leporis + Trichocephalus leporis + Eimeria stiedae + Fasciola hepatica* - 2 samples (75.0%) *Strongyloides papillosus + Eimeria magna + Fasciola hepatica + Eimeria stiedae + Passalurus ambiguous + Trichocephalus leporis* - one sample (25.0%); *Strongyloides papillosus + Dicrocoelium lanceolatum + Eimeria magna + Eimeria leporis + Passalurus ambiguous + Graphidium strigosum* - a sample (25.0%).

It was highlighted the division of parasite species according to the way of development cycles in: biohelminths (12.5%) - parasite species, whose development cycle requires a complementary host; geohelminths (87.5%) - species of parasites that do not require a complementary host in their development cycle (fig. 1).

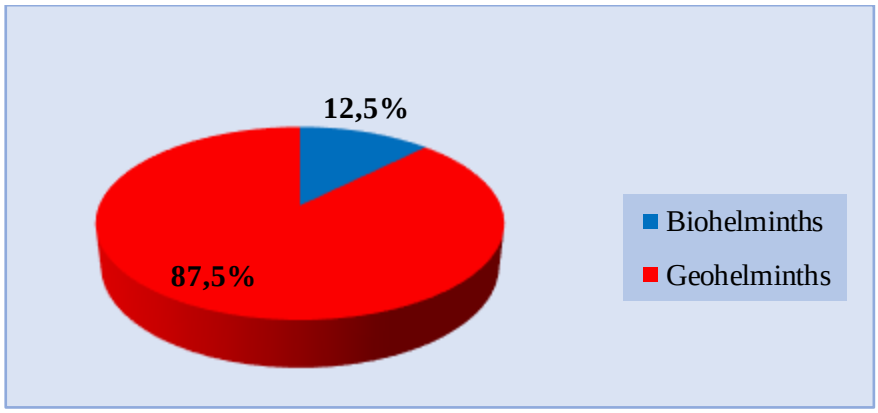

Fig. 1. Division of parasitic species, identified at hare, according to the way of development cycles

The results of parasitological research conducted on hares in the Natural Reserve "Codrii" Republic of Moldova show an increased level of infestation with various parasitic agents dangerous to both domestic animals and humans. Of the total number of parasitic species identified (16 species): 10 species (62.5%) are specific only to hares (*Trichuris leporis, Trichocephalus leporis, Passalurus ambiguous, Graphidium strigosum, Eimeria leporis, Eimeria magna, Eimeria stiedae, Eimeria perforans, Eimeria small, Eimeria intestinalis*); 4 species (25.0%) are common to other species of wild and domestic animals (*Strongyloides papillosus, Trcihostrongylus probolurus, Trichostrongylus retortaeformis, Nematodirus abnormalis*), and 2 species (12.5%), (*Fasciola hepatica, Dicrocoelium lanceolatum*), are common for both animals and humans.

Therefore, some parasitic species identified at hares from the "Codrii" Natural Reserve are common for both wild and domestic animals, as well as for humans.

These results can be explained by the fact that the hare is a herbivorous wild mammal that prefers open fields with isolated thickets for shelter. They are very adaptable and thrive on mixed agricultural lands. They need shelter, such as forest strips, ditches and permanent shelter areas. It shows a preference for agricultural lands in the areas of plains, hills and low hills, where small forest bodies are scattered. When food becomes deficient, it retreats to forests, but often approaches localities, where it enters the gardens of people who are adequate places of mutual contamination between different types of parasitic hosts (permanent, intermediate, complementary) terrestrial and aquatic.

Conclusions

1. The study of the parasitic fauna carried out on 214 biological samples collected from the hares, from the forest ecosystem of the Natural Reserve "Codrii" revealed species of parasites that fall taxonomically in 3 classes (*Trematoda, Secernentea and Conoidasida),* 9 families *(Fasciolidae, Dicrocoeliidae, Trichuridae, Strongyloidida, Trichostrongylidae, Oxyuridae, Trichuridae, Molineidae, Eimeriidae*) and 9 genera (*Fasciola, Dicrocoelium, Trichuris, Strongyloides, Trichostrongylus, Passalurimus, N* and *Passalurimus*).
2. Parasitological research has shown an increased level of infestation at hares with various parasitic agents: Tematoda class 2 species (*Fasciola hepatica, Dicrocoelium lanceolatum*); Class Secernentea 8 species (*Trichocephalus leporis, Strongyloides papillosusi, Trichostrongylus retortaeformis, Passalurus ambiguus, Trcihostrongylus probolurus, Trichuris leporis, Graphidium strigosum, Nematodirus abnormalis*) and Conoidasida Class 6 species (*Eimeria leporis, Eimeria magna, Eimeria stiedae, Eimeria perforans, Eimeria exigua, Eimeria intestinalis*).
3. It has been established that hares from the "Codrii" Natural Reserve are infested in the form of monoinvasions in 28,1% of cases, and in the form of polyinvasions - in 71,9% of cases.
4. It was found that out of the total (16) of parasitic species identified at hares: 10 species (62.5%) are specific only to hares (*Trichuris leporis, Trichocephalus leporis, Passalurus ambiguous, Graphidium strigosum, Eimeria leporis, Eimeria magna, Eimeria stiedae, Eimeria perforans, Eimeria exigua, Eimeria intestinalis*), 4 species (25.0%) *Strongyloides papillosus Trcihostrongylus probolurus Trichostrongylus retortaeformis, Nematodirus abnormalis* are common to other species of wild and domestic animals, and 2 species (12.5%) (*Fasciola hepatica, Dicrocoelium lanceolatum*), are common in both animals and humans.

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Endoparasitofauna of some wild birds of hunting interest from the Republic of Moldova

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Abstract
The result of the parasitological examination from pheasants (Phasianus colchicus L.) showed a high level of their infestation with various parasitic agents: Trematoda Class one species (Prosthogonimus ovatus); Secernentea Class 6 species (Capillaria annulata, Syngamus tracheia, Heterakis isolonche, Ascaridia galli, Heterakis gallinarum and Trichostrongylus tenuis) and Conoidasida Class 3 species (Eimeria colchici, E. duodenalis and E. phasiani). Parasitological research taken from quails (Cotrunix cotrunix L.), revealed their infestation with various parasitic agents: Trematoda Class 2 species (Echinostoma revolutum, Prosthogonimus ovatus); Secernentea Class 4 species (Capillaria caudinflata, Syngamus tracheia with, Ascaridia galli and Heterakis gallinarum); Class Cestoda with one species (Raillietina tetragona), and Conoidasida Class 3 species (Eimeria wear, E. batteries, and E. coturnicis). Parasitological research from guineafowls (Numida meleagris L.), revealed their infestation with various parasitic agents: Trematoda Class one species (Prosthogonimus ovatus); Secernentea Class 4 species (Capillaria annulata, Syngamus tracheia, Ascaridia galli, and Heterakis gallinarum); and Conoidasida Class 2 species (Eimeria numidae and E. adenoeides). Out of the total of 10 species of parasites identified at pheasant, 4 species (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum), are also specific for quail, 5 species are specific for guineafowl (Prosthogonimus ovatus, Capillaria annulata, Syngamidia tracheia, Heterakis gallinarum), 5 species are specific for chickens (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Trichostrongylus tenuis) and only 2 species are specific for turkeys (Ascaridia galli, Heterakis gallinarum). Out of the total of 10 species of parasites identified at quails, 4 species (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum) are also common at pheasants, 4 species are specific at guineafowl (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heter), 5 species are specific for chickens (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Raillietina tetragona) and 4 species are specific for turkeys (Capillaria caudinflata, Ascaridia galli, Heterakis gallinarum, Raillietina echinobothrida). Out of the total of 7 species of parasites identified at guineafowl, 4 species (Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum) are common at pheasants, quails and chickens, and 3 species are specific for turkeys (Ascaridia galli, Heterakis gallinarum, Raillietina tetragonal).
Keywords: *wild birds, endoparasitofauna, parasitic species.*

Introduction

Wild birds of hunting interest contribute essentially to the preservation of natural outbreaks of parasites, common to wild, domestic animals and to humanity. In this context, the study of their parasitofauna has a special importance both from theoretical and practical point of view. Knowledge of parasitic fauna in birds of hunting interest is especially important in order to avoid the spread of parasitic agents, both among other wild and domestic animals and in humans (1, 2, 5).

The common pheasant (*Phasianus colchicus L.)* is the most important bird from the hunting avian fauna of the Republic of Moldova, both by its numerical weight and degree of spread as well as by its hunting prospects. Analyzing the dynamics of pheasant herds during the last years in the Republic of Moldova, an ascent of its acclimatization dynamics was highlighted, thanks to the complex measures of protection and its permanent repopulation in nature from the specialized breeders. The breeding stock of the pheasant in the spring of 2018 was estimated at about 42 thousand specimens, with an annual increase of 75-90%. Notwithstanding the above, the number of pheasants from year to year is increasing by only 13-18%, signaling a drastic decrease in their number in the cold period of the year. The multiple measures aimed at increasing the number of

animals of hunting interest will not be enough, because parasitic diseases not only slow down their growth and development, but also cause their mortality [5, 9, 11, 12].

Likewise, the quail population in 2018 was estimated, at the initial phase of nesting with an average density of about 40 quails per 100 thousand ha, thus gathering a herd of over 160 thousand quails. By autumn, a local population estimated at about 400,000 quails [7, 13, 15].

The study of parasitic fauna in wild birds presents a major interest, in that they in a short period of time travel long distances from one continent to another while transporting in / on their body a rich range of external parasitic agents (malophages, fleas, mites) and internal (nematodes, trematodes, cestodes, etc.). Thus, wild birds can maintain and transport these species of parasites [2,4,12,13].

Gastrointestinal helminths (cestodes, trematodes, nematodes) are considered to be an important cause not only of productivity losses, but also of diseases and often even mortality. Associated polyparasitism is frequently recorded in wild birds of hunting interest [3, 6, 8, 14, 16].

The prevalence and abundance of infestations at birds of hunting interest can be influenced by many factors such as: distribution of intermediate and complementary hosts, age, sex, their infestation rate, number of eggs and infesting larvae, etc. It is found that birds of hunting interest are more vulnerable in their first year of life, when their mortality can reach the limit of about 90% and being determined by the association of infectious and parasitic diseases with helminthic specificity [1, 3, 6, 10].

Research methods

In order to identify the various species of endoparasites, biological samples were collected from wild birds of hunting interest (pheasants, guineafowls, quails) from the hunting grounds of Ialoveni district, Chisinau municipality and from various natural and man-made biotopes of the North-Central Republic of Moldova. Biological samples were also collected from domestic birds from 38 households (chickens, turkeys). The investigations took place during the years 2015-2019, in which a total of 354 samples were taken and examined: of which 153 samples from chickens (*Gallus gallus domesticus L.);* 78 - pheasants (*Fhasianus colchicus L.);* 39- turkeys (*Meleagris gallopavo L.);* 48 - quails *(Cotrunix cotrunix L.)* and 36 - guineafowl *(Numida meleagris L.).*

In the parasitological diagnosis were used methods copro-ovoscopic (Fulleborn, Darling), copro-larvoscopic (Popov, Baermann), partial parasitological investigations (after K.I. Skriabin) and successive washing. The parasitological evaluation was performed by determining the degree of spread (prevalence,%) using the Novex Holland B ob microscope. 20-40 WF 10x Din / 20mm. The results were statistically processed in the Excel program.

Parasitological investigations were performed in the Parasitology and Helminthology Laboratory of I.P. Institute of Zoology

Results and discussions

The result of the parasitological examination of 78 samples collected from pheasants *(Phasianus colchicus L.)* showed a high level of their infestation with various parasitic agents: Trematoda Class species *(Prosthogonimus ovatus* with EI 12.4% and II-2.8 eg.); Secernentea Class 6 species *(Capillaria annulata* with EI-5.1%, II-6.6 eg., *Syngamus tracheia* with EI-9.5,1%, II-3.7eg., *Heterakis isolonche* with EI-10.3% , II-8.4 eg., *Ascaridia galli* with EI-82.3%, II-14.4 eg., *Heterakis gallinarum* with EI-21.8%, II-11.9 eg. and *Trichostrongylus tenuis* with EI-11.1%, II-3.6 eg.) and Conoidasida Class 3 species *(Eimeria colchici* with EI-11.9%, II-19.4 eg., *E. duodenalis* with EI-27,0%, II-14.7 eg. and *E. phasiani* with EI-9.3%, II-15.2 eg.,) (tab. 1).

Table 1
Diversity of parasitic fauna at wild birds of hunting interest in the Republic of Moldova

Parasite species	The parasitic host									
	Pheasants		Quails		Guineafowl		Chickens		Turkeys	
	EI (%)	II (eg.,)	EI (%)	II (eg.,)	EI (%)	II (eg.,)	EI (%)	II (eg.,)	EI (%)	II (eg.,)
Trematode class										
Echinostoma revolutum (Frohlinch, 1802)	-	-	14.5	3.4	-	-	1.3	2.5	-	-
Prosthogonimus ovatus (Rud.,1803)	12.4	2.8	10.4	5.6	2.77	7.3	3.26	5.4	-	-
Secernentea class										
Capillaria caudinflata (Zeder,1800)	-	-	8.3	4.3	-	-	-	-	5.12	4.7
Capillaria annulata (Molin, 1858)	5.1	6.6	-	-	47.2	9.8	-	-	-	-
Capillaria gallinae (Cheng, 1982)	-	-	-	-	-	-	25.4	13.6	-	-
Syngamus tracheia (Montagu, 1811)	9.5	3.7	2.08	2.3	2.77	3.7	1.0	1.5		
Heterakis isolonche (Linstow, 1906)	10.3	8.4	-	-	-	-	-	-	-	-
Ascaridia galli (Schrank, 1788)	82.3	14.4	64.6	18.3	41.6	11.2	45.7	15.6	76.9	12.3
Heterakis gallinarum (Schrank, 1788)	21.8	11.9	60.4	14.3	16.6	12.4	44.4	16.4	94.8	14.6
Trichostrongylus tenuis (Mehlis, 1846)	11.1	3.6	-	-	-	-	1.3	2.6	-	-
Cestoda Class										
Raillietina echinobothrida (Megnin, 1818)	-	-	-	-	-	-	3.9	3.4	-	-
Raillietina tetragona (Molin,1858)	-	-	22.9	5.8	-	-	17.6	9.9	66.6	13.3

Conoidasida Class										
Eimeria numidae (Pellerdy, 1962)	-	-	-	-	51.5	15.6	-	-	-	-
E. meleagridis (Tyzzer, 1929)	-	-	-	-	-	-	-	-	46.1	13.2
E .tenella (Railliet & Lucet, 1891)	-	-	-	-	-	-	31.9	14.6	-	-
E.necatrx(Johnson, 1930)	-	-	-	-	-	-	34.1	15.6		
E. adenoeides (Moore and Brown, 1951)	-	-	-	-	32.1	16.8	-	-	28.2	13.6
E.acervulia (Tyzzer, 1929)	-	-	-	-	-	-	41.0	15.4	-	-
***E. brunetti* (Levine, 1942)**	-	-	-	-	-	-	25.4	15.5	-	-
E .maxima (Tyzzer, 1929)	-	-	-	-	-	-	20.9	17.3	-	-
E. uzura (Tsunoda and Muraki, 1971)	-	-	14.5	15.6	-	-	-	-	-	-
E. bateri (Bhatia, Panday and Pande, 1965)	-	-	20.8	12.4	-	-	-	-	-	-
E. coturnicis (Chakravarty & Kar 1947)	-	-	35.4	17.6	-	-	-	-	-	-
Eimeria colchici (Norton, 1967)	11.9	19.4	-	-	-	-	-	-	-	-
E. duodenalis (Norton, 1967)	27.0	14.7	-	-	-	-	-	-	-	-
E. phasiani (Tyzzer, 1929)	9.3	15.2	-	-	-	-	-	-	-	-
Totally researched	**78**		**48**		**36**		**153**		**39**	

From the total of 78 samples examined from pheasants, it was shown that 24 samples (30.7%) were infested in the form of monoinvasions, and 54 samples (69.3%) were mixtinvasions (fig.1).

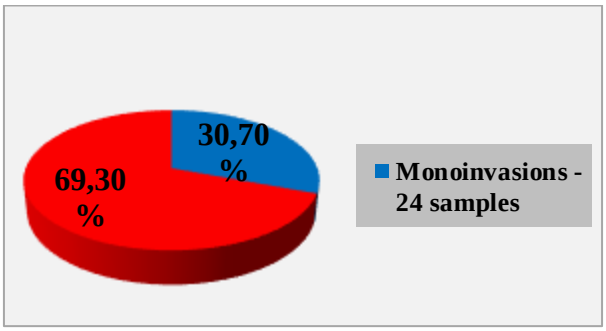

Fig.1. Monoinvasions and mixtinvasions identified at pheasant.

Monoinvasions at pheasants consisted of: *Ascaridia galli* - 8 samples (33.4%); *Eimeria duodenalis* - 6 samples (25.0%); *Prosthogonimus ovatus* - 4 samples (16.6%); *Heterakis gallinarum* - 3 probes (12.5%); *Trichostrongylus tenuis* -2 samples (8.3%) and one sample (4.2%) with *Eimeria colchici.*

Out of the total of 54 polyparasitic samples, the parasitological examination performed at pheasants allowed to highlight polyparasitic associations consisting of 2 species - 18 samples (33.3%) and composed of: *Ascaridia galli* + *Eimeria duodenalis* - 7 samples (38.9%); *Ascaridia galli* + *Heterakis gallinarum* - 5 samples (27.8%); *Ascaridia galli* + *Prosthogonimus ovatus* - 3 samples (16.6%); *Trichostrongylus tenuis* + *Ascaridia galli* - 2 samples (11.1%) and one sample (5.6%) *Ascaridia galli* + *Eimeria colchici.*

Polyparasitic associations at pheasants constituted of 6 species of parasites were identified in 2 samples (3.7%) and being composed of: *Ascaridia galli* + *Heterakis gallinarum* + *Syngamus tracheia* + *Capillaria annulata* + *Eimeria duodenalis* + *E. phasiani* - one sample (50.0%) and one sample (50.0) consisting of *Ascaridia galli* + *Heterakis gallinarum* + *Prosthogonimus ovatus* + *Trichostrongylus tenuis* + *E. duodenalis* + *E. colchici*.

Most species of helminths identified at pheasant - 5 species (71.4%) *(Syngamus tracheia, Heterakis isolonche, Ascaridia galli, Heterakis gallinarum, Trichostrongylus tenuis)* are in the development cycle without using another complementary host, belonging to the category geohelminths, however 2 species (28.6%) *(Prosthogonimus ovatus, Capillaria annulata)* achieve their development cycle through another complementary species, thus belonging to the category of biohelminths.

Out of the total of 10 species of parasites identified at pheasant, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum),* are also specific for quail, 5 species are specific for guineafowl (*Prosthogonimus ovatus, Capillaria annulata, Syngamidia tracheia, Heterakis gallinarum),* 5 species are specific for chickens *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Trichostrongylus tenuis)* and only 2 species are specific for turkeys *(Ascaridia galli, Heterakis gallinarum).*

Parasitological research performed at 48 samples taken from quails *(Cotrunix cotrunix L.),* revealed their infestation with various parasitic agents: Trematoda Class 2 species *(Echinostoma revolutum* with EI 14.5% and II-3.4 eg., *Prosthogonimus ovatus* with EI 10.4% and II-5.6 eg.); Secernentea Class 4 species (*Capillaria caudinflata* with EI-8,3%, II-4,3eg., *Syngamus tracheia* with EI-2.0%, II-2.3 eg., *Ascaridia galli* with EI-64.6%, II-18.3 eg. and *Heterakis gallinarum* with EI-60.4%, II-14.3 eg.); Class Cestoda with one species *(Raillietina tetragona* with EI-22.9%, II-5.8 eg.), and Conoidasida Class 3 species *(Eimeria wear* with EI-14.5%, II-15.6 eg., *E. batteries with* EI-20.8%, II-12.4 eg., and *E. coturnicis* with EI-35.4%, II-17.6 eg.) (tab. 1).

Out of the total of 48 samples examined from quails, it was shown that 17 samples (35.4%) were infested in the form of monoinvasions, and 31 samples (64.6%) were with mixed invasions.

Monoinvasions at quails consisted of: *Ascaridia galli* - 5 samples (29.4%); *Heterakis gallinarum* - 4 samples (23.5%); *Raillietin tetragon* - 3 samples (17.6%); *Capillaria caudinflata* - 2 samples (11.8%); *E. coturnicis*.- 2 samples (11.8%) and *E. bateri* - one sample (5,9%).

Out of the total of 31 polyparasitic samples, the parasitological examination performed at quails allowed to highlight polyparasitic associations consisting of 2 species - 12 samples (38.7%) and composed of: *Ascaridia galli* + *Heterakis gallinarum* - 4 samples (33.3%); *Ascaridia galli* + *Echinostoma revolutum* - 3 samples (25.0%); *Heterakis gallinarum* + *Raillietina tetragon* - 3 samples (25.5%); *Ascaridia galli* + *Eimeria coturnicis* - one sample (8.3%) and one sample (8.3%) *Heterakis gallinarum* + *Eimeria batteries*.

If we refer to the division of parasitic agents identified at quails, depending on how to achieve the parasitic development cycle, then 4 species (57.2%) are biohelminths, and 3 species (42.8%) are geohelminths.

Out of the total of 10 species of parasites identified at quails, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum)* are also common at pheasants, 4 species are specific at guineafowl *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heter),* 5 species are specific for chickens *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Raillietina tetragona)* and 4 species are specific for turkeys *(Capillaria caudinflata, Ascaridia galli, Heterakis gallinarum, Raillietina echinobothrida).*

Parasitological research performed to 48 samples taken from guineafowls *(Numida meleagris L.),* revealed their infestation with various parasitic agents: Trematoda Class a species *(Prosthogonimus ovatus* with EI-2.77% and II-7.3 eg .); Secernentea Class 4 species *(Capillaria annulata with* EI-47.2%, II-9.8 eg., *Syngamus tracheia* with EI-2.77%, II-3.7 eg., *Ascaridia galli* with EI-41.6%, II-11.2 eg., and *Heterakis gallinarum* with EI-16.6%, II-12.4 eg.); and Conoidasida Class 2 species *(Eimeria numidae* with EI-51.5%, II-15.6 eg., and *E. adenoeides* with EI-32.1%, II-16.8 eg.) (tab. 1).

From the total of 36 samples examined from guineafowl, it was highlighted that 11 samples (30.5%) were infested in the form of monoinvasions, and 25 samples (69.5%) were with mixtinvasions.

Monoinvasions at guineafowls consisted of: *Capillaria annulata* - 4 samples (36.3%); *Ascaridia galli* -3 samples (27.3%); *Eimeria numidae* -3 samples (27.3%) and *E. adenoeides* - one sample (9.1%).

Out of the total of 25 polyparasitic samples, the parasitological examination performed at guineafowls allowed to highlight polyparasitic associations consisting of 2 species - 9 samples (36.0%) and composed of: *Ascaridia galli* + *Eimeria numidae* - 3 samples (33.3%) ; *Ascaridia galli* + *Eimeria adenoeides* - 2 samples (22.2%); *Ascaridia galli* + *Capillaria annulata* - 2 samples (22.2%); *Ascaridia galli* + *Heterakis gallinarum* - one sample (11.1%) and one sample (11.1%) *Heterakis gallinarum* + *Eimeria numidae*.

If we refer to the division of the parasitic agents identified at guineafowls, depending on the way of accomplishing the parasitic development cycle, then 2 species (40.0%) are biohelminths, and 3 species (60.0%) are geohelminths.

Out of the total of 7 species of parasites identified at guineafowl, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum)* are common at pheasants, quails and chickens, and 3 species are specific for turkeys (*Ascaridia galli, Heterakis gallinarum, Raillietina tetragonal*).

Therefore, the parasitological results obtained, show that both domestic and wild birds are infested with various parasitic agents, dangerous for both hunting and domestic birds.

Conclusions

1. The result of the parasitological examination of 78 samples collected from pheasants *(Phasianus colchicus L.)* showed a high level of their infestation with various parasitic agents: Trematoda Class one species *(Prosthogonimus ovatus* with EI 12.4% and II-2.8 eg.); Secernentea Class 6 species *(Capillaria annulata* with EI-5.1%, II-6.6 eg., *Syngamus tracheia* with EI-9.5%, II-3.7eg., *Heterakis isolonche* with EI-10.3%, II-8.4 eg., *Ascaridia galli* with EI-82.3%, II-14.4 eg., *Heterakis gallinarum* with EI-21.8%, II-11.9 eg. and *Trichostrongylus tenuis* with EI-11.1%, II-3.6 eg.) and Conoidasida Class 3 species *(Eimeria colchici* with EI-11.9%, II-19.4 eg., *E. duodenalis* with EI-27.0%, II-14.7 eg. and *E. phasiani* with EI-9.3%, II-15.2 eg.);
2. Parasitological research performed at 48 samples taken from quails *(Cotrunix cotrunix L.),* revealed their infestation with various parasitic agents: Trematoda Class 2 species *(Echinostoma revolutum* with EI 14.5% and II-3.4 eg., *Prosthogonimus ovatus* with EI 10.4% and II-5.6 eg.); Secernentea Class 4 species (*Capillaria caudinflata* with EI-8.3%, II-4.3eg., *Syngamus tracheia* with EI-2.0%, II-2.3 eg., *Ascaridia galli* with EI-64.6%, II-18.3 eg. and *Heterakis gallinarum* with EI-60.4%, II-14.3 eg.); Class Cestoda with one species *(Raillietina tetragona* with EI-22.9%, II-5.8 eg.), and Conoidasida Class 3 species *(Eimeria wear* with EI-14.5%, II-15.6 eg., *E. batteries with* EI-20.8%, II-12.4 eg., and *E. coturnicis* with EI-35.4%, II-17.6 eg.);
3. Parasitological research performed to 36 samples taken from guineafowls *(Numida meleagris L.),* revealed their infestation with various parasitic agents: Trematoda Class one species *(Prosthogonimus ovatus* with EI-2.77% and II-7.3 eg); Secernentea Class 4 species *(Capillaria annulata with* EI-47.2%, II-9.8 eg., *Syngamus tracheia* with EI-2.77%, II-3.7 eg., *Ascaridia galli* with EI-41.6%, II-11.2 eg., and *Heterakis gallinarum* with EI-16.6%, II-12.4 eg.); and Conoidasida Class 2 species *(Eimeria numidae* with EI-51.5%, II-15.6 eg., and *E. adenoeides* with EI-32.1%, II-16.8 eg.);
4. Out of the total of 10 species of parasites identified at pheasant, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum),* are also specific for quail, 5 species are specific for guineafowl (*Prosthogonimus ovatus, Capillaria annulata, Syngamidia tracheia, Heterakis gallinarum),* 5 species are specific for chickens *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Trichostrongylus tenuis)* and only 2 species are specific for turkeys *(Ascaridia galli, Heterakis gallinarum).*
5. Out of the total of 10 species of parasites identified at quails, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum)* are also common at pheasants, 4 species are specific at guineafowl *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heter),* 5 species are specific for chickens *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum, Raillietina tetragona)* and 4 species are specific for turkeys *(Capillaria caudinflata, Ascaridia galli, Heterakis gallinarum, Raillietina echinobothrida).*
6. Out of the total of 7 species of parasites identified at guineafowl, 4 species *(Prosthogonimus ovatus, Syngamus tracheia, Ascaridia galli, Heterakis gallinarum)* are common at pheasants, quails and chickens, and 3 species are specific for turkeys (*Ascaridia galli, Heterakis gallinarum, Raillietina tetragonal*).

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Parasitofauna and the effectiveness of antiparasitic treatment at deer with various types of stress reactivity

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Abstract
So, the determination of the type of stress reactivity at deer by applying the adrenaline test formulated by Ahmadiev GM (1990), allows to select deer resistant to infestation with various parasitic agents and to obtain a higher effectiveness of antiparasitic treatment in stress-resistant deer compared to stress-reactive.Therefore, we recommend selecting deer according to the type of stress reactivity and obtaining flocks of stress-resistant animals, which have a high resistance to parasite infestation with a high therapeutic efficacy and with minimal deworming costs. So, before applying antiparasitic treatment to deer, it is necessary to establish their type of stress reactivity, and to the stress reactive to apply repeated treatment over 14 days, because its effectiveness is varied and depends on the type of reactivity of the animal's organism.
Keywords: *deer, infestation, type of stress reactivity.*

Introduction

One of the main concerns of specialists in the field of parasitology and helminthology at the present stage is the study of the relationship between the host and various species of parasites, which is manifested by irritative-mechanical, spoilage, toxic and stressful actions manifested by altering homeostatic mechanisms at various levels following the reduction of the superficial and deep energy of the adaptation of the parasitized animal (Niculescu, Didă, 1998).

The stress reaction that occurs not only at the action of extreme exciters of high intensity, but also of a lower power, but with a long and repeated duration of action, causes a typical stress reaction (Davidov, Mukhin, 1978; Pavaliuc, 1999; Gorizontov, 1983).

The adaptation of animals to current conditions depends primarily on the individual specificity of the activity of the superior and endocrine nervous system. On the reactivity and on the possibility of adaptation of the animal organism to stressors, the level of stress resistance has a decisive influence, in the essence of which is the type of superior nervous activity (Macarov, 1987; Plyanko, Sidorov, 1987; Curus, 1992; Curus; Штирбу, Струтинский и др., 1992; Olteanu, Curcă, 1993; Анисько, Анисько, 1991; Степанов, 1997). Among the stressors, an essential influence on the body has and the parasitic factor. The question arises - but how does this factor reflect on the reactivity of the animal and how is it expressed at animals with different types of reactivity.

In working with animals, it is very important to know the extent of their sensitivity, especially to parasitic and infectious factors. The terms "sensitive" and "resistant" are terms used very often by many authors, but which so far don't have an exact notion. The term "sensitivity" means the state of the organism, when under conditions of host-parasite combination, the host is able to provide such a surrounding atmosphere, in which it is possible development and maturation of parasite. The term "resistance" means the state, when the host possesses those qualities both innate and conditioned, which limit the development of the parasite to some stage of development in the host.

According to other authors, stress sensitivity is understood as a level of reactions of the animal to the action of the stress factor, and by stress resistance - the possibility of the animal to adapt to the new conditions created, without a clear loss of productivity. Knowing the possibilities of adaptation of the organism, the mechanism of these reactions and their method of activation,

they have a great significance for an effective exploitation of animals. An important practical interest is the determination of the stress reactivity and stress resistance of animals. As was determined, the manifestation of stress depends not only on the type and character of the stress factor, but also on race, age, sex, etc.

Determining the degree of sensitivity of animals to sresogenic factors is largely related to increasing or decreasing of the content of corticosteroids in the blood. Attempts have been made to determine the reactivity and stress resistance at cattle by type of constitution, productive qualities, time of complete elimination of milk, number of somatic cells in milk. For this purpose, some authors used the effort with adenocorticotropin (ACTH), on which they determined the number of leukocytes, neutrophils, eosinophils, lymphocytes and glucose. The level of variation of these indices, to some extent, reflects the adaptation reaction of the body and the functional state of the pituitary-adrenal system.

To determine the stress reactivity at cattle, it is also recommended to use such indices as: content of corticosteroids in the blood, the ascorbic acid in the adrenal glands, cholesterol, free fatty acids, creatine phosphokinase, lactate dehydrogenase, lactate, glucose, etc. (Жижикина Г., 1981; Ковак Л., 1982; Пономарь С.И., 1989).

Was researched the method of testing the stress reactivity at cattle after the level of corticosteroids in the blood, with the parallel determination of the aggression coefficient of the animals. According to other data, for this purpose it is possible to determine the activity of some ferments such as: creatinine kinase, lactate dehydrogenase, cationic lysosomal test, etc. (Плященко С.И., Сидоров В.Т., 1987; Макаревич Н.А., 1988).

Kokorina E.P. (1986) developed a method for determining the stress resistance of cattle, based on establishing the intensity of inhibition of the milk elimination reflex, as response to the action of the stress factor, which causes a foreign milking during the cow's milking.

In the technology of cattle breeding, in the last years, for determination of stress resistance the ethological method is used more widely, which allows the differentiation of sheep according to their behavior.

The galatonic test is used more widely to assess the stress sensitivity at pigs (Кузнецов А.И., 1991; Забудский utiliz.И.1991).

At cattle, the adrenaline test is successfully used to assess the sensitivity of animals to stressors (Чумаков В.И., 1978; Erhan D.C., Rusu Ş.T., Pavaliuc P.P., 2005, 2007).

Determination of the type of stress reactivity at deer is recommended to be performed in order to improve their genophone in nature, by obtaining some descendants resistant to stressors including parasites. Currently, in order to highlight the type of stress reactivity, are needed express methods, through simpler tests.

The proposed goal is to identify the type of stress reactivity of deer and them division into two groups (stress reactive, stress resistant), before applying antiparasitic treatment. At the formed batches, were established the intensity and extent of the invasion, before and after the application of the antiparasitic treatment.

Both batches of deer had the same maintenance conditions.

Materials and methods

The experiments were carried out during the years 2018-2020 at deer maintained in the Zoo from Chisinau town, Republic of Moldova and in the Laboratory of Parasitology and Helminthology of the Institute of Zoology. The researches aimed to identify the level of infestation and to determine the effectiveness of antiparasitic treatment at deer with different types of reactivity.

The determination of the type of stress reactivity at deer was performed after the adrenaline test, formulated by Ahmadiev G.M. (1990), which consists in the action of the 0.1% - adrenaline hydrochloride solution, on the immune reactivity of the deer's body.

The simplicity of the method, the minimum cost of the preparations and of the equipment allow its application, in the field and in mass, which has a major importance in determining the deer's reactivity.

In total, according to the adrenaline method formulated by Ahmadiev G.M. (1990), were tested 26 deer, after which were selected and formed the batches, including 10 deer in each batch. Thus, were formed 2 groups: group I - stress-reactive, group II-stress-resistant.

Deer from both groups formed, underwent at parasitological investigations according to coproovoscopic methods (Fulleborn, Darling), coprolarvoscopic (Popov, Baermann), special examination in sarcocystosis according to the Kakurin method, partial parasitological investigations (after K. I. Skrjabin) and consecutive washing. The intensity of the invasion with nematode larvae was established in 5 g of faeces, oocysts of *Eimeria spp*., eggs of trematodes and nematodes, in 10 visual microscopic fields (10x40).

Results and discussions

At deer from the I group (reactive stress) were established the following indices of invasion extensiveness (EI) and invasion intensity (II): *Fasciola hepatica* EI- 40% of cases, II -1.7 eg, *Dicrocoelium lanceolatum* with EI - 50.0 %, II - 2.8 ex., *Strongyloides papillosus* with EI - 100.0% and II - 22.0 ex., *Cooperia punctata* with EI- 60.0% and II-12.0 ex., *Ostertagia ostertagi* with EI - 40.0% and II - 6.2 ex., *Toxocara vitulorum* with EI - 20.0% and II-3.5 ex., *Eimeria ponderosa* with EI- 60.0% and II - 5.0 ex., *E. capreoli* with EI - 80.0% and II - 6.9 ex. and *E. bovis* with EI - 30.0 and II - 4.3 exemplaries (tab.1).

At deer from the II group (stress resistant) was established the following level of infestation: *Fasciola hepatica* with EI - 20.0%, II -1.0 ex., *Dicrocoelium lanceolatum* with EI - 30.0%, II - 2.0 ex., *Strongyloides papillosus* with EI - 70.0% and II - 8.4 ex., *Cooperia punctata* with EI - 40.0% and II - 5.2 ex., *Ostertagia ostertagi* with EI - 30.0% and II - 5 , 3 ex., *Eimeria ponderosa* with EI- 40.3% and II - 2.2 ex., *E. capreoli* with EI - 60.0% and II - 2.3 ex. and *E. bovis* with EI -20.0 and II - 3.5 exemplaries (tab.1).

As a result of parasitological investigations obtained from both groups of deer, it can be noted that the level of infestation with all parasite species identified at deer is obviously higher at the stress-responsive group compared to those from the stress-resistant group.

Table 1

The level of deer infestation before and after antiparasitic treatment

Parasite species	Before treatment				After treatment			
	The 1th group		The 2nd group		The 1th group		The 2nd group	
	EI, %	*II, ex.,*	*EI, %*	*II, ex.,*	*EI, %*	*II, ex.,*	*EI, %*	*II, ex.,*
Fasciola hepatica	40,0	1,7	20,0	1,0	-	-	-	-
Dicrocoelium lanceolatum	50,0	2,8	30,0	2,0	20,0	1,0	-	-
Strongyloides papillosus	100,0	22,0	70,0	8,4	30,0	3,0	-	-
Cooperia punctata	60,0	12,0	40,0	5,2	20,0	4,5	-	-

Ostertagia ostertagi	40,0	6,2	30,0	5,3	10,0	1,0	-	-
Toxocara vitulorum	20,0	3,5	-	-	-	-	-	-
Eimeria ponderosa	60,0	5,0	40,0	2,2	20,0	1,0	-	-
E. capreoli	80,0	6,9	60,0	2,3	30,0	1,6	-	-
E. bovis	30,0	4,3	20,0	3,5	-	-	-	-

After determination of type of stress reactivity and of level of infestation of both groups of deer, was applied the complex antiparasitic treatment in the form of briquettes. (according to the short-term Patent "Deer deworming process". MD 1303 Y 2019.01.31)

The briquettes have in their composition a mixture, which contains corn meal, oat meal, wheat meal, sunflower seed cake, soybean meal, bentonite, iodized table salt, premix for paracopity based on vitamins, trace elements and minerals, diclazuril 1%, levamisole 8%, molasses, dextrin and water, in the following component ratio, g/head: maize meal- 133.33, oat meal- 133.33, wheat meal- 111.11, sunflower seed cake - 44.44, soybean meal- 22.22, bentonite- 177.77, iodized table salt- 8.88, premix for paracopity based on vitamins, trace elements and minerals- 20.0, diclazuril- 1%, 28 ml levamisole- 8%, 7.0 - molasses, 22.22 ml – dextrin, 22.22 - water 60.88 ml, at the same time the mixture is administered in the form of briquettes of 800 g in a dose of 1 briquette/head once.

Levamisole 8% is used at cattle, sheep, goats, pigs against lung parasites: *Dictiocaulus spp., Metastrongylus spp., Protostrongylus spp.,* and against gastrointestinal nematodes: *Trichostrongylus spp., Ostertagia spp., Haemonchus spp., Cooper Bunostonum spp., Nematodirus spp., Oesophagostomum spp., Strongyloides spp., Chabertia spp., Toxocara vitulorum., Hyostrongylus spp., Trichuris spp.* and *Ascaris spp*. Levamisole 8% is also indicated for the control of immunodeficiency.

Diclazuril is a coccidiostat that belongs to the benzene-acetonitrile group. It has a coccidiostatic action on *Eimeria* species. Depending on the species of coccidia, diclazuril has an effect on the asexual or sexual stages of the parasite's developmental cycle. Treatment with diclazuril interrupts the coccidial cycle and suppresses oocyst excretion. The premix included in the briquette is a product based on vitamins, trace elements, assimilated concentrated minerals, indicated for paracopitate animals, ruminants: large horned cattle, deer.

Based on the results of coprological analyzes obtained at deer, with the detection of parasitic forms of the class Trematoda *(Fasciola hepatica, Dicrocoelium lanceolatum*), and preparations antiparasitic from the composition of briquettes (Levomisol 8%, Diclazuril 1%) doesn't have antiparasitic action, additional with the concentrates was added Brovalzen (powder) - the active substance in which is albendazole, which has an antiparasitic action also on trematodes. 1g of Brovalzen contains 75 mg of Albendazole. Albendazole belongs to the group of benzimidamides, blocks protein synthesis and as a result, disrupts intercellular transport of nutrients and exchange of substances (adenosine triphosphoric acid and glucose), reducing mitochondrial reactions, by reducing the action of fumarate reduction, which then leads to the death of the parasites. It is effective on the mature and larval forms of nematodes located in the gastrointestinal tract and in the lungs. It is a broad-spectrum anthelmintic, it is rapidly absorbed and diffuses in all organs, regardless of the species and category of treated animals. It is recommended for dehelminthization of ruminants administered in a single batch, having action on gastrointestinal and pulmonary nematodes from the families: *Anisakidae, Ancylostomatidae, Ascaridae, Dictyocaulidae, Oxyuridae, Protostrongylidae, Strongylidae, Syphacidae, Trchuridae, Trichonematidae, Trichostrongylidae.*

Brovalzen (powder) also has an action on mature forms of trematodes (*Fasciolidae, Dicrocelidae*). It also acts on cestodes from the families: *Avitellinidae, Anoplocephalidae, Taeniidae*. The recommended dose is 1g per 10 kg live weight. Respectively for each animal with a mass of about 70.0 kg, dose of 7.0 g of preparation returns, and at 20 heads we obtain the amount of 140.0 g of Brovalzen (powder). (Н. В. Демидов. Гельминтозы животных. Справочник М. Агропромиздат, 1987, с. 79).

Parasitological investigations performed on deer from group I (stressful) on the 14th day after antiparasitic treatment, showed the following results: *Dicrocoelium lanceolatum* with EI - 20.0%, II - 1.0 ex., *Strongyloides papillosus* with EI - 30.0% and II - 3.0 ex., *Cooperia punctata* with EI- 20.0% and II-4.5 ex., *Ostertagia ostertagi* with EI - 10.0% and II - 1.0 ex., *Eimeria ponderosa* with EI- 20.0% and II - 1.0 ex. and *E. capreoli* with EI - 30.0% and II - 1.6 ex. (Table 1).

At the deer from group II deer (stress resistant) after application of antiparasitic treatment, weren't identified parasitic agents at any animal (tab.1).

The result of the parasitological investigations obtained after the application of the antiparasitic treatment to both groups of deer, allows us to notice that the effectiveness of the antiparasitic treatment performed is higher in the group of stress-resistant deer compared to those in the stressful group.

After the parasitological diagnosis was performed, after 14 days from the first treatment of the deer from the stress group, was applied the repeated antiparasitic treatment.

After the application of the repeated antiparasitic treatment, parasitological investigations were performed, as a result of which it was established that the deer from both groups (stress-reactive and stress-resistant) are completely dewormed.

Conclusions

In conclusion, we can note that for the first time was realized deer selection according to the type of stress reactivity by applying the adrenaline test formulated by Ahmadiev GM (1990), which increases the effectiveness of treatment and prophylaxis of parasites at deer by selecting them with higher resistance to infestation with various parasitic agents and the application of antiparasitic treatment according to the type of stress reactivity. Because the effectiveness of antiparasitic treatment at deer is different and depends on the type of stress reactivity, the repeated application of antiparasitic treatment to the stress reactive group over 14 days is recommended. Therefore, it is proposed that in determining the efficacy of antiparasitic preparations, to take into account the type of reactivity of the animals, and in the case of stress reagents in order for obtaining a high antiparasitic efficacy it is necessary to apply the antiparasitic treatment repeatedly.

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Medicinal properties of *Thymus vulgaris* essential oil: a review

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Abstract

Globally, the interest in essential oils has been steadily increasing over the last decades due to their beneficial health effects and the wide range of applications that are directly linked to a variety of pharmacological and biological activities. Thymus vulgaris L., also called common or garden thyme is a small perennial herb, which has been used over the years as food additive, but also as a valuable cure in several pathologies. It is believed that the medicinal properties of Thymus vulgaris are attributed to its essential oil, which is a mixture of monoterpenes. Moreover, the therapeutic properties of this essential oil are due to its main compounds, namely the terpenoid thymol and its phenol isomer carvacrol. Several investigations have indicated that thyme oil possesses strong antiseptic, antimicrobial, antifungal, antioxidant effects and therefore, all the aforementioned features make this essential oil a promising remedy in human and veterinary medicine fields. The aim of the present study was to review and highlight the medicinal attributes of Thymus vulgaris essential oil, apart from its nutritional value, in order to identify novel alternative cures in the treatment of both humans and animals diseases.

Keywords: *essential oil, medicinal properties, Thymus vulgaris.*

Introduction

Thymus vulgaris L., one of the most famous aromatic plants in the *Lamiaceae* family and belonging to the genus *Thymus*, is highly recommended by researchers due to numerous therapeutic promises of its essential oil, commonly known as thyme oil (Zaidi and Crow, 2005; Maksimoviæ et al., 2008; Sokoviæ et al., 2009).

In general, essential oils may contain around 20-80 phytochemicals (Regnault-Roger et al., 2012). Concerning *Thymus vulgaris*, there are several chemotypes which are named in accordance with the major compound, for instance, thymol, carvacrol, terpineol and linalool. In addition to this, Sienkiewicz et al. (2017) reported that thyme oil consists mainly of thymol (38.1%), p-cymene (29.1%), γ-terpinene (5.2%), linalool (3.7%) and carvacrol (2.3%). Moreover, it has been stated that the biological properties of thyme oil are primarily due to its main constituents, thymol and carvacrol (Newton, 2000).

Besides the fact that thyme oil is one of the most popular essential oils in the food and cosmetics industries, many studies underlined its therapeutic potential in various pathologies (Stahl-Biskup, 2002; Burt, 2004; Shin and Kim, 2005; Politeo et al., 2007).

The present study was designed to highlight and summarize some of the medicinal properties of *Thymus vulgaris* essential oil, which lately, has gained more and more attention from researchers around the world.

Antimicrobial, antioxidant, anticancer effects

Also known as volatile oils, essential oils are natural aromatic compounds, nontoxic and nonpollutive products, presenting low risk of adverse effects and low risk of microbial resistance development (Rajkowska et al., 2014). The antimicrobial capacity of essential oils depend, in general, on their chemical compounds, mostly on their phenolic compounds, such as thymol, γ-terpinene, carvacrol (Boruga et al., 2014; Cristiani et al., 2007). Thymol has registered 30 times stronger antibacterial activity and 4 times lower toxicity compared to phenol, an antiseptic that can

be found in several herbicides (Hajimehdipoor, 2010; Weber, 2004). Furthermore, thymol appears to disrupt the membrane structure and to increase the cell permeability, leading to a proton motive force diminution and decreased ATP intracellular levels, and hence, causing the death of the pathogen cell (Liolios et al., 2009; Fong et al., 2011). Another constituent present in essential oils composition is p-cymene, which seems to exhibit an antibacterial activity only when it is used with thymol and γ-terpinene, proving synergistic effects (Rota et al., 2008).

Several studies demonstrated the efficacy of thyme essential oil against numerous pathogens (Lai and Roy, 2004; Mitsch et al., 2004; Penalver et al., 2005; Al-Bayati, 2008). In this regard, a strong antimicrobial activity was recorded against *Helicobacter pylori, Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Selenomonas artemidis, Porphyromonas gingivalis, Streptococcus mutans, Streptococcus sobrinus, Streptococcus spp., Pantoea spp., Bacillus spp., Shigella spp* (Ceyhan and Ugur, 2001; Boruga et al., 2014; Imelouane et al., 2009; Nasir et al., 2015). Additionally, it was evaluated the growth inhibition effects of thyme esential oil against various strains of both Gram-negative and Gram-positive bacteria (Marino and Bersani, 1999; Prasanth et al., 2014; Saleh et al., 2015). A strong antimicrobial activity was observed against both types of bacteria, but however, it was more pronounced against the Gram-positive bacteria.

Likewise, other researchers pointed out that thyme oil is highly effective against different types of fungi and yeast, such as *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Trichophyton spp., Microsporum spp., Rhodotorula rubra and Candida albicans* (Arras and Usai, 2001; Inouye et al., 2001a; Nasir et al., 2015; Rajkowska et al., 2014). Moreover, Jabeur et al. (2017) reported that thyme essential oil is active against the plant-pathogenic fungus *Mycosphaerella graminicola*, responsible for septoria tritici blotch (STB), one of the most economically important diseases of wheat, which threatens global food production. Furthermore, Nolkemper et al. (2006) revealed a strong antiviral activity of thyme essential oils against Herpes simplex virus type 1 (HSV-1) and acyclovir-resistant strain of HSV-1.

Antioxidants are compounds which are able to inhibit different oxidation reactions and to remove free radical intermediates and thus, preventing cell death (Dipak, 2013). The antioxidant capacity of thyme essential oil is due to the presence of its active compounds, mainly thymol and carvacrol (Ruberto and Baratta, 2000). In *vivo* and *in vitro* studies showed important antioxidant effects of thyme oil and thymol (Youdim and Deans, 2000; Nickavar et al., 2005).

Thyme oil also contains significant amounts of zeaxanthin, apigenin, lutein, luteolin and thymine, which represent a valuable source of antioxidants (Dauqan and Abdullah, 2017). The flavonoids may prevent the release of superoxide anion and protect erythrocytes from oxidative stress (Youdim and Deans, 2000). Moreover, El-Nekeety et al. (2011) evaluated the protective effects of thyme oil against aflatoxin-induced oxidative stress in male Sprague-Dawley rats. The animals were divided into six groups and treated for 2 weeks: control group; the groups treated orally with low and high doses of thyme oil; the group fed AFs (aflatoxins)-contaminated diet and the groups fed AFs-contaminated diet and treated orally with the oil at the two tested doses. The results highlighted a disturbance in serum lipid profile, a low antioxidant capacity, increased creatinine, uric acid and nitric oxide in blood serum, lipid peroxidation in liver and histological changes within the liver tissues, when applying the treatment with aflatoxins; the oil at different doses did not seem to produce any significant changes. An improvement was however observed in the investigated parameters and histological aspects, when using the combined treatment.

Available data have suggested that foods which are rich in phytochemicals, can reduce the risk of various types of cancer, due to their antioxidant, anti-inflammatory and immunomodulatory

activity and have the ability to modulate the proliferation, apoptosis, angiogenesis of cancer cells (Kapinova et al., 2017; Kapinova et al., 2018; Stewart and Wild, 2014). According to Sertel et al. (2011), *Tymus vulgaris* essential oil inhibited the growth of human oral cavity squamous cell carcinoma, with the regulation of N-glycan biosynthesis and extracellular signal-regulated kinase 5 (ERK5) and interferon signaling. Furthermore, an *in vitro* study pointed out the antiproliferative and proapoptotic activity of thyme essential oil in MCF-7 cells and MDA-MB-231 cells, which are both breast cancer cell lines (Kubatka et al., 2019). Moreover, Kang et al. (2016) reported that thymol manifests anticancer activity by suppressing cell growth, inducing apoptosis, producing intracellular reactive oxygen species, depolarizing mitochondrial membrane potential, activating the proapoptotic mitochondrial proteins Bax, cysteine aspartases and poly ADP ribose polymerase in human gastric AGS cells, a human gastric adenocarcinoma cell-line.

Gastrointestinal health benefits

Numeorus research have been carried out in order to explore the gastrointestinal health benefits of thyme essential oil and particularly two of its most important constituents, thymol and carvacrol. In this regard, indigestion which is also known as dyspepsia could be treated by oral administration of thyme extract (Mossa et al., 1987). Moreover, it was reported that thymol and carvacrol may enhance the activities of intestinal and pancreatic trypsin, protease and lipase, when administered to animals in equal amounts. These two compounds also appear to ameliorate the liver function and increase appetite (Hosseinzadeh et al., 2015; Thompson et al., 2003; Hashemipour et al., 2013). Another research conducted by Höferl et al. (2009) suggested that thymol and carvacrol act as antispasmodic agents.

Thyme essential oil proved to be highly effective in several intestinal infections and infestations, namely hookworms, ascarids, Gram-positive and Gram-negative bacteria, fungi and yeasts, due to its valuable compounds (Ceyhan and Ugur, 2001).

Oliveira et al. (2012) revealed the carvacrol gastroprotective effects on experimentally induced gastric lesions in rats, by preventing the gastric epithelium injury. Furthermore, Silva et al. (2012) demonstrated that the oral administration of carvacrol reduced the gravity of chemically induced gastric damages in rats after two weeks of treatment by comparison to controls. Other researchers investigated the effect of thymol on differential gene expression in young pig gastric mucosa and they observed that this compound stimulates genes associated with mitosis, cell division regulation and the stomach digestive function (Colombo et al., 2014).

Thyme oil and oral care

Some of the most common and widespread oral pathologies are represented by dental caries, periodontal diseases and streptococcal pharyngitis. Dental caries, also known as tooth decay is the result of a complex interaction between acid producing tooth-adherent bacteria and fermentable carbohydrates leading to demineralization of the tooth and the formation of cavities. Dental caries aetiology involves microbial factors, fermentable carbohydrates, susceptible tooth surface (Petersen, 2005; Selwitz et al., 2007). The main microbial pathogen resposible for dental caries is *Streptococcus mutans*, a Gram-positive facultative anaerobe (Bowen and Koo, 2011; Gross et al., 2012). Different studies revealed the strong inhibitory effect of thyme essential oil on *Streptococcus mutans* growth (Ghorab et al., 2014; Hammad et al., 2007; Fani and Kohanteb, 2017; Gonçalves et al., 2011).

Periodontitis, or gum disease, represents a chronic inflammatory pathology which is activated by microbial agents and triggers the destruction of the tooth-supporting apparatus, causing tooth loss. The agents most frequently involved in the aetiology of periodontitis are

considered to be *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (Kononen et al., 2007). In this regard, there are some available data in literature regarding the inhibitory activity of thyme oil on the aforementioned pathogens (Rodriguez-Garcia et al., 2010; Fani and Kohanteb, 2017).

Streptococcuspyogenes (group A *Streptococcus*) is the most common and important cause of pharyngitis, a bacterial infection of oropharynx which involves tonsils and larynx. Since increasing failure to *Streptococcus pyogenes* treatment with penicillin and erythromycin has been noticed, because of allergy and antibioresistance, respectively, it is imperative to develop alternative natural antibacterial agents (Malli et al., 2010; Ying-Huang et al., 2014). Therefore, several research have emphasized gratifying results regarding *S. pyogenes* being highly sensitive to *Thymus vulgaris* oil (Inouye et al., 2001b; Solano et al., 2006; Sfeir et al., 2013; Nikolic et al., 2014; Fani and Kohanteb, 2017).

Thyme oil and wound healing

Due to its multiple biological properties, thyme oil can be regarded as a strong candidate concerning the development of products related to tissue repair. For instance, thymol and carvacrol, two major compounds detected in thyme oil seem to act in the wound healing phases. Thus, they have exhibited modulatory effect on the inflammatory cytokines and oxidative stress, during inflammatory phase and have enhanced re-epithelialization, angiogenesis and granulation tissue development, during proliferative stage. In the remodelling/maturation phase, they have increased the collagen deposition and have promoted the fibroblasts and keratinocytes growth (Komarcevic, 2000; Costa et al., 2019).

Additionally, thymol and carvacrol are able to activate antioxidant systems, as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), modulate reactive species and reduce inducible nitric oxide synthase (iNOS), one of the reactive oxygen (Andre-Levigne et al., 2017; Guvenc et al., 2018; Xiao et al., 2018). Another study conducted by Mollarafie et al. (2015) pointed out that a chitosan film consisting of pure thymol enhanced fibroblast cell growth. Likewise, Pivetta et al. (2018) reported that a gel containing nanoencapsulated tymol did not present any cytotoxicity in human keratinocytes, maintaining cell viability above 80%.

Conclusions

As we live in an era where pathogens have become more and more resistant to our arsenal of medications, there is an urgent need to explore and develop novel natural remedies in order to control public health threats. Thyme essential oil presents a high potential for the development of new therapeutic formulas in different kind of pathologies, due to its multiple biological activities. However, further research must be carried out in order to observe the efficacy and safety of these compounds, to discover the mechanisms of action, some of them being not yet fully understood or to make adjustments regarding dosage and treatment protocol.

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The helminth fauna of some invasive fishes from various natural and artificial water bodies from the Republic of Moldova

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Abstract
This paper presents the results of the study of the helminth fauna of invasive fish species from different water bodies from the Republic of Moldova: prussian carp – Carassius gibelio (Bloch, 1782), stone moroko – Pseudorasbora parva (Temminck & Schlegel, 1846), chinese sleeper – Perccottus glenii Dybowski, 1877, pumpkinseed – Lepomis gibbosus (Linnaues, 1758), silver carp – Hypophthalmichthys molitrix (Valenciennes, 1844), bighead carp – Hypophthalmichthys nobilis (Richardson, 1845). As a result of this study 16 species were detected (Dactylogyrus sp., Gyrodactylus sp., Diplozoon paradoxum, Eudiplozoon nipponicum, Phyllodistomum folium, Diplostomum spathaceum, Botriocephalus opsariichthydis, Khawia parva, Paradilepis scolecina, Philometroides sanguinea, Pseudocapillaria tomentosa, Raphidascaris acus, Posthodiplostomum cuticola, Valipora campylancristrota, Ligula intestinalis).
***Keywords**: water bodies, helminths, invasive fish*

Introduction

Under the direct action of anthropogenic factors, as well as under the influence of climate change, significant disturbances in the structure of local ichthyocenosis can occur. In a degraded environment, negative processes take place in a much more accelerated form, with invasive fishes capable of causing chain reactions such as: alteration of native fish species habitat, destabilization of host ichthyocenosis, genetic degradation of host fish stocks, introduction of new parasites and diseases, negative socio-economic effects [1,3].

Fish, as a vital source of food for people, in the conditions of a large development gap within countries, can be treated as a desired and economically valuable species (in least developed and developing countries), and on the other side, as an invasive and dangerous species (in developed countries). This is observed in the Republic of Moldova, where silver carp – *Hypophthalmichthys molitrix* (Valenciennes, 1844), bighead carp – *Hypophthalmichthys nobilis* (Richardson, 1845), grass carp – *Ctenopharyngodon idella* (Valenciennes, 1844) and prussian carp – *Carassius gibelio* (Bloch, 1782) are always desired, and, in the USA, were grass carp, silver carp, bighead carp, and wels catfish – *Silurus glanis* Linnaeus, 1758 are considered dangerous species for the ecological security of the country [2,5,6,7].

Invasive fish species are characterized by their rapid expansion in the primary area, naturalization in the receiving territories and major ecological and socio-economic damage. There are scientific data that demonstrate the impact of the invasive fish species over the native ichtyocenosis. Thus grass carp introduced in the Czech Republic caused major damage to common carp – *Cyprinus carpio* Linnaeus, 1758 by contaminating it with *Bothriocephalus gowkongensis*, round goby – *Neogobius melanostomus* (Pallas, 1814) spread *Bucephalus polymorphus* in the Danube river, prussian carp facilitated the spread of the monogenean species *Gyrodactylus shulmani* and *Gyrodactylus sprostonae*, and stone moroko (topmouth gudgeon) – *Pseudorasbora parva* (Temminck & Schlegel, 1846) caused the decrease of belica – *Leucaspius delineatus* (Heckel, 1843) and other cyprinids by spreading the parasite *Sphaerothecum destruens* [3,4]

Therefore, the aim of this study was to determine the level of infestation of the invasive species with helminths.

Materials and Methods

The ichthyological material (prussian carp, stone moroko, pumpkinseed, chinese sleeper, silver carp, bighead carp) necessary for this study was collected between 2017-2019 from different water bodies, during the period of vegetation favourable for the development of fish and parasite hosts, (Dniester river – lower sector of Dubăsari reservoir, Lopatnic river – left tributtary of the Prut river, Costești-Stânca lake, Bâc river, Rose Valley lake from Chișinău, Muzeul Satului lake from Chișinău, the ponds from Nimoreni and Fălești district).

The parasitological researches were performed in the laboratory of Parasitology and Helminthology of the Institute of Zoology, on live fishes, according to the standard method proposed by Skryabin K.I. (examination of all internal organs of the animal) and the method proposed by Dogel V.A. and modified by Bykhovskaia – Pavlovskaia [9,11]. The microscopy of the detected helminths was performed using the stereomicroscope MBS, as well the examination at the optical microscope Novex Holland B, as fresh preparation slide-coverglass, with the objective 10x and ocular WF10X DIN/20MM. The detected helminths where identified using the keys written by Bauer [8], and stored in 70% ethanol and Barbagallo solution (formalin 3%+saline solution). For the parasitological evaluation, extensivity (%) and intensivity of invasion were used.

Results and discussions

The study of the helminth fauna of the prussian carp from the Dniester river (lower sector of Dubăsari reservoir), Costești-Stânca lake, Bâc river, revealed an infestation with helminths classified in 4 classes (Monogenea, Trematoda, Cestoda, Chromadorea), 11 families (Dactylogyridae Bychowsky, 1933, Gyrodactylidae Cobbold, 1864, Diplozoidae Palombi, 1949, Gorgoderidae Loos, 1899, Diplostomidae Poirier, 1886, Lytocestidae Hunter, 1927, Bothriocephalidae Blanchard, 1849, Gryporhynchidae Spassky & Spasskaya, 1973, Capillariidae Zedder, 1800, Philometridae Baylis et Daubney, 1926, Raphidascarididae Hartwich, 1954) and 12 genera (*Dactylogyrus, Gyrodactylus, Diplozoon, Eudiplozoon, Phyllodistomum, Diplostomum, Khawia, Bothriocephalus, Paradilepis, Pseudocapillaria, Philometroides, Rhaphidascaris*).

The specimens of prussian carp collected from Dniester river (lower sector of Dubăsari reservoir) were infested with monogeneans – *Dactylogyrus* sp. (EI-38.57%, II-1-64 ex.), *Eudiplozoon nipponicum* (EI-4.28%, II-1 ex.), and trematodes – *Phyllodistomum folium* (EI-2.85%, II-1-3 ex.), *Diplostomum spathaceum* (EI-21.4%, II-1-6 ex.). Monoinvasions were present in 47,07% of the examined specimens, and mixed invasions in 52.93%.

The detected monogeneans (*Dactylogyrus* sp. and *Eudiplozoon nipponicum*) are geohelminths which parasitize on the gills. They are hermaphrodites, oviparous, and pass from one fish to another without the need for an intermediate host. The biohelminths were represented by trematodes *Phyllodistomum folium* and *Diplostomum* spathaceum. *Phyllodistomum pholium* parasitizes in the ureters, and the intermediate host is the aquatic mollusk *Dreissena polymorpha*. *Diplostomum spathaceum* is a trematode that parasitizes in the fishes eye's lense, and the intermediate hosts represented by mollusks of the genus *Radix* and *Lymnaea*.

The result of the study of helminth fauna of the prussian carp captured from the Costești-Stânca lake, revealed an infestation with helminths from Monogenea class - *Dactylogyrus* sp. (EI-27.27%, II-1-5 ex.), Cestoda class - *Paradilepis scolecina* (EI-11.36%, II-2-6 ex.), *Khawia parva* (EI -2.27%, II-1 ex.), *Bothriocephalus opsariichthydis* (EI-2.27%, II-1 ex.); Chromadorea class - *Raphidascaris acus* (EI-6.81%, II-1 ex.). Monoinvasions were present in 25.0% of cases, and in 75.0% of cases mixed invasions were detected 75,0%. Mixtinvasions consisted of associations of two species (*Dactylogyrus* sp. + *Paradilepis scolecina*).

Out of the total helminths detected in the prussian carp from Costești-Stânca lake, 80.0% were represented by biohelminths (*Paradilepis scolecina*, *Bothriocephalus opsariichthydis*, *Raphidascaris acus*) and 20.0% only by geohelminths (monogeneans). *Bothriocephalus opsariichthydis* (syn. *B. gowkongensis*) (fig.1) is an alogene helminth wich was introduced with Asian carps (silver carp, bighead carp and grass carp) in 1961 [10].

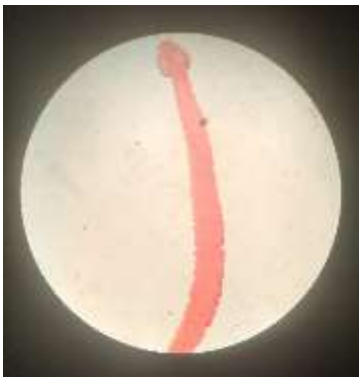

Fig.1 *Bothriocephalus opsariichthydis* found in intestine of a prussian carp (Carmine stain)

The helminth fauna of prussian carp from the Bâc river included parasites from Monogenea class - *Dactylogyrus* sp. (EI-100%, II-29-153 ex.), *Gyrodactylus* sp. (EI-57.89%, II-4-15 ex.), *Diplozoon paradoxum* (EI-36.84%, II of 1-7 ex.), Trematoda class - *Diplostomum spathaceum* (EI-63.15%, II-1-3 ex.), Enoplea class - *Pseudocapillaria tomentosa* (fig.2) (EI-18.94%, II-2-5 ex.) (fig.2).

a b c

Fig.2 *Pseudocapillaria tomentosa*
a) general view b) anterior part of the body c) posterior part of the body

Out of the total helminths detected in the prussian carp from Bâc river, 60% were represented by geohelminths (*Dactylogyrus* sp., *Gyrodactylus* sp., *Diplozoon paradoxum*) and 40% by biohelminths (*Diplostomum spathaceum*, *Pseudocapillaria tomentosa*). Mixed invasions were represented by 2, 3, 4 associations of helminths (*Dactylogyrus* sp. + *Gyrodactylus* sp.;

Dactylogyrus sp. + *Diplozoon paradoxum*; *Dactylogyrus* sp. + *Gyrodactylus* sp. + *Diplozoon paradoxum*, *Dactylogyrus* sp. + *Gyrodactilus* sp. + *Pseudocapillaria tomentosa*).

The research of the helminth fauna of stone moroko from various water bodies from the Republic of Moldova (Costești-Stânca lake, Bâc river, Lopatnic river, lake Muzeul Satului), revealed its infestation with helminths systematically classified in 2 classes (Trematoda, Cestoda), 3 families (Diplostomidae Poirier, 1886, Gryporhynchidae Spassky & Spasskaya, 1973, Capillariidae Zeder, 1800) and 5 genera (*Posthodiplostomum* Dubois, 1936, *Paradilepis* Hsü, 1935, *Valipora* Linton, 1927, *Pseudocapillaria* Freitas, 1959, 19)

Stone moroko from Bâc river was infested with cestodes *Paradilepis scolecina*, *Valipora campylancristrota* and nematodes *Pseudocapillaria tomentosa*. *Paradilepis scolecina* and *Valipora campylancristrota* were found in larval stage. The prevalence and intensity of invasion of stone moroko with these parasites were: *Paradilepis scolecina* (EI-30.0%, II-1-2 ex.); *Valipora campylancristrota* (EI-30.0%, II-1 ex.); *Pseudocapillaria tomentosa* (EI-70.0%, II-1-3 ex.). Parasitic invasions in 90,0% of cases were represented by monoinvasions (in one case *Pseudocapillaria tomentosa* was detected, and in another case *Valipora campylancristrota*), and in 10.0% of cases by mixed invasions (*Paradilepis scolecina* + *Valipora campylancristrota* + *Pseudocapillaria tomentosa*).

The parasitic invasions of stone moroko from the Lopatnic river and the lake Muzeul Satului was represented by monoinvasions. The captured specimens were sporadically infested with *Posthodiplostomum cuticola* and *Hepaticola petruschewski*. In the case of infested stone moroko from the Lopatnic river, the metacercariae of *Posthodiplostomum cuticola* inside the cyst were not viable, possible due to host's immune system that increases with age.

The helminthological study of 133 specimens of chinese sleeper (*Perccottus glenii* Dybowski, 1877) from the Lopatnic river revealed the presence of the nematode *Hepaticola petruschewskii* Schulman, 1948 which was detected inside the liver and intestine. The prevalence was 53.58% and the intensity of invasion 5-20 parasites. This nematode is very dangerous for pond fish (common carp, silver carp, grass carp), and can cause mass infestations with a prevalence above 90%. In hepaticolosis pathologic changes are observed in infested fish – disorders of the structure and consistency of the liver, its mosaicism, presence of punctate hemorrhages, destructive changes in hepatocytes. The importance of detecting the nematode *Hepaticola petruschewskii* lies in the fact that chinese sleeper and other invasive fishes act as a vector in the spread of this parasite, thus being dangerous for economically valuable fishes.

As a result of the study of the helminth fauna of the pumpkinseed (*Lepomis gibbosus*, Linnaeus, 1758) from lake Muzeul Satului, it was established that it was infested with *Diplostomum spathaceum* and *Hepaticola petruschewski*. In the specimens captured from the lake Muzeul Satului, the level of infestation with these helminths was: *Diplostomum spathaceum* located in the lens of the eyeball (EI-2.98%, II-1 ex.); *Hepaticola petruschewski* (EI-58.20%, II-1-7 ex.). Parasitic invasions consisting of a species of helminths were present in 5.13% of cases (infestation infestation with *Hepaticola petruschewski*), and in 94.87% of cases, parasitic invasions were represented by mixed invasions.

The pumpkinseed from Rose Valley lake, unlike the one from the Muzeul Satului lake, was infested only with *Diplostomum spathaceum* (EI-81.25%, II-1-7 specimens).

The study of the helminth fauna of silver carp from the pond from the "Codrii" natural forest reserve highlighted an infestation with trematodes from the genus *Diplostomum* von Nordman, 1832 (fig.3) parasitizing in the lens of the eyeball. The extensivity of invasion with this helminth was 60.0%, and the intensity of the invasion 1-130 specimens. The disease caused by metacercariae of these helminth is called diplostomiasis. Specific for diplostomiasis are the acute

form and the chronic form. The acute form, caused by *cercariae,* is very dangerous for juveniles. Punctate hemorrhages on the skin and mass death of juveniles are specific for the acute form. The chronic form, which is also called *worm cataract,* is caused by *metacercariae,* and due to local inflammatory processes, eye cataract occurs, as a result of which fish feeds poorly, becomes cachectic and dies.

Fig.3 *Diplostomum* von Nordman, 1832 found in the lens of the silver carp

In a pond of Fălești district, a case of massive infestation of the silver carp with plerocercoids of the *Ligula intestinalis* was detected (fig.4). The prevalence was high, above 90%, with intensity of invasion of 3 and more plerocercoids per fish. In ligulosis, fish swim more on the surface of the water, being prone to be eaten by fish-eating birds. The plerocercoid, which parasitizes in the abdominal cavity, by the pressure it generates, causes atrophy of the internal organs, such as gonads, becoming sterile, or penetrates the wall of the abdominal cavity that leads to the death of fish.

Fig.4 Plerocercoids of *Ligula intestinalis* found in the abdominal cavity of a silver carp

The helminth fauna of bighead carp from Nimoreni pond (Pescăruș S.A) was represented by helminths systematically classified in 3 classes (Monogenea, Trematoda, Cestoda), 2 families (Dactylogyridae Bychowsky, 1933, Diplostomidae Poirier, 1886, Gryporhynchidae Spassky & Spasskaya, 1973) *Diplostomum* von Nordman, 1832, *Paradilepis* Hsű, 1935, *Valipora* Linton, 1927).

As a result of the study of the helminth fauna of bighead carp, it was established its infestation with helminths from Monogenea class - *Dactylogyrus* sp. parasitizing on gills (EI-91.67%, II-2-28 ex.), Trematoda class - *Diplostomum spathaceum* in the lens of the eyeball (EI-

44.44%, II-2-38 ex.), Cestoda class - *Paradilepis scolecina* in liver (EI-8.33%, II-1 ex.), and *Valipora campylancristrota* located in the gallbladder (EI-44.44%, II-1-6 specimens). The parasitic invasions represented by monoinvasions were present in 27.27%, of cases and in 72.73% of cases the parasitic invasions were represented by mixed invasions. Mixed invasions consisted of 2 and 3 associations of helminth species (*Dactylogyrus* sp. + *Diplostomum spathaceum*; *Dactylogyrus* sp. + *Valipora campylancristrota*; *Dactylogyrus* sp. + *Diplostomum spathaceum* + *Valipora campylancristrota*; *Dactylogyrus* sp.+ *Valipora campylancristrota*+ *Paradilepis scolecina*).

Conclusions

The study of the helminth fauna of the invasive fishes from various water bodies from the Republic of Moldova, revealed a different level of infestation with helminths. The most infested species were prussian carp (*Dactylogyrus* sp., *Gyrodactylus* sp., *Diplozoon paradoxum*, *Eudiplozoon nipponicum*, *Phyllodistomum folium*, *Diplostomum spathaceum*, *Botriocephalus opsariichthydis*, *Khawia parva*, *Paradilepis scolecina*, *Philometroides sanguinea*, *Pseudocapillaria tomentosa*, *Raphidascaris acus*) followed by stone moroko (*Posthodiplostomum cuticola*, *Paradilepis scolecina*, *Valipora campylancristrota*, *Pseudocapillaria tomentosa*, *Hepaticola petruschewski*). The less infested species were: bighead carp, silver carp, pumpkinseed and chinese sleeper. In future research it will be necessary to expand surveillance on protists and parasitic crustacean fauna.

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Observations regarding the values of immunocompetent cells at lambs according to age

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Abstract
The scientific researches reflected in this study aimed to investigate some immunological aspects regarding the values of the indices of immunocompetent cells at lambs in different age periods. The obtained data indicate that the cellular defense mechanisms are performed by lymphocyte cells endowed with immune defense functions and are responsible by cellular and humoral mechanisms. These determines the importance of cellular mechanisms in triggering and controlling immune reactions. The results of the study reveal the evaluation of cell indices at lambs by finding significant values of both T and B immunocompetent cell activity. These researches allow us to conclude on the fact that the installation of the immunological reactivity of the animal organism takes place and in the same time, the adaptation to the changes of the environmental conditions.
Key words: *Immunity, Lambs, Immunocompetent cells, T lymphocytes, B lymphocytes.*

Introduction

The immune system represents the fight against microbial pathogens. The functions of the immune system are determined primarily by generating a specific immune response against the invasive pathogen and controlling infection; second, by recalling this first "conflict" and triggering an accelerated immune response following re-exposure to the same microbial pathogen. At the same time, the immune system consists of a complex network of cells, tissues and organs that participate together in the defense of the animal organism [1, 10].

Knowing the complex structure and the function of the immune system helps to understand the basis of immune deficiencies and to perceive the potential ways in which the immune system can be modulated in specific diseases. Immunocompetent cells of the immune system are represented by T lymphocytes, B lymphocytes, NK cells, granulocytes, macrophages or monocytes, fibroblasts, epithelial cells, dendritic cells [3,11].

Most of the pathogens agents have a very high proliferative capacity, and the prevention of systemic infections requires adequate and rapid responses from the inborn immune system and the acquired immune system. For these reasons, non-specific or inborn immunity provides the first line of defense against pathogenic microbial agents [4, 6].

It is characterized by nonspecific, rapid and equally intense immune responses, regardless of the type of pathogen, which are rarely enough to completely eliminate cellular microbial infections and by a lack of immunological memory or long-lasting protective immunity. It is ensured through anatomical barriers (skin, membranes, mucous membranes), physiological barriers (temperature, pH), chemical factors (interferons, complement system), cells with phagocytic activity (macrophages / monocytes, dendritic cells, neutrophils, fibroblasts, epithelial cells), cell-mediated cytotoxicity (NK cells) [2].

Bibliographic studies confirm that one billion human lymphocytes are produced daily by humans and animals. Circulating and recirculating through the network of blood and lymph vessels, the cells and molecules of the immune system ensure the surveillance of the body, the recognition of molecules and non-self cells, to eliminate them. Investigations into the activity of immunocompetent cells in correlating immune status have allowed us to determine a number of features in the development of cellular and humoral immunity. The elements of the immune system - macrophages, T and B lymphocytes are developing until the birth of the animal, after which they

begin to function intensively. B lymphocytes are the predecessors of plasma cells, and T lymphocytes favor the possibility of developing the immune response in the first days of life of the newborn animal [5,8].

Currently, the problem of immunodeficiency has become more and more acute and has become widespread in medical clinical practice. This is explained by the result of immunodeficiencies of T and B cells of the immune system. Therefore, the stimulation of T and B lymphocytes that act on the mucous membranes thus increases the specific resistance of the entrance gates and prevents infectious processes from the first days of animal life [7,9].

The study of the cells of the immune system reflects the cooperation between them, in the mutual formation on the necessity of reaction, which control each other in their activity through molecules secreted by lymphocytes or macrophages, with an extremely important role in organizing and controlling immune reactions. From this point of view, the main objective of this research is to study the activity of cellular immunity determined by the indices of immunocompetent cells at lambs at different periods of age.

Material and method

The investigations were performed in a private immunology laboratory in Chisinau. To perform the investigations were used blood samples from lambs from the household of a private sheep farm.

Blood samples were taken from the jugular vein with heparin based on the calculation of 0.3 ml heparin per 10 ml blood for anticoagulation. The samples were used to identify leukocyte, lymphocyte and immunocompetent T and B cells.

Results and discussions

The results obtained regarding the immune investigations on the immune system regarding the values of immunocompetent cells at lambs in dependence of age show that the level of leukocytes and lymphocytes varies at different stages of animal age (Table 1).

Significant results of leukocyte and lymphocyte indices were registered at lambs of 10 and 20 days age, constituting leukocyte values of 7.85 ± 0.81 and 6.82 ± 0.81 compared to the values obtained at 30 days old lambs, where the indices constituted the level of 7.33 ± 0.81. At the same time, the number of lymphocytes at newborn animals was determined and assessed, which denotes appreciable values at the age of 10 days, constituting 3.71 ± 0.81 compared to 20- and 30-days-old lambs constituting 3.82 ± 0.8 and 3.41 ± 0.81.

Table 1

Leukocyte and lymphocyte dynamics at lambs depending on age, %

Age (days)	Leukocytes (thousands/mcl)	Lymphocytes (thousands/mcl)
10	7,85 ± 0,81	3,71 ± 0,81
20	6,82 ± 0,81	3,82 ± 0,81
30	7,33± 0,81	3,41± 0,81

As a result of cellular activation, complex processes are initiated characterized by the initiation and realization of the functions of the cells involved in the immune response. Cells go through the stages of the cell cycle. Activation of T cells is achieved by antigen signals and a costimulatory molecule represented by a cytokine (IL-1). At the same time, activations of B lymphocytes are triggered as a result of the recognition of the antigen by the BCR molecules. Following the activation of B cells, the proliferation and synthesis of antibodies is performed.

Table 2

Dynamics of T and B lymphocytes at lambs depending on age, %

Age (days)	T-active lymphocytes	Total T lymphocytes	B
10	22,0 ± 0,08	13,3 ± 0,08	10,0 ± 0,81
20	25,0 ± 0,08	15,0 ± 0,08	12,0 ± 0,81
30	23,6 ± 0,08	14,1 ± 0,08	13,28 ± 0,81

The research results reveal important values of T and B lymphocytes in various age periods (Table 2). Therefore, the level of T-active lymphocytes indicates values of 22.0 ± 0.08 and 25.0 ± 0.08 at the age of 10 and 20 days, compared to the age of 30 days, where these indices constituted 23.6 ± 0, 08. Simultaneously at the age of 10 and 20 days, the values of total T-lymphocytes also determined important characteristic values constituting 13.3 ± 0.08 and 15.0 ± 0.08 compared to the age of 30 days where the level of these T-total lymphocytes constituted 14.1 ± 0.08.

The comparative aspects of B lymphocytes at lambs determined comparative characteristics regarding their concentration in different age periods. Thus, at the age of 10 days, the level of B lymphocytes was 10.0 ± 0.81, compared to the age of 20 and 30 days, where the values of B lymphocytes were 12.0 ± 0.81 and 13.28 ± 0.81.

Research conducted to determine the immunobiological characteristics of lambs from birth to the age of 30 days has revealed some peculiarities in the emergence and development of cellular and humoral immunity. Thus the main factor of cellular immunity is represented by T and B lymphocytes with the respective subpopulations, which determine the organism's immune reactions. Between these immunocompetent cells there is a certain interaction of the T system, which promotes the immunocompetence of lymphoid cells and regulates the system. The elements of the immune system, macrophages, T and B lymphocytes are developing in the organism until the birth of the animal, after which they begin to function intensively.

At all ages, from the 10th day of life until the 30rd day of life, significant increases in T and B lymphocyte indices were determined, which allows us to conclude that it has place the installation of the immunological reactivity of the newborn organism and the adaptation to the changes of the environment conditions and especially to the action of the pathogenic microorganisms.

The mechanisms of cellular defense against bacteria are performed by effector cells with phagocytic functions (neutrophils, macrophages, etc.), cytotoxic cells, etc.

Through various cell-mediated mechanisms, macrophages undergo an activation process performed by lymphokines secreted by T lymphocytes. Therefore macrophages have an important

role in triggering and controlling cellular reactions, which will activate B lymphocytes for antibody synthesis.

That is why it is important to know the specific prophylaxis methods of some diseases of the youth, regardless of the species, which requires the study of the optimal age, doses, inoculation pathways and other parameters depending on the response capacity of the animal.

Conclusions

1. Immunological investigations have shown that cellular immunity gradually develops at lambs during certain periods of growth and development. Therefore, the T and B values of lymphocytes reveal the well-defined functions of the cellular reactions characteristic to the installation of the organism's resistance.
2. The study and interpretation of the mechanisms of formation of the immune system of the animal organism offers the possibility to analyze the evolution of cellular and humoral reactions, which maintain the organism's immune homeostasis, being considered the main ones in the regulation of the immune system.

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Boar freeze-dried semen in medium with antioxidants evaluated based on DNA integrity after a long-time preservation

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Abstract
Sperm freeze-drying is considered an alternative method to preserve male gametes in refrigeration or at room temperature condition. In order to preserve sperm integrity special protection is required. The aim of our research was to examine the effect of vitamin C (0.5 mM) and rosmarinic acid (105 µM) on the DNA spermatozoa integrity after freeze-drying and 36 months of preservation at refrigerator temperature. Our results indicates that more than 90% of DNA boar spermatozoa integrity is not affected by long-time preservation with small differences between experimental groups: with +0.59% higher DNA integrity in AR group from Duroc boar, with +2.83% higher DNA integrity in AR group from Landrace boar and with no differences regarding DNA integrity in group supplemented with vitamin C. The main conclusion of these preliminary results is that DNA integrity of boar freeze-dried semen is not affected by long-time preservation and it can be used further for intracytoplasmatic sperm injection technique.
Key words*: DNA-integrity, freeze -dry, antioxidants, boar semen*

Introduction

There are several methods by which semen can be preserved. Some of them are used in current practice, others used only in research. Among the cryopreservation methods there are: slow freezing, fast freezing, vitrification (ultra-fast freezing), freezing of a small number of sperm and lyophilization. Lyophilization (freeze-drying) is a technique that does not require liquid nitrogen, the samples can be stored at refrigerator temperature, they can be transported at room temperature. DNA damage is much lower than in conventional freezing techniques, but sperm are immobile, so they can only be used in fertilization by the intracytoplasmic sperm injection (ICSI) technique (Hazavehei et al.,2018).

Semen lyophilization was applied to several species, such as mouse, rat, cat, rabbit, boar, monkey with different results of the percentage of blastocysts obtained and gestation rates (Patrick et al, 2017). In 2019 Wakayama and Yanahimachi were the first researchers to obtain new-borns through ICSI with lyophilized mouse sperm (Wakayama și Yanahimachi, 2019).

Negative effects on DNA during lyophilization can occur due to the activation of endogenous nucleases, but also oxidative stress (Shahabna et al., 2016). Different solutions such as EGTA, EDTA, but also different antioxidants are used to ensure sperm protection (Shahabna et al. 2016; Olaciregui et al., 2017).

Boar semen is more sensitive to cryopreservation than other species, due to the high content of unsaturated phospholipids and the low level of cholesterol in the plasma membrane. Thus, during cooling-thawing procedures, changes in the sperm membrane lead to a destabilization, affecting calcium homeostasis, acrosome integrity, but also a disorganization of the lipid membrane (Yeste et al., 2015).

The imbalance between the presence of ROS and the antioxidant activity of sperm is the main cause of the effects of cryopreservation on sperm. To support this balance, antioxidants are used, such as rosmarinic acid, which added to the diluent improves motility and prevents peroxidation of boar sperm (harvested from the epididymis), there is a significant correlation between it and the concentration of malonaldehyde (MDA - highlights lipid peroxidation)(Malo et al., 2011). Studies on bull semen have shown that rosmarinic acid in a concentration of 10g/L added

to the diluent increases the viability, motility and speed of sperm after thawing (Daghigh et al., 2014).

Vitamin C (ascorbic acid), another antioxidant, plays an important role in the integrity of sperm and their fertility by increasing testosterone levels and preventing agglutination. Physiologically, in the seminal plasma, it is 10 times higher than in the blood serum and contributes up to 65% of the total antioxidant capacity of the seminal plasma. In human medicine it is used to improve the quality of semen, in treatment for 3 months with 500 mg /day vitamin C along with zinc and vitamin E (Cyrus et al., 2015).

The aim of the research is to evaluate the antioxidant effects of vitamin C and rosmarinic acid on the integrity of sperm DNA in diluted boar semen preserved by lyophilization, after a period of 36 months, kept at $+3^0$C.

Materials and methods

The research was carried out on four samples of diluted and refrigerated sperm obtained from four different breeds (Pietrain, Large White, Duroc and Landrace).

Sperm samples originated from Semest-BVN Targu-Mures and were transported within 24 hours under appropriate conditions (15-17^{0}C) at the CLC Assisted Reproduction Laboratory, USAMVB Timisoara. Each semen sample was divided in 3 groups: control group (M group), vitamin C group (C group) and rosmarinic acid group (RA group). In control group was no antioxidant, in group C we added 0.5 mM vitamin C and in group RA we added 105 µM rosmarinic acid. Totally we analyzed 24 samples.

The concentrations was chosen based on literature data regarding their use as antioxidants for animal semen (Olaciregui et al., 2017; Varo et al., 2014; Fanaei et al., 2014)

Lyophilization was performed with Ilshin FD 5512, GF3100, at -52°C and 5 mTorr. After 36 months from lyophilization, each sample of sperm was reconstituted in 10 ml DPBS (D-8662, Sigma Aldrich),

Evaluation of sperm DNA integrity was performed using the Halomax kit. This assessment is based on the differential response of sperm chromatin with or without fragmented DNA to a protein depletion treatment. In the absence of massive DNA breakage, the removal of nuclear proteins produces intensely stained nucleoids with very small haloes of DNA loops emerging from a central and compact core. However, nucleoids from sperm containing fragmented DNA show a big and faintly stained halo of diffusion of DNA fragments emerging from a residual central core.

The DNA fragmentation analysis in all groups was performed following the manufacturer's instructions. In brief, the lysis solution was placed at room temperature (22°C). Then, an Eppendorf tube containing agarose was placed in a water bath at 95°C–100°C for five minutes, and then transferred in a water bath at 37°C for five minutes. Meanwhile, 25 µl of each diluted sperm sample was added to an empty eppendorf tube, and 50 ml of liquefied agarose was then transferred into the tube and gently mixed. The temperature of the tubes was maintained at 37°C. Then, a drop of 2 µl of the cell suspension was placed onto marked wells and each drop was covered with a 24 × 24 mm glass coverslip. The slides were held in a horizontal position throughout the entire process. The slides were placed on a cold surface precooled at 4°C in a fridge to solidify the agarose. After 5 min the slides were taken out of the fridge and the coverslips were gently removed. Then, the slides were fully immersed horizontally in 10 ml of lysis solution for five minutes. Subsequently, the preparation was introduced into a bath of distilled water for 5 min and then dehydrated by immersion in 2 successive baths of ethanol at 70% and 100% for 2 min each. Finally, the slides were allowed to air-dry before staining. All the slides were stained using a commercial kit for red fluorescence staining (Fluored, HT-FR100, Halotech DNA SL, Spain). 2 µl

of red fluorochrome and mountain medium (1:1; vol/vol) was placed into the well of slide for fluorescent staining of sperm chromatin. The samples were evaluated using fluorescent microscopy (Leica DMI 4000) at magnification 20X and a minimum of 200 spermatozoa were counted per semen sample. Five replicated trials were carried out for each group. Sperm showing a small and compacted halo around a compacted nuclear core contained intact DNA and sperm that displayed a large and spotty halo around the nuclear core corresponded to those sperm with fragmented DNA.

Results and discussions

The results presented in figure 1 shows that over 90% of the sperm analyzed have intact DNA after lyophilization even after a period of 36 months, kept at $+3^0$C. There are individual variations, depending on the breed, but these were not significant. The most important observation found after analyzing these samples is that the semen can be stored for a long time at a temperature of 3^0C without affecting the sperm DNA, which can ensure a fertilizing capacity during use in the technique of intracytoplasmic sperm injection (ICSI). The rate of sperm DNA damage was between 10.58% in the Large White sample and 2.29% in the Landrace sample.

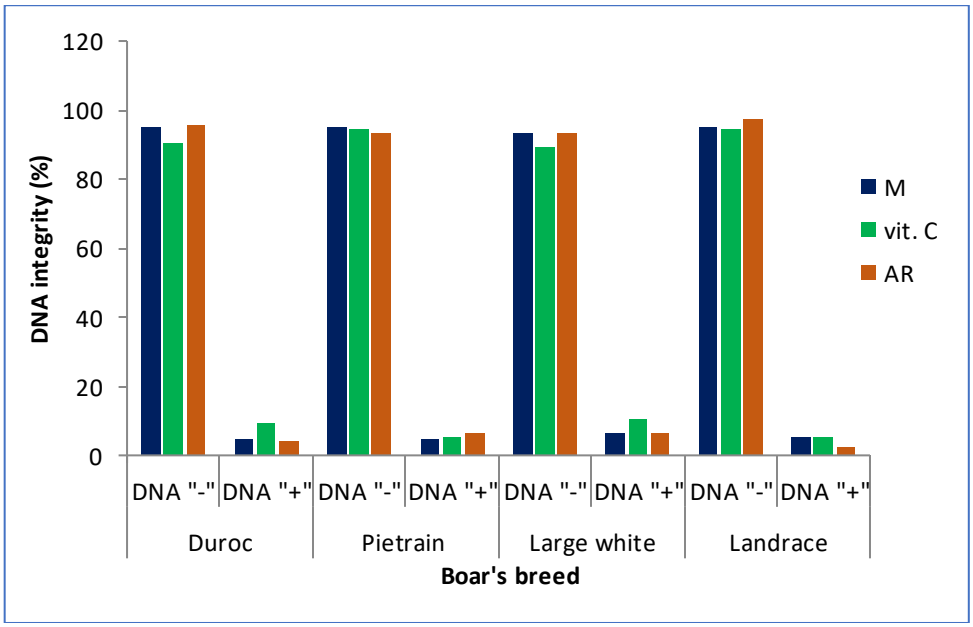

Fig.1. Boar sperm DNA integrity evaluation after 36 months from liophilization (DNA"-" = unafected DNA integrity, DNA "+" = affected DNA integrity)

The protective effect of rosmarinic acid is probably due to the blockade of hydrogen peroxide, which reduces the motility of sperm through the xanthine-xanthine oxidase system. (Malo et al., 2011). Good results were obtained with the help of rosmarinic acid (2.5g/100 ml, 5g/100 ml or 10g/100ml) and on boar spermatozoa harvested from the epididymis. Boar spermatozoa harvested from the epididymis have a higher resistance to cryopreservation probably due to the lack of seminal plasma which is rich in polyunsaturated acids. By determining the concentration of malonaldehyde (MDA) it was observed that there is a significant correlation, so a decrease in MDA was observed when rosmarinic acid was added in higher concentration (Malo et

al., 2011). Similar studies in the literature support the antioxidant effect of rosmarinic acid (105 μM) on the rate of DNA oxidation in boar samples after thawing (Luno et al., 2014).

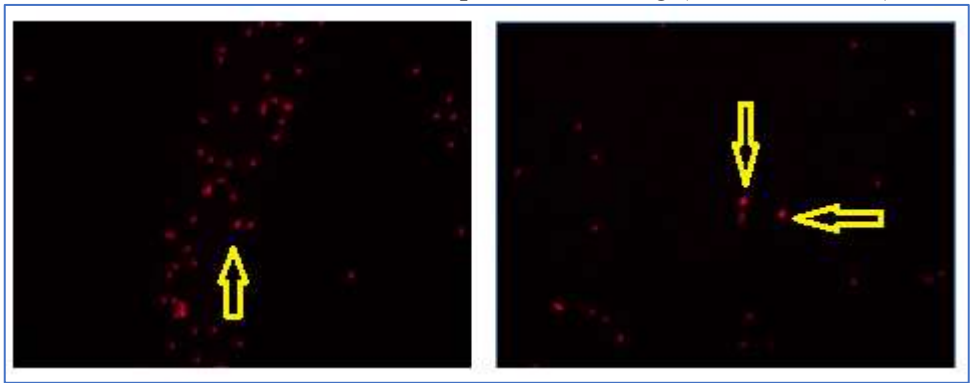

Fig.2. Lyophylized boar spermatozoa stained with Fluored after 36 months of preservation at 3^0C (arrow indicates spermatozoa with DNA integrity affected)(20X)

In 2019, among the few existing studies worldwide on the influence of rosmarinic acid on sow oocytes, Zhang et al. (2019) showed beneficial effects, but in much lower doses (5 μM), effects quantified by tracking developmental competence, bastocyst formation rate, blastocyst hatching rate, blastocyst diameter, total number of blastomeres in the blastocyst, rate of embryos obtained by nuclear somatic cell transfer. Beneficial effects of rosmarinic acid (105 μM) on the quality of sow oocytes after *in vitro* maturation for 44 h, we also observed by quantifying the gene expression of PTX3, p53, BAX, BCL-2, results being published. Similar results were obtained by Luno et al. (2014) with the help of rosmarinic acid in different concentrations (0 μM, 26.25 μM, 52.5 μM and 105 μM) added to boar semen diluted with egg lactose-egg yolk and observed after thawing that the rate of DNA oxidation at 120 and 240 minutes after thawing, respectively, was the lowest in the group supplemented with 105 μM, even if immediately after thawing there were no differences between groups.

Conclusions

1. examining the integrity of sperm DNA is a necessary and more accurate parameter for assessing the quality of semen
2. lyophilized semen can be stored at refrigerator temperature (+3^0C) without significantly affecting DNA integrity
3. there are individual variations between boar breeds regarding sperm DNA integrity
4. lyophilization can be considered a suitable method for preserving boar semen used in intracytoplasmic sperm injection (ICSI) techniques

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In vitro bovine embryos evaluation based on OCT4, SOX2, IGF1R and IGF2R expression level

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Abstract
In vitro production of bovine embryos comprises a lot of factors that can influence the successful of this technique, oxidative stress being one of them. These factors can influence the evolution of important development processes such as the maternal to zygotic transition and the embryonic genome activation. Adding antioxidants to in vitro culture media exerts the key role to reduce the effects of reactive oxidative species produced during assisted reproduction technique, influencing in a positive way also the early embryonic developement. The objective of this study was to determine the effect of antioxidant rosmarinic acid (105 µM), added to in vitro bovine oocytes maturation media, on the quality of embryo produced based on gene expression level of OCT4, SOX2, IGF1R and IGF2R. For this purpose, we used 35 bovine ovaries taken from slougtherhouse from which we obtain 202 cumulus-oocyte-complexes and 127 of them were maturated in vitro based on morphological aspects. The cumulus-oocyte-complexes were divided in two groups: control (M1, M2, M3) and with acid rozmarinic (AR1, AR2 and AR3). The levels of OCT4, SOX2, IGF1R and IGF2R were the highest in group AR1, embryos obtain from oocytes class I supplemented with rozmarinic acid, where OCT4 expression was 4.08, SOX2 was 27.66, IGF1R and IGF2R were 53.44 and 25.10.
***Key words**: cumulus-oocyte-complexes, antioxidants, gene expression, bovine*

Introduction

Curently the *in vitro* fertilization technique has become a routine technique with good results, but nevertheless there are a large number of oocytes matured *in vitro*, about 60-70% which are lost due to the inability to reach the stage of blastocyst after they have been fertilized (Meirelles et al., 2004). There are a lot of factors that interfere with the development of bovine embryos *in vitro* and cause them to stop dividing between cell cycle 4 and 5 (Betts and King, 2001). This is a very important moment because in bovine specie the transition from the maternal genome to the embryonic genome takes place in this perios, in other words the activation of the embryonic genome, and the embryo must rely on its own mRNA transcripts to continue its development. Activation of the embryonic genome is a process that takes place gradually. Embryos must go beyond the stage of transcriptional repression to initiate transcriptional activation of the genome. The impossibility of some embryos to overcome the transcriptional blockade in the fourth cell cycle is also demonstrated by the existence of nuclear fragmentation and blastomeres after this state of arrest (Betts and King, 2001). The incriminated mechanisms in blocking embryonic development are: the inability to overcome chromatin repression and activation of transcripts of important genes in development, the inability to react to the influences of the development environment.

When discuss about the influence of medium on embryonic development we have to discuss also about reactive oxygen species (ROS). Sources of ROS during *in vitro* fertilization procedures could be either endogenously or exogenous environmental factors (Agarwal et al., 2014). Although ROS are produced during cell metabolism, excessive quantities can cause DNA damage, mitochondrial dysfunction and others negative effects. Reducing the ROS activity during ART can be done by using antioxidants and rosmarinic acid is one of them. Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid derived from hydroxycinnamic acid, that belongs to polyphenols group and is found as an active compound in several medicinal plants

(*Rosmarinus officinalis, Salvia officinalis, Mentha arvense, Ocimum basilicum, Thymus vulgaris* etc)(Krajcovicova et al, 2013). In *Rosmarinus officinalis* the antioxidant activity is support by rosmarinic acid, carnasol and diphenol rosemary and other phenolic compounds that have various biological functions such as oxidation inhibitory function, preventing DNA from mutation and oxidation and anti-thrombotic effects (Huang and Zheng, 2006). Antioxidant activity of rosmarinic acid is supporting by the enhancement of superoxide and hydroxyl scavenging (Krajcovicova et al, 2013) and also by its chemical structure where the carboxylic acid group together with the catechol elements in the aromatic ring are responsible for neutralizing free radicals (Borjizadeh et al., 2019). The rosmarinic acid has a preventive effect on Sertoli cells apoptosis caused by electromagnetic fields (Hajhosseini et al, 2013). Other researchers observed that rosmarinic acid used together with ascorbic acid in the vitrification solution, improved significantly the survavial, maturation, fertilization rate and development to 4 cell stage in mice studies (Borjizadeh et al., 2019). Possitive effects on bovine oocytes maturated *in vitro* based on their morphological examination was observed also in our previous experiments (Marc et al., 2017).

There are molecular markers that can predict the developmental competence of oocytes and also the quality in embryos. Most of them are related to the functions of cell cycle regulation (CCNBI), transcription control (OCT4, YEAF1), DNA packaging (H2A, H3A), glucose transport (GLUT1), signaling (BMP15, IGF2, IGF1R, IFNT), oxidative stress (SOD2) etc (Orozco-Lucero and Sirard, 2014). With great importance are SOX2, OCT4, IGF1R and IGF2R. SOX2 is part of the SOX protein family which has 20 genes, genes that contain a DNA-binding HMG domain, nuclear import-export signals and act as transcription factors (Wegner, 2010). In bovine blastocysts SOX2 is localized in the inner cell mass (ICM) and its downregulation negatively impacts preimplantation development (Goissis et al., 2014). OCT4, a transcription factor from POU (Pit-Oct-Unc) class with gene CDX2 are essential for early development in bovine embryos (Sakurai et al., 2016). IVM is very important step for further development of the oocytes and for early bovine embryos development. Buruszewska et al. (2015) based on evaluation of embryos developmental competence-related factors (OCT4, SOX2, IGF2R), apoptosis genes (BAX, BCL2) and other genes involved in ovulatory and oocytes competence (PFKP, GLUT1, AREG, EREG and others) observed that lysophosphatidic acid, a transmembrane phospholipid, supplementation sustains the expression of developmental competence at the blastocyst stage. Other factors that are important for growth potential of the embryos and not only, are the insulin-like growth factor (IGF). The insulin-like growth factor (IGF) system is essential for pre- and postnatal growth and development and consists of two growth factors (IGF1, IGF2), type 1 and 2 receptors (IGF1R, IGF2R), the insulin receptor (IR) with short and long isoforms (IR-A, IR-B), six major IGF binding proteins (IGFBP1–6) and several lower-affinity binding proteins (IGFBP7 to IGFBP10) with increased expression from embryo to fetal stage and decreased expression from fetal to juvenile stage (Ghanipoor-Samami et al., 2018).

The main purpose of this research was to observe if rosmarinic acid added to the maturation environment of cow oocytes can improve *in vitro* fertilization results and for this we quantified gene expression of OCT4, SOX2, IGF1R and IGF2R. These genes are particularly important in the early stages of embryo development because they have roles in differentiating cell lines, maintaining the pluripotency of embryonic germ cells (OCT4, SOX2), regulating cell survival, proliferation and differentiation (IGF1, IGF2).

Materials and methods

Chemicals reagents

Culture media for *in vitro* production of bovine embryos were purchased from Minitube (Germany) and these were: TCM199 maturation medium (19990/0010), TL fertilization medium (19990/0030) and TL sperm capacitation medium (19990/0020). All chemical reagents for *in vitro* culture were purchased from Sigma Aldrich unless otherwise stated. Plastic dishes and four well plates were obtained from Nunc (Thermo Scientific).

Cumulus-oocyte complexes (COCs) collections

Bovine ovaries (n=35) were collected from slaughterhouse and transported within two hours to the laboratory in containers consisting of 0.9% NaCl solution supplied with PenStrep (17-602F, Lonza), at 35^0C. The handling medium for COC was Dulbecco-PBS (100 ml) (D8662) supplemented with 100 µl Pen/Strep; 3.6 mg sodium piruvate, 30 mg BSA (A9647), 100 mg glucose (G7021). Cumulus-oocyte complexes (COCs) were aspirated by puncturing the follicles with 3-8 mm diameter with a 18G needle attached to a 5 ml syringe.

The classification of COC's based on morphological aspects was made with stereomicroscope (Stemi 2000-C, ZEISS) with hot plate (33.4^0C) after the criteria of Hawk and Wall, (1994) as follows : *class I* - CI (COCs with cumulus compact and unexpanded, with full or at least 5 layers of cumulus cells, cytoplasm clearly seen, dense and homogenous), *class II* – CII (COCs with cumulus compact, thick, 2-4 layers of cumulus cells, covering all zona pellucida, cytoplasm dense, with uniform granulation) and *class III* - CIII (oocytes partially denuded of cumulus cells, or with 1-2 complete layers of cumulus cells and/or with irregular shrunken cytoplasm)(Figure 1). COC were washed one time in Dulbecco-PBS and three times in TCM199 maturation medium.

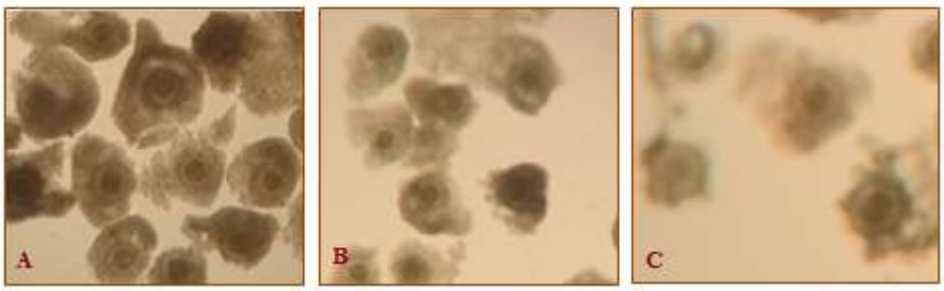

Fig 1. Classes of bovine COCs based on their morphological aspects (2.5X)
(A - class 1, B –class 2, C- class 3)

In vitro embryo production

Brefiely the TCM199 maturation medium (400 µl) was supplemented with 10% fetal bovine serum (FBS, S1400-100, Biowest, France), 0.02UI/ml PMSG (Folligon, Intervet), 0.01 UI/ml hCG (Chorulon, Intervet), covered with mineral oil and allowed to equilibrate for 6h in the incubator at 38.5^0C, 5% CO_2, humidified air atmosphere, then the oocytes (15-20/well) were cultivated for 24h in these 4 well dishes (Nunc, Germany) prepared. After 24h since *in vitro* maturation, all oocytes were examined for maturation and signs like expansion and presence of mucus in cumulus cells were observed, then the COCs were fertilized *in vitro* and cultured. TL fetilization medium (19990/0030) was supplemented with 10µg/ml heparin, 20 µM sodium

piruvate and 0.6% BSA. For *in vitro* fertilization frozen-thawed semen from different bulls was used. After thawing, motile spermatozoa were isolated with Swim-up method using TL sperm capacitation medium (19990/0020) supplemented with 1µM sodium piruvate, 0.6% BSA and 0.6 mg/ml gentamicin and incubated for 1h at 38.5^0C, 5% CO_2, humidified air atmosphere. After incubation, the upper two-thirds of the capacitation medium were recovered, centrifugated at 1000 RPM for 10 min, the supernatant removed. Above the sediment was added 1 ml TL sperm capacitation medium and one more time centrifigated at 1000 RPM for 10 min. The sperm pellet was diluted with 40µl TL fetilization medium. Groups of 10-15 COCs were co-incubated with 10 µl spermatozoa in 60 µl of fertilization medium under mineral oil for 18-22h at 38.5^0C, 5% CO_2, humidified air atmosphere. The day of *in vitro* fertilization was considered the Day 0. After fertilization the embryos were washed two times in culture medium (TCM199 supplemented with 10%FCS) and cultured under mineral oil at 38.5^0C, 5% CO_2, humidified air atmosphere. Every 48 hours the embryos were feeded with 200 µl TCM199, 20% FCS, till the Day 7.

Rosmarinic acid antioxidant for COCs *in vitro* maturation

6 groups of 12-15 COCs each were used to establish all experimental groups: control (C1, C2 and C3) and rosmarinic acid (AR1, AR2, AR3). Using of rosmarinic acid as antioxidant in bovine oocyte maturation medium was based on previously studies on boar spermatozoa (Luno et al., 2014; Luno et al., 2015), on ram spermatozoa (Olaciregui et al., 2017) and on sow oocytes (Zhang et al., 2019). The conentration used of rozmarinic acid was 105 µM.

RNA isolation reverse transcription and real time PCR

Prior to proceeding with RNA isolation, the cell samples were washed with PBS buffer and sediment by centrifugation at 3000 x g for 5 minutes. For each sample approximately $1,5x\ 10^3$ cells were harvested, this corresponding to a 50 mg quantity as required by the protocol. Total ARN was isolated and from sedimented cells using SV Total RNA Isolation System (Promega, US) commercial kit according to manufacturer's protocol.

From isolated RNA, the cDNA was synthesized using High-capacity cDNA Reverse Transcription (Thermo Scientific, Lituania) following the manufacturer indications and oligo dT(8) primer, also provided with the kit. Obtained cDNA was used as template in qPCR reactions using GoTaq qPCR Master Mix Kit (Promega, U.S.A). According to provided protocol with a Stratagene Mx3000P (Agilent) real time PCR equipment. The primers sequences (Table 2) used in this study were obtained from the reference literature and were synthesized by Eurogentec (Belgium).

Table 2.
Sequences of primers used for real-time PCR

Gene	Primer sequence
OCT4 fw	GAGAAAGACGTGGTCCGAGTG
OCT4 rew	GACCCAGCAGCCTCAAAATC
SOX2 fw	TGGATCGGCCAGAAGAGGAG
SOX2 rev	CAGGCGAAGAATAATTTGGGGG
IGF1R fw	GAGTGGAGAAATCTGCGGG
IGF1R rev	AAATGAGCAGGATGTGGAGGT
IGF2R fw	ACCTCCGATCCTCAATCCCA
IGF2R rev	TGTAGTTGAAGTGCCGGTCC
Reference gene β-actin fw	GTCACCAACTGGGACGACA
Reference gene β-actin rew	AGGCGTACAGGTGACAGCA

Each sample was analyzed in duplicate. For normalization of gene expression in terms of number of copies β - Actin gene was used. For each primer a sample without DNA template considered as negative control was run. For the relative quantification the Δ (ΔCt) method was used. For all the samples the number of cycle's threshold (Ct) was determined. For relative quantification the Δ (ΔCt) method was used. According to this method the R (the relative ratio between the control and stressed variant) is calculated with the following formula: $R = 2^{-\Delta\Delta Ct}$. (Livak and Schmittgen, 2001).

Results and discussions

Following the experiment, we observed a high value of OCT4 gene expression in group AR1 compared to all other groups, namely 27.66. High values were also recorded in groups AR2 and AR3 (Figure 2), which highlights the positive effect of rosmarinic acid added in IVM on quality of bovine embryos . Similar possitive effects of acid rosmarinic were observed by researchers on sow oocytes that were maturated in medium supplemented with 5 µM acid rosmarinic based on measurement of intracellular ROS levels in cumulus cells and oocytes and of intracellular free thiols levels, one marker of cytoplasmatic maturation of the oocytes at the end of IVM (Zhang et al., 2019). Our previous good results observed by quantifying other genes, such as PTX3, p53, BAX2, BCL2, genes involved in the regulation of cellular apoptosis in both sow and cow oocytes emphasize the importance of this antioxidant in *in vitro* fertilization technique (data being published).

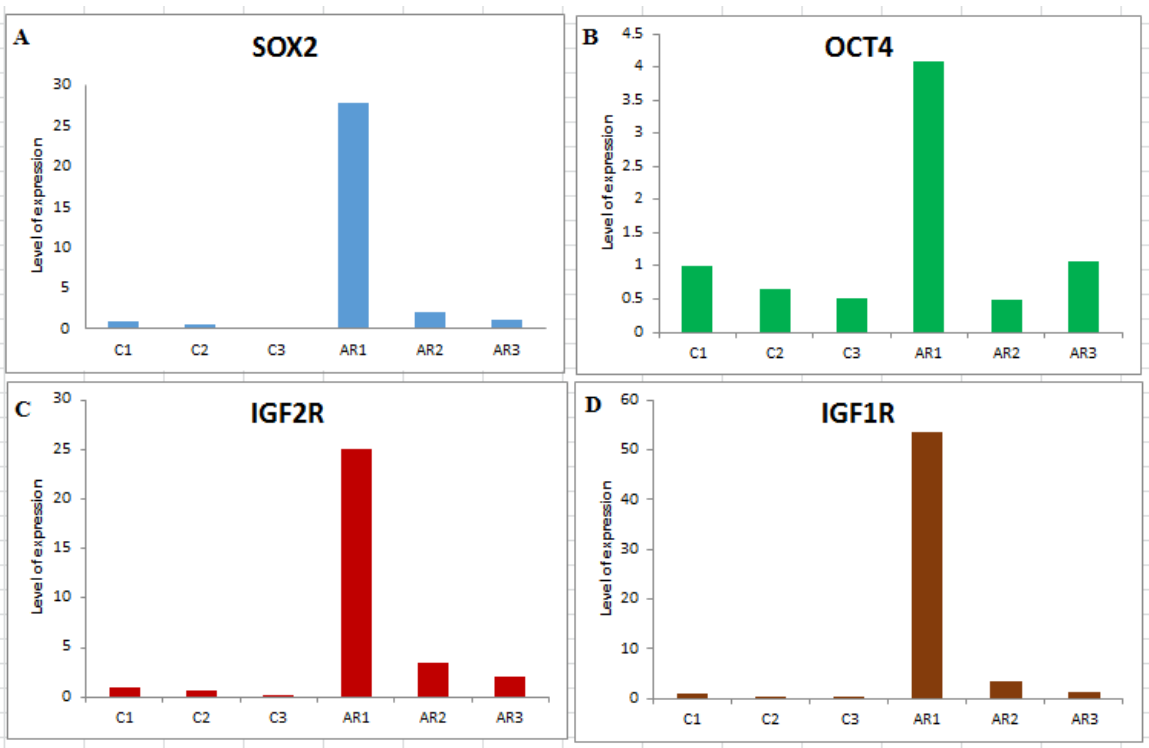

Fig 2. The effect of rosmarinic acid supplementation of maturation medium on mRNA abundance of embryos related factors: A. *SOX2*, B. *OCT4*, C. *IGF1R* and D. *IGF2R*

The information of the OCT4 gene, which is also called POU5F1, ensures the expression of a transcription factor that binds the octamer sequence "ATTTGCAT" from regions which acts as activator or silencer of some genes. The expression of these genes is very important in early embryonic development because it ensures the pluripotency of the cells in the internal mass of the embryo, so it has a role in differentiating cell lines (Kehler et al., 2004). Proper expression of the OCT4 gene is also important for the functioning of other genes such as GATA6 and FGF4 (Frum et al. 2013). Studies on relationships between NANOG, OCT4 and SOX2 gene expression and changes in two histones together with transcriptional activation (H3K4m3 and H4K16ac), but also with transcriptional repression (H3K9m2 and H3K27me3), during reprogramming of donor cells needed to obtain of somatic cell nuclear transfer (SCNT) embryos have shown that the low percentage of embryos obtained by SCNT is due to histone changes, together with the abnormal expression of the OCT4 gene (Hall, 2013)

Like the evolution of the OCT4 gene, the level of SOX2 gene expression is highest in the AR1 group. The SOX2 gene is another transcription factor that contributes to the induction of cellular pluripotency, being closely related to the OCT4 gene, ensuring the latter adequate functionality (Sakurai, 2016). The proper functioning of the two genes, SOX2 and OCT4, also has an influence on the promotion of transcription of fibroblast growth factor 4 (FGF4), which is expressed in the embryonic cell mass of the blastocyst (Hall, 2013). In group AR1 the value of this transcription factor was 27.66, and in groups AR2 and AR3 it was 2.01 and 1.13, respectively, values compared to group C, where the reference value is 1. Regarding C2 and C3 groups, the values expressed for SOX2 were below 1 (Figure 2). The evolution of IGF1R and IGF2R gene expression is similar to SOX2 and OCT4 genes. The IGF1 and IGF2 genes are part of the insulin-like growth factor (IGF) family, being essential for fetal growth and development, but also for placental development, being very strong mitogens that act as regulators of cell survival, proliferation and differentiation (Farmer, W.T.Q, 2015). The good results from AR1 group based on these four genes expression, genes important in differentiating cell lines, maintaining the pluripotency of embryonic germ cells (OCT4, SOX2), regulating cell survival, proliferation and differentiation (IGF1, IGF2) sustained the positive effect of rosmarinic acid added in IVM on bovine embryos development competence.

Conclusions

1. the morphological quality of the oocyte has a very important role in ensuring *in vitro* fertilization
2. the use of rosmarinic acid in the *in vitro* maturation environment of oocytes together with the quality of oocytes (group AR1), determined the best results regarding the expression of OCT4, SOX2, IGF1R, IGF2R genes
3. the intensified expression of the OCT4 gene in group AR1 compared to all other groups and the highlighting of increased values in the other groups as well - AR2 and AR3, highlights the positive effect of rosmarinic acid on oocytes
4. the evolution of IGFR1, IGFR2 and SOX2 gene expression, similar to that of OCT4, highlights the positive effect of rosmarinic acid on oocytes

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Traceability - an important step in food safety

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Abstract
Traceability is a topical issue used in the food processing industry, ensuring the economic operator the safety of purchasing raw materials from the external and internal market for their transformation into safe food products that reach the consumer's table. Traceability also emphasizes customer brand protection requirements. Traceability and withdrawal procedures are currently the most important in every segment of the food industry, as it must be ensured that their traceability efforts are at the highest standard to protect the brand image on the market. These growing requirements are pushing food processors to maintain traceability upstream and downstream in the supply chain. Given the expansion of the global market and the speed with which goods move around the world, it is understandable that the authorities are increasingly concerned with developing rules on traceability. There are priority approaches in the field of food, which have the greatest impact on consumers, given the frequency of consumption and the imperative nature of the need for food. , respectively: European Food Safety Authority - EFSA (Europe) and the National Sanitary Veterinary and Food Safety Authority - ANSVSA (Romania) [12,13].
Key words: traceability,food safty, consumer safety

Introduction

The European food safety system is everyone's responsibility, from the economic operator to the final producer and consumer, so the quality and safety of food must be based on the efforts of all people involved in this complex process that begins and ends. By the time they reach the table, to the consumer [6,7]. . In the food industry, throughout the technological processes, various procedures and control mechanisms are implemented, which ensure the consumer that the food that reaches his table, is edible and that the risk of contamination is reduced to a minimum [1]. However, we cannot talk about a zero risk in food and that is why European legislation and the best process management systems cannot fully protect us. An important step in the food safety system, through which end consumers can be protected from the harmful effects that may occur from the existence of contaminated food on the market, is traceability.

Research on traceability has specifically focused on tracking the animal from calving, including offspring, to finished food, to check the risk of animal diseases, to track food shipments, to reduce the risk of handling and for consumer information systems on the attributes of food. such as animal welfare, country of origin and genetic composition [8]. Given that the food market has expanded worldwide and the speed with which goods move from one part of the world to another, it has increased the attention of the authorities to develop rules relating to traceability. is a hazard management device that allows experts to withdraw or review items that have been identified as hazardous.

Thus, there are priority approaches in the field of food, which have the greatest impact on consumers, given the frequency of consumption and the imperative nature of the need for food. Past food crises have revealed that documents kept on file are not always sufficient to allow full traceability of suspicious foods. During the implementation of Regulation (EC) no. 178/2002, Regulation (EC) no. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin and Regulation (EC) No 853/2004 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on

products of animal origin intended for human consumption, experience has shown that food business operators generally do not have the necessary information to ensure that their systems for identifying food handling or storage are adequate, in particular in the food sector of animal origin. This situation has led to significant economic losses in this sector which could have been avoided and which were due to the lack of complete and rapid traceability of food [9,10,11].

Therefore, certain rules have been laid down for the specific sector of food of animal origin in order to ensure the correct application of the requirements laid down in Article 18 of Regulation (EC) No 1234/2007. These rules should allow (to some extent) flexibility in the format in which the relevant information should be made available. Each country has a set of specific rules, very dynamic in evolution, which emanate from specialized bodies. In the case of European countries, care is taken to align these rules with the relevant European regulatory framework. At European level, there are two reference rules for traceability:

 Directive 2001/95 / EC of the European Parliament and of the Council of 3 December 2001 on general product safety) and

 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, applied from 15 January 2004 and 1 January 2005, respectively, in all Member States of the European Union. The provisions of the Community Regulation no. 178/2002 are taken over in Romania, entirely by Law no. 150 / 14.05.2004.

In addition to the legislative forms perceived at European level, traceability is also managed by the quality standards, 22005: 2007, ISO 22000: 2005 and ISO / TS 22004: 2006 on traceability in the food chain. Complementarily, at regional or branch level there are other initiatives, such as: International Food Standard (IFS), founded in January 2003 by several important names in German retail to unify the procedures in the field and to provide transparency to the entire food chain. supply; subsequently, companies/associations from other European countries joined. The British Retail Consortium (BRC) is a global food standard that includes information on guidelines for meeting HACCP standards, quality and document management, and control and transparency in the food industry. FPA-SAFE-Standard (Food Products Association / Supplier Audits for Food Excellence), is a voluntary, industry-independent supplier audit program to create transparency and efficiency in production.

Traceability has developed as a concept, within the quality system. Although it is a notion that appeared before the 1990s, the importance given to traceability has increased considerably, especially in the food field, in the last decade. Traceability was defined in 1987 according to the quality standard ISO 8402 and ISO 9000/2000 as "the ability to find the history, use or location of an entity using registered identifications." The aim of developing traceability was to increase safety and security in the food chain and to establish a model for traceability, acceptable for the supply of raw materials, food manufacturing, trade and consumption. In this sense it is necessary to define two essential notions: traceability and traceability system (*Fig.1*)

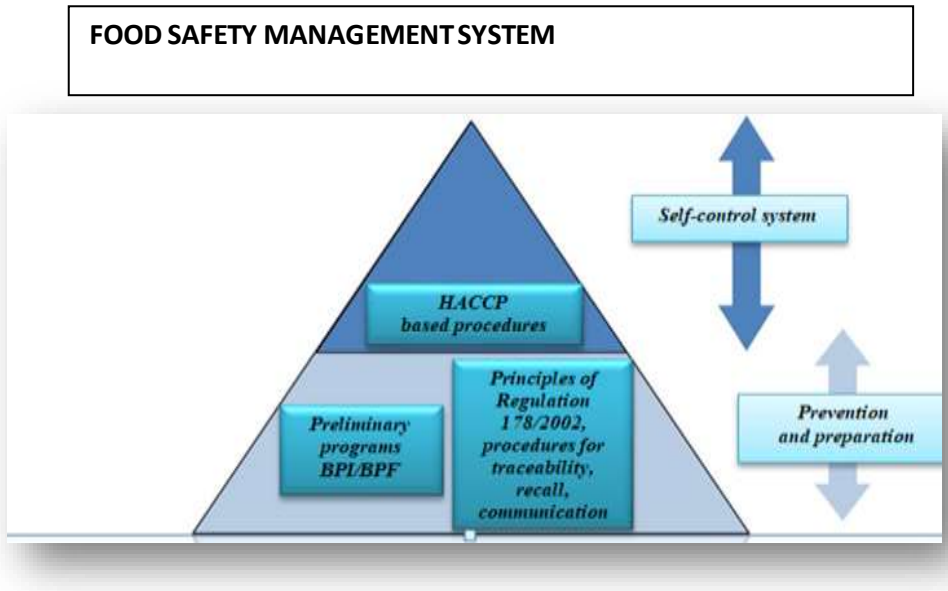

Fig 1 - Elements of a food safety management system
(taken from the Commission Communication on the implementation of food safety management systems comprising Preliminary Programs (PRPs) and procedures based on HACCP principles, including ease/flexibility of implementation in certain food companies C / 2016/4608)

Traceability is defined as the ability to identify and track, throughout all stages of the production, processing and distribution of food, feed, animal intended for food production, or a substance that follows, or which may be incorporated into a food or feed - EC Regulation 178/2002. The traceability system represents the totality of data and operations capable of maintaining the desired information about a product and its components during a part, or of the entire production and use chain (according to SR.EN.ISO22005).

Traceability, as formulated and used in the practice of food production, is a key element of transparency and allows tracking a product on its path from raw material to exposure for marketing, including the consumer and therefore, the flow of a food product by identifying and tracking the accent with documentation. Article 18 of Regulation 178/2002 of the European Parliament states the traceability requirements: The traceability of a food, feed, food-producing animal and any other substance which is intended or expected to be incorporated into food or feed must be determined at all stages of production and processing.

Food and feed operators must identify any person from whom the supply of food [2]. feed, food-producing animals and any substance to be incorporated into food or feed has been made, and must also have systems/procedures in place that will allow that the information be made available to the authorities requesting it. Food and feed business operators must have systems/procedures in place to identify other operators to whom these products have been delivered. The information will be provided to the competent authorities at their request.

Foods/feeds that are placed on the market or that will be placed on the market within the European Union will be appropriately labelled/identified to facilitate their traceability. According to Regulation 178/2002, the main responsibility for ensuring the compliance of food/feed at all levels (production, processing and distribution), belongs to the economic operator.

Product traceability is an effective means of identifying health problems, isolating contaminated products, reducing the risks of intentional contamination and food fraud.

Traceability can be applied:

1.For foodstuffs, where the traceability system links the raw materials, their origin, processing, distribution and location after marketing.

2. For data, where the traceability system refers to calculations and data along the quality path and through which a link is made with the initial quality requirements

3. In calibration, where the traceability system refers to the equipment for measuring physical quantities or properties or with reference to materials included in national and international standards.

4. In IT and programming, where the traceability system refers to the design and implementation of processes in accordance with the system requirements According to CAC 60-2006 (CAC = Codex Alimentarius Commission), respectively in the ability to track the movement of a food product in different specific stages of production, processing and distribution.

The composition of the traceability system in the food field involves the joining of a series of entities that are alternated by stages that are identifiable in the realization and circulation of a good. In REGULATION (EU) NO. 931/2011 OF THE COMMISSION traceability requirements refer to:

- Food business operators who must ensure that information on consignments of food of animal origin is made available to the economic operators to whom the food is supplied and, on request, to the competent authority;
- An accurate description of the food;
- The volume or quantity of food;
- The name and address of the food business operator from whom the food was dispatched;
- The name and address of the consignor (owner), if he is other than the food business operator from whom the food was dispatched;
- The name and address of the food business operator to whom the food was sent;
- The name and address of the consignee (owner), if this is other than the food business operator to whom the food was sent;
- A batch or transport identification reference, as appropriate;
- The date of dispatch of the food.

The information referred to in this Regulation shall be made available in addition to other information required under the relevant provisions of Union legislation on the traceability of food of animal origin, and shall be updated daily and kept at least up to date. in which it can reasonably be assumed that the food in question has been consumed. At the request of the competent authority, the food business operator shall provide the requested information without undue delay.

The appropriate format in which the information is to be made remains at the choice of the food supplier, it must be clear and obtainable by the economic operator to whom the food is supplied.

Ensuring traceability within a distribution chain (composed of entities and stages) means associating an organized (systematic, rigorous) flow of information for each link in the chain; the link consists of the two entities placed at the ends of a stage (route sequence) and the space between them (distance in time and space from one entity to another). Each entity in the distribution chain belongs both to the upstream stage (where the raw materials/products/inputs come from, etc.) and to the downstream stage (where the products/goods/process results go). This means that at any of

the entities in the route of a distribution chain will be recorded data from the previous stage ("input data"), from the stage carried out at that entity ("own data") and data related to the subsequent stage ("data"),output.

On the traceability side in the food industry, great emphasis is placed on risk analysis, more precisely on biological, physical, and mechanical hazards, which may occur in the composition of a food product in the technological flow, in certain stages of its processing.

In 2016 in Germany about 50% of food products withdrew from the market, this situation being similar internationally. The product withdrawn from the market was minced meat in which pieces of plastic were identified.If there is a threat to recall the products in large companies, they must know exactly where and to whom they delivered the products [14].

That is why the EU is involved in "solidifying" the legislative part on the traceability side, especially in the food field, where the main pawn that can be directly affected is the consumer. For this purpose, only 5 important steps can be followed regarding the transparency of the food market supply (warehouses, supermarkets, processing units, etc.).

The 5 steps are represented by:

1. Defining food target groups

In some companies in the food industry, a good concept of traceability with clearly defined objectives can be a success. First, it will be followed and determined what is the real situation within the respective company/unit, the points will be identified where there could be a risk of contamination of the products and the measures that must be taken to reduce it. Thus, this aspect will be taken into account when making this traceability system for each food product. There is a need for more security in the food sector, in order to recall from the market the products that constitute a real danger for the consumer. In creating a team on traceability, the emphasis is primarily on IT staff, on the staff in the department of production and quality assurance, as well as members of management.

2. Defining the dimensions of the lot/lots

The quality of traceability depends on the definition of the lot and the size of the lot. Smaller and more homogeneous batches allow a more precise traceability process. However, in units that work with smaller and more homogeneous batches, the traceability system can be better kept under control, the work processes associated with data entry lead to increased costs. Therefore, when defining or restricting batches, experts recommend a compromise between individual risk management on the one hand and profitability on the other. A frequently sensitive and internationally proven practice is the formation of day batches or smaller batches. The more advanced general recommendations are less sensitive because the differences in the organization of the structure and organization chart in companies are too large. An example: milk from large-scale agricultural operations, which is marketed and distributed through a single dairy farm, can be more easily tracked, despite the large batch size than organic cheese produced in small batches, which is marketed through organic shops specialized.

3. Selecting the type of traceability identification of a product

The precondition for complete traceability is the identification and clear identification of the products concerned - ideally automated. This is only possible with the right tools, such as identification numbers, barcodes, or RFID according to GS1 standards. Common standards in this context are GS1 128, transport unit number (NVE or SSCC), and EPCIS. The receipt of the goods is decisive for all subsequent identification processes. Ideally, the raw materials received are already identified by the supplier. Otherwise, the goods should, for example, receive a GS1 128 upon delivery. It lays the groundwork for the transfer of information from stock, production, packaging, and prices borne by IT to the collection. In principle, traceability documentation is also

possible on paper. However, with the increase in production volume, the number of departments involved, and the people involved in the documentation process, and a large number of batches of raw materials in the product, the documentation requirements are also increasing.

4. Recording the correct data in the right place

The organization of traceability becomes more complicated whenever different batches of raw materials are mixed in food production. Here new batches are formed which must be administered and passed to the next steps in production or packaging. For this reason, it is recommended to install on the technological flow, devices for capturing IT data, which can be processed online later. Regardless of the type of device used (mobile terminals, computers, barcode readers), it is important that the data be passed directly into the process. Only in this way can you perfectly ascertain which batch and which ingredient were introduced into the finished food product. This includes documentation of the processing quantities re-entering the production process. By capturing and examining data at all levels of production, problems can be detected quickly and even avoided before a process failure occurs.

5. Using data and creating added value

Each traceability system is as good as the quality of its data. Finally, it is about the possibility to analyze and visualize the data with the software established by the company/food processing unit, and only then can the recall processes be organized and automated effectively - which to a certain extent it is already required by EU legislation, directives, and audits. In addition, the obligation to provide supporting documents at the push of a button may be fulfilled, indicating that the attributes of the product printed on the labels are correct.

The significance of traceability systems will certainly continue to increase in the future. In EU countries, many companies are already transferring their data to consumer information systems. These databases will most likely play an international role. In this case, traceability will not only provide relevant added value for sales but will also become a basic requirement to participate in business relationships in the first place. Finally, traceability systems also offer a huge opportunity to optimize and benefit from processes as well as from an economic point of view. Purchasing optimization, up-to-date stock information, secure planning bases, meaningful evaluations and statistics, accurate batch calculations - these are all things that companies in the food industry will ultimately benefit from.

The system is called, in practice, "a step forward, a step back".At a certain degree of development of the activity, the flow of information on the movement of goods is subject to a separate management, the manager being even independent of the entities that are involved in the distribution chain.

Under these conditions, in practice, but also in the specific literature, several types and categories of traceability have been established:

- Traceability (upstream): searching for information prior to a reference moment (in the opposite direction to the route and time flow); commonly referred to as "backward traceability";
- Traceability (downstream): searching for information subsequent to a reference moment (in the direction of the route and the flow of time); often referred to as "forward traceability";* internal traceability; searching for information at the level of the reference entity, where one or more operations on the goods took place (processing, preservation, etc.);
- Product traceability: following the aspects related to the product (transformations, characteristics, quality) and at the time and place of a non-conformity - suppliers, transport, own space; it is also called qualitative traceability;

- Logistical traceability: tracking aspects of an accounting nature - quantitative compliance, specifying suppliers and beneficiaries;
- Data traceability: tracking data quality (consistency, accuracy, clarity, integrity) and compliance of data recording;
- Close traceability: specific in the case of continuous flows of goods, for which it is significant to follow the movement of goods on short sequences of time and space and which ensures the knowledge of the links between the lots that make up the supply flow;
- Track and trace (or tracking and tracing): process of determining the current and past places (and other information) of a good in a distribution chain / logistics process.

In developing a food chain traceability system, it is necessary to identify the specific objectives to be achieved, such as:

a) To support food safety and/or quality objectives;
b) Meet the specification of the beneficiary;
c) To establish the history or origin of the product;
d) To facilitate the withdrawal and / or recall of products;
e) To identify the responsible organizations in the food chain;
f) To facilitate the verification of the specific information regarding the product;
g)To communicate information to relevant stakeholders and consumers;
h)To comply with any local, regional, national or international regulations or policies, as the case may be;
i) To improve the efficiency, productivity and profitability of the organization.

If an organization participates in a traceability system with other organizations, the elements must be coordinated. Links must be established in the food chain because each organization identifies its immediately previous source and its next recipient.

Applying the principles of traceability to the food supply chain, allows specialists to monitor food and know the transformations undergone "from the farm to the fork"In the context in which in recent years we have witnessed several incidents regarding the origin of some food products, both at national and European level, traceability, based on Romanian standards that adopt European and international standards, is a solution that guarantees food safety and implicitly consumer safety.

Conclusions

1. Traceability along the food chain is an important tool for ensuring food safety. European Union regulations require every actor involved in the food chain to be able to identify both the origin of the raw material used and the destination of its final products along the food chain.
2. The actor involved must be able to demonstrate what raw material he used and in the manufacture of which the final products were included.
3. The European Union does not establish a specific system or program for traceability. Food business operators have the responsibility to develop and implement evidence and traceability systems to meet the standards set in the European Community.
4. Producers, processors and distributors, as well as food safety experts, should be kept informed of future developments in this field, in order to help them implement traceability systems appropriate to their areas of activity.

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Investigations regarding on the clinical expression of *Bluetongue* virus infection of domestic ruminants in the South-East region of Romania

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Abstract

Bluethonge has a significant economic impact, mainly due to the effect of the disease on animals (morbidity, mortality, reproductive insufficiency, reducced of milk production and animal weakening) and, in particular, disruption of international trade in animals and animal products. In the South-East region of Romania, in the period 2014-2017, animals with clinical signs specific to Bluetongue virus were identified.1437 domestic ruminants with a suspected disease were examined. Of these, 418 (29,08%) had clinical features similar to those of Bluetongue. The incidence of clinical signs in animals with suspected disease varied by species. Thus, the clinical aspects were present in 22,21% cattle, 68,21% sheep and 1,54% goats. Following clinical examination, the simultaneous presence of several clinical signs specific for Bluetongue virus infection was observed in cattle and sheep. In cattle, the incidence of clinical signs was variable, most frequently reported conjunctival mucosal hyperemia, epiphora, sialorrhea, gingival ulceration, swelling and cyanosis of the tongue, nasal edema with ulcerations of the nasal mucosa and muco-purulent jet, circumcised necrosis at the level of the udder, accentuated weakening, congestion of the coronary area, torticollis, digestive disorders, exongulations and inability to move. The pathogenesis of Bluetongue is similar in cattle and sheep, so that the clinical evolution was similar in the two species. In goats, the disease evolved inapparently or with diminished clinical expressions: prostate condition, mucopurulent discharge, edema and nasal ulcers, cyanosis of the mucous membranes, curls and limping. In addition to the immediate losses, the onset of the disease can generate long-term effects, which can decisively affect the Romanian animal breeding sector. The ease with which the Bluetongue disease has spread, shows that the territory of Romania is affected by climate change caused by global warming, which allowed the transmitting vectors to proliferate and spread the Bluetongue virus to the receptive animals.

Key words: *Bluetongue, clinical aspects, domestic ruminants*

Introduction

In recent decades, Europe has become an increasingly exposed space for *Bluetongue*. The causes underlying the spread of the disease are diverse and as a result, the distribution has been almost uniform across the continent, including northern Europe (Wilson et al., 2009).

In regions where the disease is endemic, "sentinel" monitoring programs involve actively taking blood samples from animals in sentinel groups to monitor the presence and circulation of the virus (EFSA, 2017).

The etiological agent is a double-stranded RNA virus of the genus *Orbivirus* with 27 serotypes that causes different clinical manifestations depending on the species (Mellor et al. 1995). In most animals, *Bluetongue* virus (BTV) infection develops unnoticed, but sometimes causes abortion and mortality in sheep, deer and other wild ruminants.

The pathogenesis of this disease is not fully known but it is known that the etiological agent is transmitted for sure by females of hematophagous insects that become infected with *Bluetongue* virus by consuming the blood of infected animals. In the body of culicoids, the virus is located in the salivary glands, so, during feeding, culicoid females transfer the virus to the body of host animals. Once in the bloodstream, viremia occurs, and at this stage, the viral genome can be identified in the blood. Cattle have a prolonged period of viremia and most viral serotypes do

not produce clinical manifestations, so cattle have a particularly important role in the epidemiology of the disease.

Bluetongue virus has tropism for epithelium, located in the Malpighian and scaly layer of the tongue, lips, esophagus, rumen and skin. An inflammatory degeneration phenomenon occurs, sometimes necrotic, and following the detachment of tissue platelets, erosions and ulcers form. The virus also crosses the placenta and affects the fetus by acting on the process of organogenesis and especially on the formation of the brain and neuronal precursor cells, causing hydrocephalus and cerebral hypoplasia (Perianu, 2012).

In cattle, the disease frequently develops without obvious clinical aspects, except for serotype 8 (BTV8) which causes fever, catarrhal or ulcer-necrotic inflammation of the nasal, oral and coronary mucosa (Moga Mânzat, 2005; Perianu, 2012) . This serotype was first reported in Europe and which, in 2006-2009, caused the largest outbreak of *Bluetongue* in France (Courtejoie et al. 2019). There was an increase of abortions number in cattle in this outbreak, and in Belgium and the Netherlands, calves as well as aborted lambs had hydrocephalus and cerebral hypoplasia (Desmecht et al., 2008; Wounda et al. 2008; Van der Slujs et al. 2016) which led to the hypothesis that the circulating BTV8 serotype has the ability to cross the placenta (Courtejoie et al. 2019).

The main objective of this study was to identify the clinical signs and symptoms specific to the infection with *Bluetongue* virus serotype 4, which evolved in domestic ruminants in the South-East region of Romania.

Materials and methods

During 2014-2017, 1437 domestic ruminants from some counties located in the South-East region of Romania were examined. The animals showed specific clinical signs of *Bluetongue* virus infection. (Figure 1.)

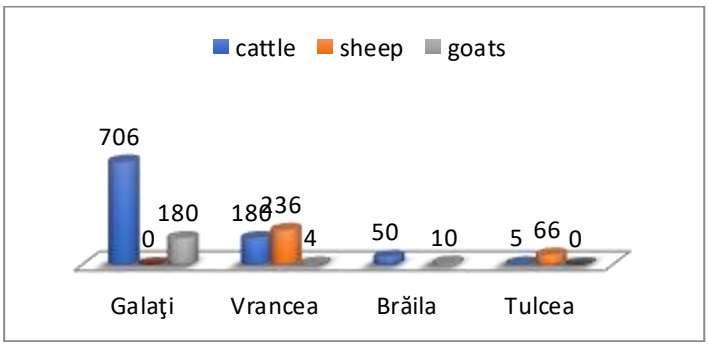

Fig 1. The numerical distribution of ruminant with suspicion of *Bluetongue*

During the study, the numerical distribution of cases was different, taking into account the zootechnical specifics of each county. Thus, in Galati County, 886 suspected animals were reported, 706 cattle and 180 goats. In Vrancea County, from 420 suspected animals 180 were cattle, 236 sheeps and 4 goats. In Brăila County, there were 60 suspicions of disease, of which 50 cattle and 10 goats; and Tulcea County there were 71 suspicions of disease, of which 5 were cattle and 66 sheeps.

For each reported case, epidemiological investigations were performed and all the defining elements of the classical epidemiological reasoning were used: surveillance, investigation and epidemiological evaluation. Monitoring the health status of receptive ruminants is a tool of the passive surveillance of *Bluetongue* disease (Ord.ANSVSA 35/2016)

The clinical examination was performed only on animals with suspected disease, and the clinical signs and lesions observed were mentioned in the animal observation sheets.

Results and discussions

Suspicion of *Bluetongue* depends on the typical clinical signs, the prevalence of the necessary vector insects and, in particular, the areas where the disease is endemic. Diagnosis in *Bluetongue* encounters special difficulties in areas where the disease has not evolved and is relatively easy to specify in endemic regions (Perianu, 2012).

During 2014-2017, 1437 suspected domestic ruminants were reported, and of these, 418 (29.08%) presented clinical aspects specific to *Bluetongue* virus infection.

The incidence of clinical signs varied from one species to another, with specific clinical manifestations being present in 22.21% cattle, 68.21% sheep and 1.54% goats (table 1).

Table 1

Incidența rumegătoarelor domestice care au manifestat semne clinice specifice infecţiei cu virusul *Bluetongue*

Specie	Examined domestic ruminants	
	Clinical signs/tested	%
Cattle	209/941	22,21
Ovines	206/302	68,21
Goats	3/194	1,54
TOTAL	148/1437	29,08%

In *Bluetongue* epidemiology, cattle have a particularly important role, due to prolonged viremia in the absence of clinical signs of the disease (Perianu, 2012). However, there is no explanation for the very high morbidity rate in cattle in Romania.

Clinical examination in cattle revealed the simultaneous presence of several clinical signs specific to *Bluetongue* virus infection (table 2.).

Table 2

The incidence of clinical signs specific to *Bluetongue* virus infection in cattle

Clinical signs specific for *Bluetongue* virus infection	Cattle		
	TOTAL	No	%
Hyperemia of the conjunctival mucosa	209	209	100
Epiphora		206	98,56
Sialorrhea		206	98,56
Nasal edema		200	95,70
Mucopurulent discharge		186	88,99
Ulcerations of the nasal mucosa		189	90,43
Lip edema		50	71,77
Gingival ulcerations		204	97,60
Swelling and cyanosis of the tongue		200	95,70
Ulcers and necrosis of the udder		168	80,38
Congestion of the coronary area		153	73,20
Exongulation		92	44,01

Diarrhea	101	48,32
Increased weight loss	159	76,07
Immobility	96	45,93
Nervous phenomena	121	57,90
Abortions	11	5,26
Ulcers in the region of the posterior train	29	13,88

The incidence of clinical signs in cattle varied according to the clinical course of the disease, so that 100% (n=209) cattle had hyperemia of the conjunctival mucosa, 98.56% (n=206) epiphora (fig. 2), 98.56% (n=206) sialorrhea and 95.7% (n=200) nasal edema (fig.3). 90.43% (n=189) animals presented ulcerations of the nasal mucosa (fig.4) and 97.6% (n=204) gingival ulcerations (fig.5), 95.7% (n=200) showed swelling and cyanosis of the tongue (fig.6), 80.38% (n=138) circumscribed necrosis, ulcerations and crusts on the udder (fig.7).

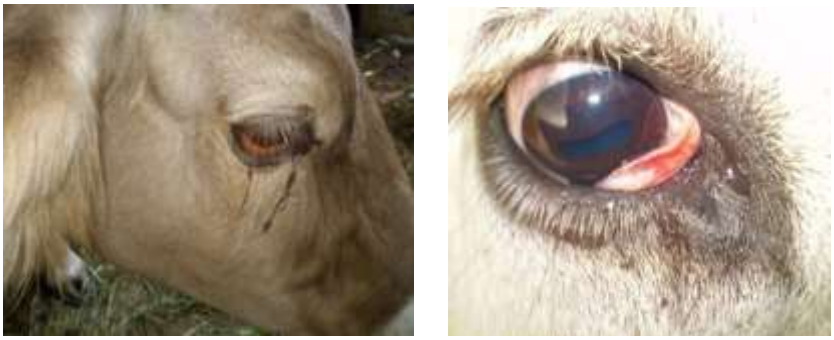

Fig.2.Hyperemia of the conjunctival mucosa and epiphora

Fig.3 Sialoree and nasal edema in cattle

Fig. 4. Edema and nasal ulcers

Fig.5 Gingival ulcers in cattle

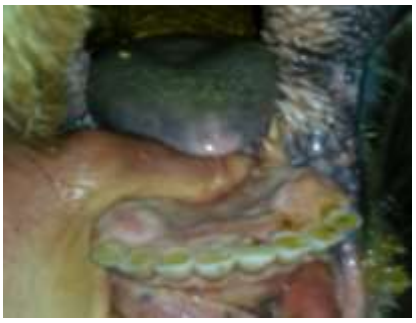

Fig. 6 Swollen and cyanosis of the tongue

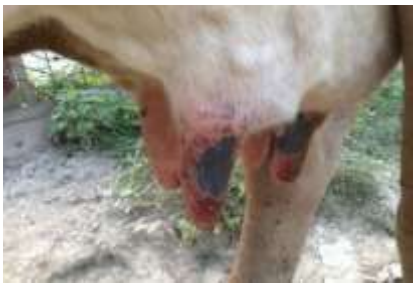

Fig.7 Ulcerations, necrosis and scabs located on the udder

Due to muscle pain and damage to the coronary area, 45.93% (n=96) of cattle avoided moving and quadrupedal station preferring decubitus (fig. 8) and also 57.9% (n=121) showed nervous signs (torticollis).

Fig.8 Decubitus and torticollis

Digestive disorders manifested by diarrheal discharges were reported in 48.32% (n=101) of cattle with clinical signs of *Bluetongue* and due to the prolonged decubital position, the posterior region showed inflammation with necrosis lesions and hemorrhagic ulcers (fig. 9).


Fig. 9 Decubital ulcers, diarrhea and vulvar inflammation

In *Bluetongue,* cutaneous hyperemia may spread throughout the body, including the axillary and inguinal areas (Perianu, 2012), but this symptomatology was not identified in cattle in our study.

The clinical aspects in sheep were similar to those in cattle, but their incidence was different (table 3).

Table 3

The incidence of clinical signs and symptoms specific to *Bluetongue* virus infection in sheeps

The clinical signs specific to *Bluetongue* virus infection	Sheeps		
	TOTAL	No	%
Prostration	206	195	94,66
Hyperemia of the conjunctival mucosa		195	94,66
Epiphora		110	53,40
Sialorrhea		205	99,50
Nasal edema		206	100
Mucopurulent discharge		206	100
Ulcerations of the nasal mucosa		206	100
Lip edema		205	99,50
Gingival ulcerations		206	100
Swelling and cyanosis of the tongue		205	99,50
Oral mucous cyanosis		200	97,10
Ulcers and necrosis of the udder		200	97,10
Congestion of the coronary area and lameness		179	86,9
Increased weight loss		132	64,10
Immobility		199	96,60

Of the 206 sheeps with clinical signs produced by *Bluetongue* virus, examined during the study, 94.66% (n=195) showed a state of prostration, 100% (n=206) nasal muco-purulent discharge, 100 (n=206) edema and nasal ulcers, 97.10% (n=200) cyanosis of the oral mucosa and 100% (n=206) with lip ulcers (fig.10). All the animals (n=206) presented circumscribed ulcers and necrosis on oral mucosa, 99.5% (n=205) sialorrhea, 99.5% (n=205) swelling and cyanosis of the tongue, (fig.11), 53.4% (n=110) epiphora, 96.6% (n=199) were unable to move and 64.10% (n=132) showed marked weakening.

Fig.10 Deviation and torticollis, mucopurulent secretions, edema and nasal ulcers in sheep with *Bluetongue*

Due to congestion and edema of the lips and tongue, the animals had a half-open oral cavity, from which abundant saliva dripped with foam. On the inflammatory background of the oral mucosa, ulcers with a diameter of 2-4 mm were observed, slightly bleeding, which is why sometimes the saliva was bloody and smelly. The tongue and connective tissue in the head region, and especially in the lips, showed swelling, which impaired the ability to eat properly. The examined sheep were caught performing rhythmic movements of the tongue, and after 24-48 hours, the tongue became strongly swollen, purple, a lesion that was the basis of the name of *Bluetongue* (fig.12).

Fig.11 Sialorrhea, oral mucosa cyanosis and lips ulcerations

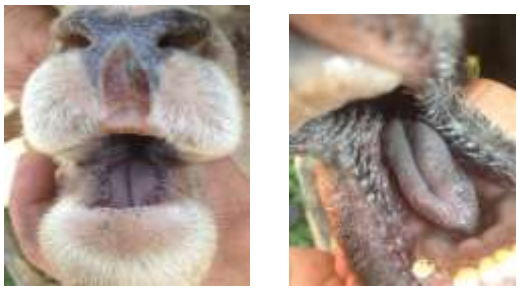

Fig.12 Swelling and cyanosis of the tongue(*blue tongue*)

Due to the muscular locations, the animals had stiff limbs, refusing to move or trying to move on their knees. Local edema was observed in the coronary band, but did not end with exongulation. No abortions were reported in pregnant sheep.

According to the literature, in this clinical form, the mortality rate can vary between 20-60% (Perianu, 2012).

The symptoms of *Bluetongue* infection were much more attenuated in goats than in sheeps. In general, the disease progressed inapparently or with moderate specific symptoms, which is why no representative clinical picture for *Bluetongue* virus infections was shown, but all examined goats showed prostration, muco-purulent discharge, edema and ulceration. nasal and lameness and 66.6% (n=2) were identified with cyanosis of the oral mucosa (table 4).

Table 4

The incidence of clinical signs and symptoms specific to *Bluetongue* virus infection in goats

The clinical signs specific to *Bluetongue* virus infection	Goats		
	TOTAL	Nr	%
Prostration	3	3	100
Mucopurulent discharge		3	100
Nasal edema		3	100
Nasal ulceration		2	66,6
Oral mucosa cyanosis		2	66,6
Lameness		3	100

The results obtained in serological surveillance correlated with the clinical and lesional aspects observed in ruminant species from Vrancea, Brăila, Galaţi and Tulcea counties, unequivocally demonstrate the presence of the circulating *Bluetongue* virus and highlighted the ability to spread continuously in the studied area.

All the clinical manifestations described above evolved in the South East region of Romania only during 2014-2015. The infection with the *Bluetongue* virus is not persistent. Due to the pathogenicity, *Bluetongue* virus infection may not be diagnosed for a certain period of time, but during this time the disease exists and evolves, with infected animals being sources of infection for culicoid insects. However, according to the World Organization for Animal Health (OIE, 2020), from 2015 to 2020, no new outbreaks of *Bluetongue* have been reported in this region, most likely due to specific prophylactic measures previously carried out, under the supervision of the National Sanitary Veterinary and Food Safety Authority (ANSVSA).

However, being a vectorial disease and because of the vectors species distribution changing periodically due to climate change, since 2015 the virus has spread to other regions of Romania (OIE, 2020). *Bluetongue* virus can resist and circulate for a long time among susceptible animal populations if climate, host, and vector conditions are met (Samy, 2016). Also, on 02.09.2020, a singular case of *Bluetongue* was confirmed on clinical suspicion in an ovine animal (male), out of 241 animals from a non-professional holding (backyard) in Turcesti, Valcea county. There were tested 63 animals (60 ovines and 3 cattle) and the infection was confirmed by the Real Time RT-PCR test in only one animal. Disease confirmed at Institute for Diagnosis and Animal Health, the National Reference Laboratory. (Https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/).

In wild ruminants, the disease can develop inapparently, but the virus persists in the body for months or years. Through these animals, the virus can reach infected areas, such as Valcea County, to disease-free areas through seasonal migrations. As a result, wild ongulates can act as a reservoir for *Bluetongue* virus and play an important role in its transmission (Niedbalski, 2015). Also, the presence of outbreaks reported in the immediate vicinity of Romania (Bulgaria, Hungary, Serbia,) as well as in the countries from which domestic ruminants are purchased (Greece, Italy, Albania, Cyprus, etc.) (OIE, 2020), shows that, the recurrence of a *Bluetongue* epidemic is imminent and vigilant surveillance of known (*Culicoides*) or alternative vectors suspected of being involved in the transmission of *Bluetongue* virus is required, as well as continued passive and active surveillance of susceptible animals.

Conclusions

1. *Bluetongue* has evolved clinically in all species of domestic ruminants in the South East region of Romania.

2. The specific signs and symptoms of *Bluetongue* virus infection that evolved in the three species were similar, but the cattle presented the most complex clinical picture.

3. The signaling of a new *Bluetongue* outbreak in 2020 shows us that the period of six years, carried out from the moment of *Bluetongue* signaling in Romania, is not enough for the total regression of the viral circulation.

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14. XXX The World Organisation for Animal Health (OIE), 2020- - Report No.1(04.09.2020) https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/

Hepatotoxic and nephrotoxic effect of acrylamide from potato chips in mice

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Abstract
Potato chips are considered to be potentially health risk products because they contain several substances with toxic potential effect upon various organs. These snacks contain acrylamide, a multi-organ carcinogenic effect substance and monosodium glutamate, used to improve the taste quality, but which has toxic effects upon several organs. In order to test the effect of potato chips diet upon different organs, two experiment were conducted. In an experiment were used adult mice aged between 4-6 months and in the other young mice of 17-20 days. They were fed for 60 days with potato chips representing 80% of the daily diet. In the first 30 days of experiment the adult mice gain weight, but at the end of the experiment they lost 10-15% from the initial weight measured at the beginning of the experiment. Histopathological modifications were noticed in internal organs of both young and adult mice. Liver presented changes in architecture, necrosis areas, hepatocytes with macrovesicular steatosis, and hydropic degeneration. Into the renal cortex, enlarged glomeruli, mesangial cell proliferation, and reduced urinary spaces were observed along with vascular congestion. Also, in the kidney were noticed renal tubules degeneration, narrow lumens and swelling epithelia. Degenerations were also present in most of intestinal tunics where villi fusion, villi atrophy, modifications in epithelia, in subepithelial connective tissue, and changes in smooth muscle fibers were observed.
Keywords: potato chips, acrylamide, glutamate, multiorgan degeneration, mice

Introduction

Potato chips are familiar foods for children all over the world being well appreciated because their attractive taste and aspect (Konings et al, 2003). Some research reported that potato chips contain high levels of substances that induce neurotoxicity (Abou-Donia et al, 1993; Gad-Allah et al, 2013), genotoxicity (Paulsson et al, 2003; Besaratinia & Pfeifer, 2005; Katen et al, 2016) and carcinogenicity effect (Maronpot et al, 2015) such as acrylamide and some food additives (salt, monosodic glutamate, sugar, etc) which are not present in uncooked food (Exon, 2006; Foot et al, 2007; Riboldi et al, 2014; Sawicka and Mohammed, 2018). Chronic dietary exposure of children was estimated to be on average between 0.5 and 1.9 µg/kg b.w. per day and the 95% was between 1.4 and 3.4 µg/kg b.w. per day (*). In the case of adolescents, adults, elderly and very elderly, the chronic dietary exposure was estimated to be on average between 0.4 and 0.9 µg/kg b.w. per day and the 95% was between 0.6 and 2.0 µg/kg b.w. per day depending on the survey and age group (*). Acrylamide is converted in glycidamide, and Mice are more proficient in doing that compared with either rats or humans (*). In commercial foods that are processed at different temperatures, especially in carbohydrate-rich foods, compounds with cancer risk in humans are formed (Mitka, 2002; El-Sayyad et al, 2011). The acrylamide concentration in fried potato chips ranged from 376 to 2348 microg/kg being in direct correlation with the thermal process and brown coloring (Amrein et al, 2006; Mojska et al, 2008, Friedman & Levin, 2008). The toxic potential of potato chips compounds may produce severe disorders mainly in the liver (Altinoz et al, 2015; Mahmood et al, 2015), kidneys (Mucci et al. 2003, Mucci et al., 2004), heart muscle and cardiovascular system (Naruszewicz et al., 2009), nervos system, and others both in humans and animals.

Material and methods

The study was performed on conventional mice (Mus musculus), 20 individuals, both males and females which were divided into 3 groups: the control one consisted of 4 individuals

and 2 experimental groups of 8 individuals each. In an experiment were used young mice of 17-20 days (first experimental group) and adult mice (the second experimental group) aged between 4-6 months. The experiment lasted for 60 days. During this time, the experimental groups were fed with potato chips, representing 80% of the daily diet, the rest of it consisting of vegetables and cereals. The potato chips come from the distribution network of the local stores, being used several assortments from several producing companies. The control group was fed with vegetables, cereal and mice food. Water was administrated ad libitum and the accommodation to microclimate conditions were ensured in according with national Law no. 43/2014 and to DIRECTIVE 2010/63 / EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The end of the experiment, provided "as without recovery of animals", used the euthanasia of mice according to Art. 16, of Law 43/2014.

Kidney and liver samples were collected from each mouse. Tissue samples from these organs were fixed with Bouin solution, dehydrated with alcohol, cleared with xylene, and paraffin embedded. From each sample 5µm sections were obtained using a Slee microtome. After deparaffination and rehydration the sections were stained with hematoxylin for 10 minutes and eosin for 1 minute. Slides were examined using a Leica microscope and LAS V4.9 soft.

Results

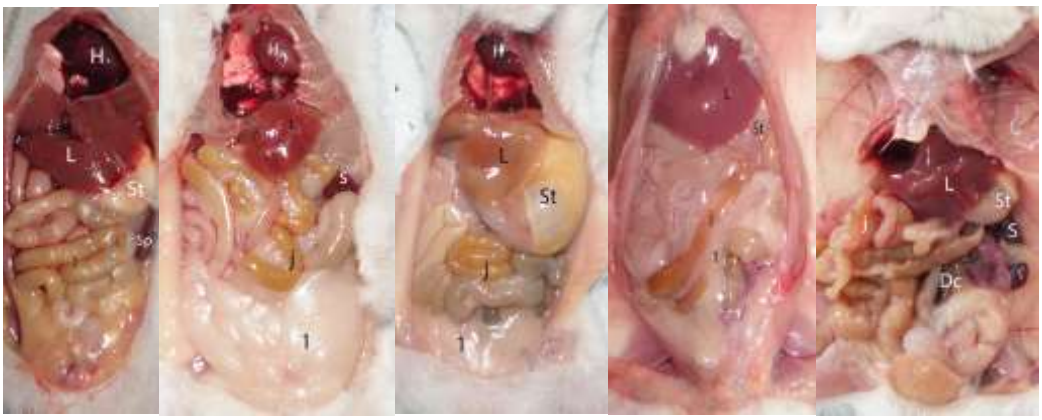
By repeated weight measuring during the experiment, changes in the weight of the mice were found, both in the young mice group (first experimental group) and in adult mice group (second experimental group). At 30 days, in both experimental groups it was observed an increase in weight between 2-4 g in adult mice (approximately 10%) and between 3-6 g in young animals, which represents approximately 15-35% of the initial weight, compared to the control group in which there was registered of approximately 0,5 g.

After 60 days of experiment, there is a reduction in weight between 2-3 g in experimental adults and variable between 0-4 g in youth compared to the initial weight recorded, the weight of control mice increasing during this time by about 1-2 g.

A 30-day necropsy examination found an increase in the volume of mesenteric fat mass in both males and females of young and adult experimental groups (both experimental groups). The stomach and intestines were slightly enlarged and full of food. The liver was intensely colored, in adults it was slightly enlarged in size with evident lobulation (Fig. 1, B, D).

At 60 days of experiment, there was an obvious reduction in the mass of abdominal mesenteric fat in both experimental groups. In 3 of the 4 young mice, the stomach was much dilated with gas compared to the adult stomach, which appeared much smaller in size and predominantly empty (Fig. 1, C, E).

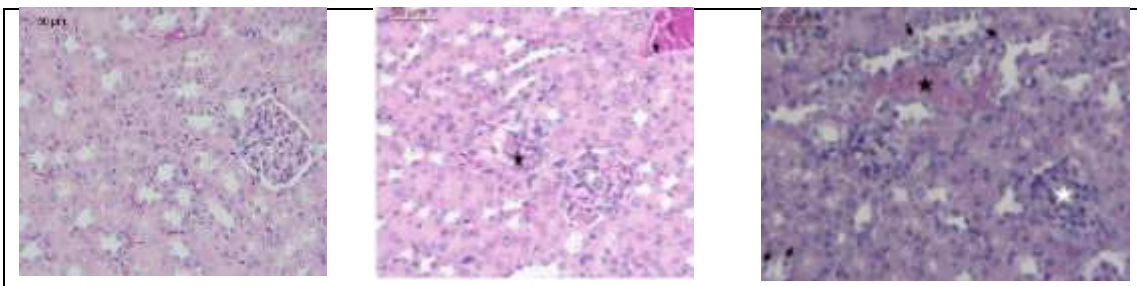
In young mice there was a slight reduction in the size of the liver, which had a light yellowish clay color, without any lobular design, and the gallbladder was rather dilated (Fig. 1, C). In experimental adults, the liver generally appeared intense clay color, the hepatic lobulation being visible through the capsule (Fig. 1, E). On inspection of the kidneys they were intensely colored and surrounded by an obvious fat mass in both experimental groups at 30 days of experiment, after 60 days the kidneys having a lighter color compared to their appearance in the control group, without any visible changes with the naked eye.



A. B. C. D. E.

Fig. 1. The macroscopic aspect of thoraco-abdominal organs in mice used in experiment
A. control mouse, B- experimental young mouse, the 30th days of experiment, C. experimental young mouse, the 60th days of experiment, D- experimental adult mouse, the 30th days of experiment, E. – experimental adult mouse, the 60th days of experiment
Dc- descending colon, J- jejun, H- heart, L- liver, S- spleen, St- stomach, 1- mesenteric mass of fat.

At light microscopy evaluation, the control group kidney samples showed the normal cytoarchitecture (Fig. 2, A). In the kidneys of both experimental group mice, were observed swelling of the renal tubule epithelium, congestion in the glomeruli and between the renal tubules, enlarged glomerulus with reduced urinary space, and hyperplasia of the mesangial cells (Fig 2 B, C). Degeneration of the renal tubule was noticed due to the cloudy swelling of the renal tubule epithelial cells.



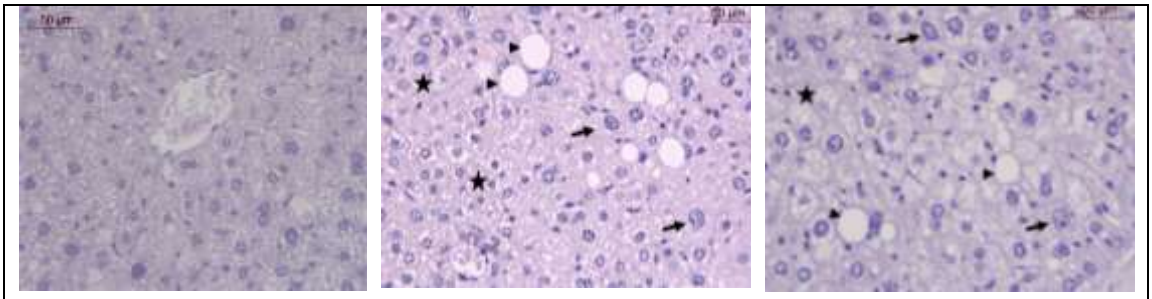
A. B. C.

Fig. 2. Kidney. HE. x400. The histopathological aspects of kidney in mice. Histological normal appearance of kidney (A). Renal congestion (black star) in the glomeruli and between the renal tubules (B, C). Enlarged glomerulus caused by the hyperplasia of mesangial cells (white arrow) (B, C). Degenerated epithelium (arrows) in the renal tubules (C).

Vascular modifications were noticed because of the congestion present between the renal tubules (Fig 2, B, C). Congestion was also noticed in some glomeruli of renal corpuscles in both experimental groups (Fig 2 B, C). The modified renal corpuscles presented enlarged glomerulus with reduced urinary space, caused by hyperplasia of the mesangial cells (Fig 2 B, C).

The liver in control group showed no modifications (Fig 3.A). The liver of both experimental groups presented diffuse areas with severe macro vesicular steatosis, hydropic degeneration, and necrosis. In experimental groups, steatosis areas with hepatocytes presenting

large lipidic vacuoles in the cytoplasm and peripheral nuclei were observed (Fig 3 B, C). Some hepatocytes presented hyperhydration with protidic coagulation in the cytoplasm and large hyperhydrated nuclei (Fig 3 B, C). Necrosis was present in several areas in which hepatocytes with pyknotic nuclei were noticed.



A. B. C.

Fig. 3. Liver. HE, x400. The histopathological aspects of liver in mice. Normal histological appearance of liver (A). Diffuse areas with macro vesicular steatosis (arrowhead) (B, C). Hydropic degeneration (arrow) in hepatocytes presenting enlarged nuclei (B, C). Necrosis (star) present in diffuse areas (B, C).

Discussions

Variations in body weight observed for the mice in our study were described in experiments in which acrylamide was administrated (Sengul et al., 2020).

Vascular congestion in the kidney both in the glomerulus and between the renal tubules was described in several experiments in which acrylamide was administrated to animals (Ghorbel et al., 2014; Sengul et al., 2020). The effect of acrylamide in the kidney structure was observed in the form of tubular degeneration with cytoplasmic hyperoesinophilia, enlarged hyper chromatic nuclei and nucleoli along with hyaline droplets and enlarged Bowman's capsule (Rahej and Al-Daheri, 2017). Degeneration of the renal tubular epithelium with vacuolization, loss of brush borders, rupture of cells, necrosis and hyperemia was described by other authors too (Kandemir et al., 2020; Mahmood et al., 2015). Studies have shown that acrylamide also induces leukocyte infiltration between the tubules and glomerulus fragments (Ghorbel et al., 2016).

Degenerated hepatocytes, with large lipid vacuoles observed in diffuse areas were described by Altinoz et al., 2015. The fatty deposits were observed in hepatocytes with variable size vacuoles, along with chromatolysis and congestion of central veins (Allam et al., 2010). Studies showed liver modifications such as congested blood vessels and degenerated hepatocytes, mostly suffering from necrosis, and mononuclear inflammatory cells infiltration in portal areas (AL-Mosaibih, 2013; Mahmood et al, 2015).

The damaging effect of acrylamide upon both the liver and kidney was showed in several experiments (Kandemir et al., 2020; Mahmood et al., 2015). The exposure to acrylamide increases the generation of free radicals and hydroperoxides which leads to lipid peroxidation (Prasad and Muralidhara, 2012). About 50% of ingested acrylamide is metabolized in liver. The cytochrome P450 biotransforms it into glycidamide, and both are conjugated with glutathione by enzymes from the family of glutathione S-transferase (Tareke et al., 2008). The reaction with glutathione makes acrylamide and its derivatives easily to be eliminated from the organism by excretion in urine (Friedman et al., 2003). Glutathione is a cell antioxidant, but large quantities of ingested acrylamide induces higher activity of antioxidative system and exposure for long time periods induce symptoms of oxidative stress (Semla et al., 2017). Oxidation of biological molecules (lipids,

enzymes, DNA) leads to damage of organelles, impaired cell metabolism, DNA fragmentation and cell death (Greń, 2013). These microscopical changes are translated into clinical symptoms of numerous diseases including diabetes, neurodegeneration, diseases of cardiovascular system (Rahman et al. 2012,). The oxidative stress induced by acrylamide explains the modifications observed in both kidney and liver collected from mice fed with potato chips in our experiment.

Conclusions

Modifications induced by acrylamide were observed in the bodyweight variations and macroscopic and histologic aspects. Degenerations observed in liver and kidney of mice feed with potato chips correspond to the hepatotoxic and nephrotoxic effect of acrylamide.

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Contributions to the study of rooster orchidectomy

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Abstract
Castration of the rooster, also called claponage is an operative intervention that contributes to the quantitative and qualitative increase of the meat obtained. The capon is calmer, being concerned only with feeding, and the "crowing of the rooster" disappears because it no longer relates. The meat obtained is superior in terms of quantity and taste, as the slaughter of the capons is performed at an early age compared to the uncastrated ones. These beneficial aspects are reduced by the fact that the intracavitary topography of the testicles exposes to intraoperative accidents which, in most cases, are fatal. (8.3). Due to this fact, the study performed in this surgery aimed to identify the factors that contribute to the occurrence of hemorrhagic accident. In this sense, the hemorrhagic accident was correlated with the age of the rooster at the time of clapping and the operative access used. In the rooster, the testicles are in the abdominal cavity, just before the kidneys (6,10,9). They are oriented parallel to the ceiling of the cavity, at a distance of 0.5 - 1 cm from each other, with the anterior pole reaching the level of the last two ribs, and with the posterior one slightly exceed the costal wall (1, 9). The shape of the testicles is ovoid, resembling a bean, the left one being larger than the right one. Their color is yellowish white. The testicles are held in place by the testicular ligament (mesorchium). which is short and crossed by the spermatic artery and vein. Between the two testicular ligaments, under the spine, the aorta and the caudal vena cava have passed, vessels of vital importance having thin walls as they are protected by the testicles in this area. In roosters over six months of age, these vessels are so developed that damage to their integrity causes heavy bleeding. Due to this fact, the capping operation can be accompanied by the danger of a fatal hemorrhage (1, 2, 4, 7, 8).

Material and method

The study aimed to correlate the hemorrhagic accident with the age of the rooster at the time of surgery, with the practice of laparatomy to create operative access and how to apply the loop of the polypotome on the testicular ligament and tightening it. 32 roosters were subjected to the orhidectomy operation, of which, at 22, the laparotomy was performed in the last intercostal space, and at 10 behind the last rib. The age of the roosters varied between 8 and 14 weeks.

The preoperative preparations were those indicated for the success of any surgery. In this sense, the roosters were subjected to a 24-hour diet, during which time they received only water at their discretion. The diet helped to empty the intestines so that they could be easily moved out of the operating field. The instrumentation was represented by the scalpel for the capping, the automatic spacer, the hook for opening the air sacs, the clamping cap, the polypotome with a handle, to which are added the necessary materials for suturing. The lack of a polypot can be replaced by a hemostatic forceps with a more pronounced curvature.

The operative peace was ensured by the intramuscular administration of xylazine in a dose of 2 mg / kg body weight, and after ten minutes Ketamine was administered in the same way in a dose of 20 mg / kg. The anesthetic combination ensured the comfort necessary for the execution of the operative act, the bird being able to be placed on the operating table in lateral decubitus, with the limbs in extension and the wings stretched far back and forth, cost-abdominal region. The wings can be held in place by applying a clamp that secures the plumage.

The place of choice was represented by the last intercostal space or the posterior face of the last rib, in relation to the operator access used. The skin area plummets bilaterally. Anoperatively, anisepticize with sanitary alcohol.

The creation of the operative access by laparatomy in the last intercostal space was made by making a skin incision, in this space, vertically, at a distance of 3-4 cm. The incision starts near the cost-vertebral joint and descends as close as possible to the anterior edge of the last rib, thus avoiding the injury of the intercostal vessels, which follow the posterior contour of the anterior rib. Also, during the execution of the skin incision, the skin moves slightly forward so that the skin

incision does not overlap with that of the muscles. Laparatomy continues with the incision of the muscles in the operating field, creating access to the abdominal cavity. By applying the automatic divider and fixing one arm on the slider of the other arm, the created access is kept open. The absence of the automatic diverter can be replaced by a blepharostat.

With the sharp tip of the hook, the wall of the posterior air sac is perforated, in the operating field appearing the intestinal loops. With the widened end of the hook, the intestinal loops move from the operating field, revealing the testicle. The identification of the testicle was facilitated by the light from the headlamp. On palpation the testicle has low consistency as the testicular albuginea is very fine compared to other species.

Orchidectomy is the important intraoperative moment responsible for triggering fatal hemorrhage. The loop of the polypot is inserted in the abdominal cavity and benefiting from the projected light, the loop is fixed at the base of the testicle, respectively on the testicular ligament. The loop should not be too close to the testicle as tightening can rupture fragments of the testicle due to the fragility of the testicular albuginea. However, the application of the loop too low, respectively towards the dorsal region of the abdominal cavity, appears the danger that during the tightening of the loop or the forceps and their torsion to be affected the vessels sheltered at this level, respectively the posterior vena cava or aorta triggering fatal hemorrhage. These accidents can also occur when using curved hemostatic forceps. The forceps are applied to the ligament, and the ablation is performed by digital twisting of the testicle, without crushing or tearing, accidents that cause bleeding.

In case of using the operative access by laparatomy behind the last rib, the incision of 3-4 cm. it is practiced starting from the cost-vertebral joint, descending to 0.4-0.5 cm. by the posterior contour of the last rib. Through this access, the posterior pole of the testicle appears in the operating field. The application of the loop of the polypot is more difficult, as it must be passed under the last rib to cover the entire testicle.

The orchidectomy operation is completed by closing the wound for operative access. The edges of the wound are faced in a single layer, using non-absorbable thread passed in separate points or in the surface. Friction with iodine tincture is performed around the suture, and the threads are removed after two weeks. The rooster is turned on the other side and restrains itself. The local preparation being performed at the beginning of the operation, the plucked place is decontaminated and the orhidectomy is performed, following the same technique. Postoperatively, the rooster is accommodated in a limited space, but to allow it to move, as waking up from anesthesia is done in about 45-60 minutes.

Possible complications are hemorrhage and subcutaneous emphysema. Hemorrhage is considered the most serious complication. If it comes from the aorta or posterior vena cava, death occurs within minutes. Hemorrhage following the injury of the internal spermatic artery causes death within 10-20 minutes. The main sign of slow bleeding is the pallor of the ridge. Injury to the intercostal artery-venous cord causes a slow hemorrhage that is combated by tamponade.

Subcutaneous emphysema occurs as a result of suture mistakes, respectively by incorrect coping of the muscle layer allowing air to enter the air sacs between the facing edges and subcutaneous stationary. The complication is combated in relation to the volume of air accumulated. If the emphysema is reduced in area and tension, aseptic needles can be made with a needle for intramuscular injections, and the skin is evacuated by skin compressions. In case of accumulation of a larger quantity, 1-2 stitches can be removed, in relation to the location, evacuation of air and restoration of the suture.

Results and discussions

Rooster orchidectomy is an intervention that requires a lot of attention in observing the surgical technique, both in creating the operative access and during the ablation of the testicle. To this statement is added the observance of the age of the rooster subject to the intervention, the data obtained are presented in the table.

The results obtained in orhidectomy

Age at castration in weeks	Intercostal operator access			Retrocostal operator access		
	Operated roosters	Mortality		Operated roosters	Mortality	
		Nr.	%		Nr.	%
8	8	-	-	3	-	-
9-10	6	-	-	2	1	50
Total	14	-	-	5	1	20
11-12	4	1	25	2	1	50
13-14	4	1	25	3	1	33
Total	8	2	25	5	2	41,5

The analysis of the data in the table highlights the fact that the orchidectomy is successfully performed at the age of 8-10 weeks using intercostal operative access, the recovery being 100%. In the case of using retrocostal operator access, at the same age category, accidents due to mortality represent 20%. Although the testicles are small, the weight of holding the testicular ligament in the loop of the polypot, or the arms of the forceps, requires many maneuvers that can injure the vessels in the ligament.

In 11-14 week old roosters, mortality due to intra and postoperative hemorrhage can reach 25% through intercostal access, respectively 41.5% in the case of retrocostal operative access.

C o n c l u z i i

1. Chicken orchidectomy is a surgery in which the results depend on the age of the operated, the operative access path and the testicular excision maneuvers.
2. It is recommended that the orchidectomy be performed in the age range of 8-10 weeks, using intercostal operative access, the recovery being 100%.
3. The advanced age of the cock leads to the shortening of the growing testicular ligament difficulties in ensuring hemostasis in the testicular ablation work.
4. Place of application of the loop of the polypot or forceps for excision on the ligament testicular should be at equal distance between the ceiling of the abdominal cavity and the testicle, thus avoiding complications of hemorrhage or spread of testicular parenchyma on the abdominal serosa.

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Immune mediated glomerulonephritis (immune GN.) – a literature review

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The kidney is often the target of many aggressions of extra-renal origin. Its structure and function make it vulnerable and part-taking to the pathological changes than develop inside the living organism.

The increased blood presure inside the glomerular capillaries, its purpose in ultrafiltration and the negatively charged glycoproteins from the structure of the glomerular filtration barrier participate in increasing the sensitivity to the toxic action of exogenous or endogenous circulating substances.

Based on their pathogenesis, immune mediated glomerulonephritis may be divided into two categories:

1. glomerulonephritis caused by immune complexes deposited inside the glomerulus;
2. glomerulonephritis caused by anti-basal membrane antibodies.

1. Glomerulonephritis caused by immune complexes deposited inside the glomerulus

Studies regarding spontaneous and experimental glomerulonephritis have demonstrated that the immunological mechanisms play a very important role in glomerular pathology.

Based on recent reseach, in human pathology about 70-80% of immune glomerulonephritis are caused by the precipitation of immune complexes inside the glomerulus and in the pathology of companion carnivores about 77% of proteinuria cases are caused by immune mediated glomerulonephritis (Jergens A.E., 1987).

In the pathogenesis of glomerulonephritis caused by immune complexes there are two basic mechanisms involved in producing the structural changes inside the glomerulus, both of them triggering type III hypersensitivity reactions:

- circulating Atg-Atc immune complexes (preformed);
- Atg-Atc immune complexes formed "in situ" (inside the glomerulus).

Generally, immune based glomerulonephritis are characterized by the precipitation of immune complexes and complement fractions on the basal membranes and the mesangium as discontinuous deposits with a granular aspect that may be visualized through immunofluorescence and immuneperoxydasis methods.

These immunofluorescent granular deposits have an opposite aspect than those with a smooth, liniar, diffuse aspect caused by the precipitation of anti-basal membrane antibodies.

The pathogenicity of circulating immune complexes derives from their capacity to trigger a series of phenomena that have as a result the degradation of basal membranes (membranous and membrano-proliferative GN.) and mesangial cells (proliferative GN.).

Clinical symptoms are mainly characterized by severe proteinuria.

The pathogenesis of glomerulonephritis caused by the precipitation of circulating immune complexes

In causing immune mediated glomerulonephritis the circulating Atg-Atc immune complexes represent the main factor.

These complexes may contain bacteria, viruses, parasitic or tumoral antigens.

The pathogenicity and precipitation pattern of the immune complexes on the glomerular structures depend on their quantitative and qualitative aspects:

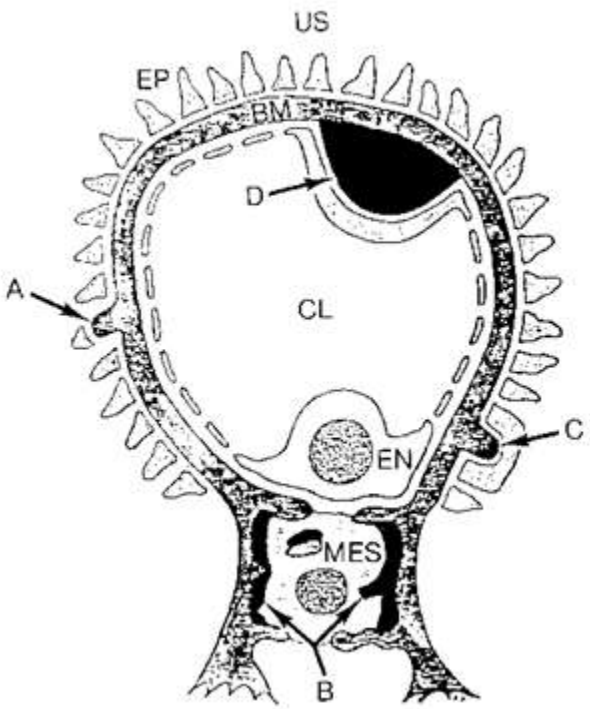
- the quantity of Atg-Atc complexes;
- the size of the complexes;
- molecular configuration;
- the afinity between the antibody and the antigen;
- electrical charge;
- solubility.

Large, insoluble complexes that are excessively formed are rapidly removed from the blood stream inside the kidney and phagocytosed by the monocytic-macrophage system (SMM) or partialy taken by the mesangial cells.

On the other hand, intermediary sized immune complexes formed in the presence of excess antigens remain in solution and may be deposited on the basal membrane of the glomerular capsule, of the vascular bundle and on the mesangium.

Through electron microscopy precipitated immune complexes may be visualized as irregular, electron-dense deposits, located under the endothelium (*subendothelial GN.*) or under the epithelium (*subepithelial GN.*), within the thickness of the basal membrane (*intramembranous GN.*) or the mesangium (*mesangial GN.)* (Slauson and Cooper, 2002) **(Schematic 1)**.

Schematic 1



THE MECHANISMS OF PRECIPITATION OF IMMUNE COMPLEXES IN THE GLOMERULUS

(after *Comeford*, 1968)

A – epithelial nodules;
B – mesangial deposits;
C – subepithelial deposits;
D – subendothelial deposits;
BM – basal membrane;
CL – capillary lumen;
EP – podocytic processes;
MES – mesangium;
US – filtration space;
EN – endothelial cells;

The subendothelial precipitation is common for circulating immune complexes with a high anionic charge (which does not allow them to pass through the glomerular basal membrane) and high afinity of the antibody for the antigen (which makes them difficult to be dissociated).

In contrast, the subepithelial precipitation is seen in immune complexes that have a cationic charge and a low afinity of the antibody to the antigen, which allows for the dissociation of the immune complex, the separate migration of the antigen and antibody through the glomerular basal membrane and the reconstitution of the complex on its subepithelial side.

The intramembranary depositing of the immune complexes is less common, in some cases representing an intermediary phase of the migration through the thickness of the glomerular basal membrane.

The depositing in the glomerular mesangium is particular for immune complexes with a neutral charge (Slauson and Cooper, 2002).

Finally, these antigen rich complexes don't determine a significant activation of the complement, hence they manifest a lower capacity in causing glomerular damage.

Also, it is important that any disease with a chronic evolution that prosuposes a prolongued exposure to antigens may stimulate the continuous formation of circulating immune complexes involved in the pathogenesis of immune mediated imflammations.

"*In situ*" formation of immune complexes

In contrast with the precipitation of "preformed" immune complexes we may also see the formation of "in situ" immune complexes directly within the walls of the glomerular capillaries.

This pathogenetic variant is supported by the observations done on spontaneous or experimental glomerulonephritis.

In situ, the formation of immune complexes is initiated by the circulating antigenic molecules of small or medium size that are able to penetrate the fenestrated endothelium of the capillary or small antigens that can easily pass through the *lamina densa* reaching the *lamina rara externa*, close to the visceral epithelial cells.

Also, the formation of immune complexes may be initiated by antibodies targeting structures of the glomerulus or antibodies that will be paired with antigens that are already fixated on the glomerular structures.

Antigens that are deposited inside the glomerulus may be specific for some glomerular components or nonspecific, trigerring an atraction for circulating antibodies that migrate through the basal membrane all the way to the already fixated antigens. Recent observations showed that the fixation of antigens on the glomerular structures is based on electrical phenomena. The positively charged antigens interact with the anionic glomerular structures (basal membrane, podocytic processes of the epithelial cells, mesangium).

The basal membrane has a strong negative charge due to the presence in its structure of polyanionic molecules such as syalo-glycoproteins and heparan-sulphate, thus facilitating the

passage of cationic molecular proteins, no matter of their nature and restricting the transit of anionic proteins (albumin) (Walker, F., 1973).

Antigens located inside the glomerular structures may be of endogenous or exogenous nature.

Endogenous antigens are represented by histone-DNA complexes, IgA and other immunoglobulin isotypes. Exogenous antigens are represented by various drugs and infectious agents (Slauson and Cooper, 2002).

The fixation of antigens on the glomerular antigens depend on the size, electrical charge, molecular arrangement and their carbohydrates content.

The selective permeability of the capillary wall given by the electricaly charged structural molecules has been demonstrated using feritine macromolecules (480 000 daltons) as markers. In normal conditions feritine anionic particles can pass through 100 nm wide endothelial pores, but cannot pass through the glomerular basal membrane due to the polianionic charge of the *laminei rara interna*.

When experimentaly cationized feritine is capable to penetrate the trilamellar basal membrane reaching the subepithelial space and even the filtration space.

The strong negative charge of the glomerular basal membrane works as a selective electrical filter, permissive for neutral and cationic molecules and nonpermissive for anionic molecules (albumin). Even more, the electrostatic barrier represented by the glomerular basal membrane is meant to permanently maintain the distance between the podocytic processes of the epithelial cells.

We may conclude that the electrical charge of the molecules (particles) that need to pass through this barrier is more important than their size, thus transiting can be done more easily for the larger cationic molecules than for the smaller anionic ones.

When the negative electrical charge of the barrier is lost podocytes increase their volume, the podocytic processes are reduced and the membranary pores are destroyed. Thus, from a functional point of view, the barrier against the positively charged molecules is destroyed leading to proteinuria.

Loss of polyanions from the glomerular basal membrane determines the accumulation inside the mesangium of immunoglobulin and complement macromolecules.

The hypothesis of glomerulonephritis caused by the formation of immune complexes "*in situ*" is supported by observations made following experimental immunization with *Dirofilaria immitis* in dogs. *Dirofilaria immitis* antigens were administered directly in the renal artery after wich the changes of the glomerular basal membrane were observed. Secondarily, circulating antibodies reacted with the antigens deposited on the basal membrane, finally forming Atg-Atc immune complexes ("in situ") (Grauer and col., 1988; Muramatsu and col., 1988).

The role of the complement system

A special part in triggering glomerular lesions is due to the classical path of activation of the complement system.

The major immunopathogenetic effect is represented by the chemotactic atraction of neutrophils and monocytes to the site where immune complexes are fixated on the surface of the basal membrane through C3a and C5a fractions of the complement.

Neutrophils are capable to phagocytose immune complexes but also produce alterations of the glomerular basal membrane through the release of their one lysosomal enzymes (elastasis, G catepsin, collagenasis and oxygen free radicals). Also, oxygen free radicals (singlet oxygen, superoxyde) may determine the activation of mesangial cells.

Circulating monocytes that are atracted through chemotaxis in the damaged glomeruli and mesangial cells with phagocytic properties actively participate in the glomerular inflammatory process. Both cell types produce and release chemokines, activation factor for blood plateles, prostaglandines (PGE2), metabolites of arachidonic acid, oxygen free radicals and platelet procoagulating factors.

Using experimental models it has been shown that the complement fraction C5b-C9, the complement membranary atack complex participated in the alteration of the glomerular basal memebrane, thus increasing its permeability (Gharaee-Kermani and col., 1996).

Fractions C3a and C5a (anaphylatoxins) of the complement determine the release of histamine from the basophilic granulocytes and increases the vascular permeability which favours the precipitation of an even larger amount of immune complexes in the capillary wall.

This process in which the vasoactive amines induce an increase in the vascular permeability and subsequently the depositing of a larger amount of circulating complexes is called "anaphylactic atraction" (Slauson and Cooper, 2002).

Another consequence of the damages brought to the glomerular basal membrane is the activation of the blood platelets. Exposed membranary collagen initiates the activation of blood plateles with the release of excessive quantities of vasoactive amines such as histamine or serotonin.

Blood plateles amplify even more the glomerular lesions, the basal membranes loosing all their anionic charge, determining the precipitation of an even larger number of immune complexes at this level, the continuous atraction of neutrophils and the passage of proteins in the urinary filtrate becomes unstopabble (Wyers M., 1976).

Another important event is the activation of the XII factor (Hageman Factor) by exposing the glomerular basal membrane which initiates the intrinsic coagulation in such a way that fibrin deposits may be visualized in some forms of glomerulonephritis.

The activated Hageman Factor determines in its turn the activation of the complement system which increases the capillary permeability and the atraction of neutrophils (through mechanisms already discussed).

In conclusion, the cascade of inflammatory mechanisms triggered after the precipitation of circulating immune complexes or after their formation "*in situ*" following different causes is perpetuated endlessly affecting the integrity of the glomerular basal membrane and its permeability.

Still, the glomerular response to these injuries is somewhat monomorphic, being represented mostly by the thickening of the glomerular basal membranes, cellular proliferation and finally sclerosis.

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The morphology of immune mediated glomerulonephritis – a literature review

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1. Membranous glomerulonephritis

Membranous glomerulonephritis have as a characteristic the precipitation of immune complexes [Ig G, Ig M, C3, membranary atack complexes (C5b-C9)] in all segments of the glomerulus (Trautwein and Hewicker-Trautwein, 2000).

The name of this type of lesion is borrowed from the anglo-saxone literature and illustrates the particular aspect of the basal membranes of the glomerular capillaries. Still, in some situations it has been possible to identify the antigen within the structure of the immune complex retained within the glomerulus. Following this ideea, membranous glomerulonephritis have been observed following infection with adenoviruses in dogs, infestation with *Dirofilaria immitis* in dogs (Grauer and colab., 1989) and infection with feline leukosis virus in cats (FeLV). Still, in 70% of cases these lesions are idiopathic (Lees and col., 1997).

Immune complexes, no matter of their origin, cause glomerular lesions following a similar mechanism. Through elecron-microscopy studies performed on dogs suffering from idiopathic membranous glomerulonephritis the evolutionary steps of this lesion could be described.

In the early phases, very discrete, electron-dense immune deposits could be observed on the epithelial surface of the glomerular basal membranes. Later on, a slight thickening of the basal membrane can be seen as well as a fusion of the podocytic processes on top of the membranary immune deposits with a diffuse or sometimes focalized aspect. Podocytes look swollen and the podocytic processes contain a granular material.

Subsequently, the thickening of the basal membranes becomes evident through the newly synthetized membranary material, visible between the membranary deposits and the basal membrane. This material may be visualized as small dots and spikes on the epithelial side of the glomerular basal membrane imprinting a pectinated aspect, easily recognizable through silver impregnation (Moreau G., 1989, Vilafranca and col., 1994).

Studies regarding membranous glomerulonephritis in humans have pointed out that the spikes are actually extensions of the basal membrane formed of $\alpha 3$ and $\alpha 5$ chains of type IV collagen with laminin and fibronectin (Nevins T.E., 1985, Verlander J.W., 1998).

In the late evolutive stages the membranary spikes fuse with the membranary immune deposits, thus determining the thickening of the capillary wall. Also, we may notice a slight proliferation of the mesangial cells and an increase in the quantity of the mesangial matrix.

Finally, the glomerular basal membrane becomes distorted, folded and loses its polyanionic charge, thus becoming very permissive, especially for seric proteins, leading to proteinuria and nephrotic syndrome.

Histologically, the glomerular basal membrane appears thickened (5-6 times), especially at the level of the peripheral glomerular capillaries (solitary), being distorted and folded (*wire loop* aspect). The lumen of the damaged capillaries appears normal or even enlarged and empty. Also, a slight proliferation of the mesangial cells could be noticed and an increase of the mesangial

matrix. Steatosis and tubular granular dystrophy were also common, as signs of alteration of the glomerular membranes (fig. 1, fig. 2).

In 15% of cases the evolution in favorable, the lesions being reversible in most part, but there are also cases where a slow, progressive evolution towards glomerular sclerosis was noticed, as a consequence of the obliteration of the capillaries. (Moreau G., 1989).

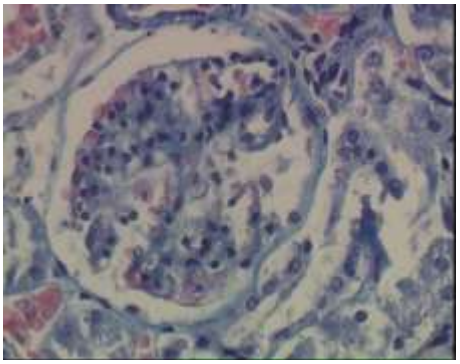

Fig. 1. Membranous glomerulonephritis. Masson trichromic stain, x1000

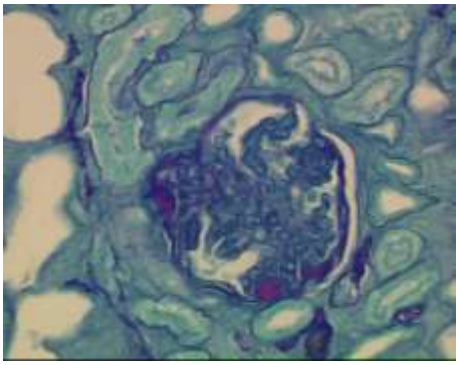

Fig. 2. Membranous glomerulonephritis. PAS stain – light green, x1000

From a clinical point of view, membranous glomerulonephritis are frequently found associated with nephrotic syndrome. Most membranous glomerulonephritis have a generalized and global evolution, are idiopathic (the structure of the circulating immune complexes could not be determined) or the expression of various pathological processes (infections, neoplasia, poisonings or autoimmune diseases) (Carpenter and col., 2002; Scott and col., 2001).

Membranous glomerulonephritis may also be an expression of old age (continuous synthesis of membranous material), heavy metal poisoning (gold, mercury), chronic septic diseases (pyometra in bitches), parasitic infestations, interstitial nephritis (in dogs), systemic metabolic disorders (diabetes, thyroiditis) or idiopathic (nonidentified immune complexes).

2. Mesangial glomerulonephritis (mesangio-proliferative)

Histologically, mesangio-proliferative glomerulonephritis is dominated by cellular proliferations which lead to an appearence of pluricellularity or polynucleosis of the Malpighi corpuscle (Oprean O.Z., 2002; Paul I., 1991) (fig. 3).

This type of immune glomerulonephritis is the most frequently encountered, being dominated by the cellular and matriceal changes of the glomerular mesangium, translated morphologically through a more or less evident increase in the number of cells inside its structure, due to the proliferation of the mesangial and endothelial cells associated with inflammatory type cells (polymorphonuclear cells, mononuclear cells), and an increase of the mesangial matrix and the accumulation of mesangial deposits. The capillaries have thin basal membranes, their lumen is collapsed, they are emptied of blood and sometimes non-existent (Pașca and col., 2006) (fig. 4).

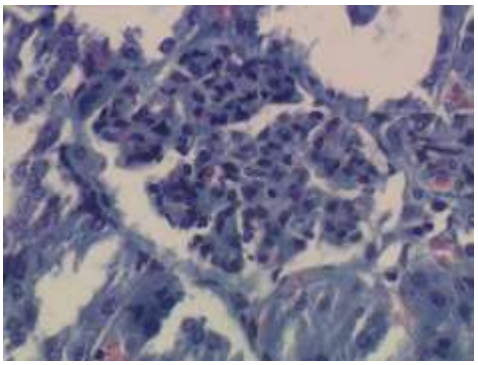

Fig. 3. Mesangio-proliferative glomerulonephritis. Mesangial proliferations lead to the tendency of separation between the glomerular lobules
Masson trichrome stain, x900

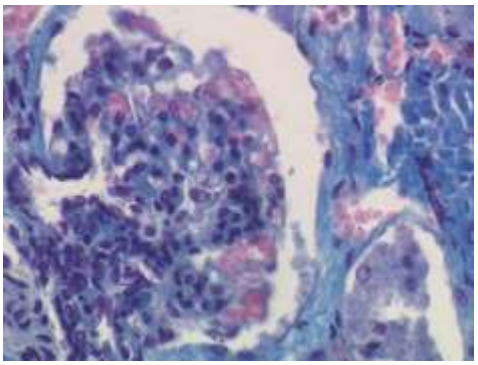

Fig. 4. Mesangio-proliferative glomerulonephritis.
Masson trichrome stain, x900

Proliferative glomerulonephritis may be generalized, affecting the whole glomerulus or segmentary, causing damage to only a few glomerular lobules.
In the case of pure mesangio-proliferative glomerulonephritis, the mesangial and matriceal changes are exclusively limited to the axial region of the glomerulus, without altering the lumen of the glomerular capillaries (Moreau G., 1989).
In segmentary glomerulonephritis, the hyperplasia that occurs within the glomerulus (glomerular polynucleosis) may cause a severe reduction in the caliber of the glomerular capillaries through external compression, leading to complete stenosis, thus determinig a reduction of the glomerular blood perfusion up to sclerosis (Jergens A.E., 1987).
Using electron microscopy we can observe the fusion of the podocytic pedicles. The extra-membranary deposits may be seen with extreme clarity using electron microscopy and using immunohistochemistry we may also highlight regulated, homogenous or granular deposits located in the subendothelial space, along the glomerular basal membrane, deposits that contain IgG and IgM, without the C3 fraction of the complement (Trautwein and Hewicker-Trautwein, 2000).

Most part of cellular proliferations are mediated by factors derived from the complement and platelets. It has been pointed out that the increase in numbers of the cells and in quantity of the mesangial matrix is accompanied by an increase in the level of expression of PDGF (platelet derived growth factor) produced by the endothelial cells and that of the receptor proteins for PDGF, demonstrating the fact that the cellular proliferation is based on an autocrine mechanism.
These proliferative phenomena have as a consequence segmentary glomerular sclerosis, or sometimes even a generalized such transformation, along with the collapse of the glomerular capillaries and the formation of adhesions between the vascular bundle and the Bowman capsule.

The quantitative and qualitative outcomes of these lesions are in indispensable in formulating a histologically based prognosis.

The appearence of segmentary or generalized proliferative glomerulonephritis was observed in infections with *Leishmania sp*. in dogs, mink plasmacytosis, horses and chikens infected with *Streptococcus zooepidermicus*, etc.

3. Membrano-proliferative glomerulonephritis (mesangio-capillary)

Membrano-proliferative glomerulonephritis (MPGN), also called mesangio-capillary, represent a morphoclinical entity characterized through the proliferation of the mesangial cells and at the same

time the thickening of the glomerular basal membrane,thus reuniting both aspects of membranous and mesangio-proliferative glomerulonephritis (fig. 5, fig. 6).

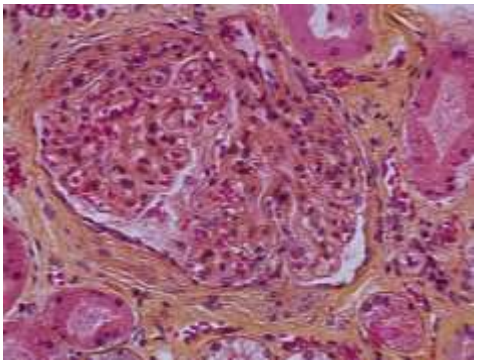

Fig. 5. Membrano-proliferative glomerulonephritis; Adherences glomerulus – capsule. Masson trichrome stain, x200

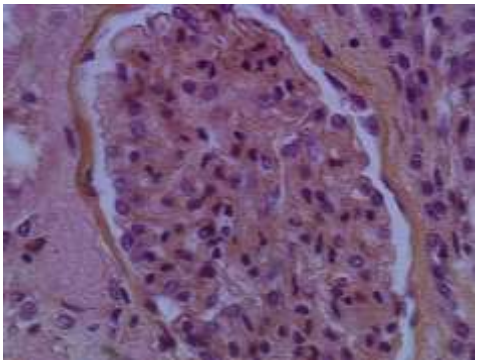

Fig. 6. Membrano-proliferative glomerulonephritis. Masson trichrome stain, x400

These morphological aspects are considered intermediary lesions that become final through generalized chronic glomerulonephritis, leading to chronic renal insuficiency.
Structural changes within this type of lesion explains why these animals manifest with hematuria and nephritis concomitant with proteinuria and nephrotic syndrome.

Membrano-proliferative glomerulonephritis may be associated with systemic illnesses or with a well determined etiology (secondary GN.) or, like in most cases, may be idiopathic (primary).
Membrano-proliferative glomerulonephritis, as a dominant-recessive type autosomatic disease, was described in 20 Bernese Mountain dogs, with ages between 2 and 5 years who showed a renal insufficiency syndrome with severe proteinuria. It appears that the infection with *Borrelia burgdorferi* represents a triggering factor for this pathological process (Gough and Thomas, 2004).
Based on the clinical and pathological aspects in humans and animals we can recognize 3 types of membrano-proliferative glomerulonephritis: type I, II and III, the last being the least known and controversed.

a. MPGN type I

Type I membrano-proliferative glomerulonephritis are characterized, from a histological point of view, by an accentuated lobulation of the glomerulus caused by the proliferation of the mesangial cells and an increased amount of mesangial matrix.

The process of epithelial and membranary proliferation may be extended to the whole glomerulus, from the vascular pole to the urinary one, or may be localized solely to a few capillary branches (glomerular lobules).
In the first situation the glomerulus appears shrunken, more or less atrophic, partially thrombotic or sclerotic.
In the second situation, the affected glomerular branches appear compacted, the rest of the capillaries remaining permeable.

As a whole, this category of glomerulonephritis is characterized by severe lesions that do not ensure healing without sequelae.

The walls of the glomerular capillaries appear thickened due to the subendothelial deposists composed of immune complexes containing immunoglobulins (Ig G, Ig M) and the C3 fraction of the complement (C3 nephrotic factor).

Highlighting within the structure of the immune complex deposits of C1q and C4 complement

fractions indicates that the classical path for activating the complement plays an important role in the pathogenesis of type I MPGN.

Another characteristic aspect of this type of glomerulonephritis is the so called "mesangial interpositioning" that regards the interpositioning of the cells and the mesangial matrix between the endothelium and the basal membrane. This strucure confers a double contour to the glomerular basal membrane also known as a "tram track" aspect, easily observable through silver impregnation and PAS stain.

In dogs, type I membrano-proliferative glomerulonephritis evolve mainly as an idiopathic disease (Trautwein and Hewicker-Trautwein 2000). Still, some data suggest that sometimes it is possible to associate this lesion with systemic diseases, bacterial (leishmaniasis and boreliosis in dogs) or viral infections (feline infectious peritonitis, feline leukosis, equine infectious anemia, african swine fever) and neoplasia (Brostoff and col., 1991, Dambach and col., 1997; Osborne and Finco, 1995).

b. MPGN type II (*Dense Deposit Disease*)

The characteristic histological aspects are represented by the dense thickening of the PAS-positive glomerular basal membrane. The mesangium will appear distended due to the proliferation of the mesangial cells or the matrix.

The definitive diagnosis may be established after highlighting through electron-microscopy the deposits formed by a dense, osmophilic material in the lamina densa of the glomerular basal membrane and the glomerular mesangium.

The same characteristic aspect of "mesangial interpositioning" may also be used to describe the type II membrano-proliferative glomerulonephritis (Cotran and col, 1999).

Also, through immunohistochemistry it has been observed that these deposits are mainly made out of the C3 fraction of the complement and properdine.

Within the pathology of glomerulonephritis in humans it has been noted the intense participation of Ig G auto-antibodies against C3-convertasis, also called C3 nephritic factors (C3NeF). The basal memebrane stabilizes the C3-convertasis, the C3bBb enzyme of the alternative path for the activation of the complement, thus continuing the activation of the C3 fraction with the fomation of C3b and C3a.

The continous activation of the complement leads to its comsumption (hypocomplementemia). The seric level of the C3 fraction is very low, but at the same time the C1q and C4 fractions remain within normal limits.

c. Type III MPGN are characterized by the presence of immune deposits on both sides of the basal membrane at the same time (sub-endothelial and sub-epithelial). The lesion seems to be a variant of the type one (Cheville N., 1994).

Ig A Nephropathy (Berger's Disease)

IgA nephropathy is the most common type of glomerulonephritis in humans and a frequent cause of asymptomatic hematuria. It can also be seen in dogs and experimentaly in mice (Osborne C.A., 1995)

From a morphological point of view it is characterized by IgA mesangial deposits, the proliferation of mesangial cells and the expansion of the mesangial matrix (Brostoff and col., 1991).

Histologically, this glomerular lesion falls into the category of membrano-proliferative glomerulonephritis.

The reseaches of the Béné collective (1984) have established that the disease occurs due to an abnormal synthesis of IgA or the synthesis of IgA with an abnormal structure (Béné and col, 1984).

In the first case, the increased synthesis of IgA is due to a prolongued exposure of the mucosas to an antigen represented by the pathogen during respiratory and digestive infections, which overcomes

the elimination capacity of the monocytic-macrophage system, thus leading to the precipitation of IgA deposits in the renal glomerulus, in the glomerular mesangium (Day and Penhale, 1988).

The amount of circulating Ig A immune complexes is directly proportional with the severity of the infection in the patient due to this abnormal synthesis.

In the second situation, the immune deposits located in the mesangium are composed of IgA1-glycosylate with an abnormal structure. Due to this abnormal structure of IgA1, syaloglyco-proteins (receptors form macrophages) cannot couple with the macrophages of the monocytic-macrophage system to initiate phagocytosis, thus leading to mesangial precipitation (Brostoff and col., 1991).

Glomerular lesions owed to the depositing of immune complexes containing IgA have been observed with an increased frequency in patients with liver cirrhosis. Studies on mice with experimentally induced cirrhosis through poisoning with carbon tetrachloride helped highlight the presence of IgA with an abnormal structure circulating in the blood stream but also located on the glomerular basal membranes.

The presence within the mesangial deposits of the C3 fraction and the final components of the complement, except for C1 and C4, suggests that the activation of the complement through the alternative path mediated by IgA plays an important role in the pathogenesis of this type of mesangio-proliferative glomerulonephritis **(**Brostoff and col., 1991; Slauson and Cooper, 2002).

The diagnostic of IgA nephropathy is based on highlighting through immunofluorescence the Ig A deposits present in the glomerular mesangium (Mc Cluskey R., 1983).

The highest prevalence of glomerular IgA deposits was seen in dogs with enteritis and hepatitis.

In the examined patients deposits containing IgM and IgG could also be observed.

4. Anti basal membrane glomerulonephritis

The name of this category of glomerulonephritis is reserved for the glomerular lesions caused by antibodies targeting intrinsical anti-antigens of the basal membrane which, through interaction lead to the accumulation of membranary immune deposits.

The experimental prototype is called the *Masugi nephritis* or *toxic nephritis* and has been achieved on mice through the administration of mouse anti-kidney antibodies, prepared on rabbit, after immunization with mouse renal tissue.

The glomerulonephritis caused by the anti-basal membrane antibodies is an autoimmune disease with severe and rapid evolution due to the formation of anti-basal membrane antibodies and their depositing inside the renal parenchyma or other extra-renal tissues, more frequently in the glomerulus and sometimes, but not always, in the walls of the pulmonary alveoli. The cause leading to the formation of these antibodies is still unknowkn.

This disease was experimentally induced in sheeps and dogs in order to understand the evolution of immune based glomerular lesions.

Following this lead, the circulating antibodies produced through the experimental immunization using heterologus anti-basal membrane antigens also react with their endogenous antigens.

The diagnostic of this type of glomerulonephritis is based on highlighting the lineary deposits located on the external side of the glomerular capillaries, in the subepithelial space, constituted by anti-basal membrane antibodies and IgG. Much more rarely IgM and IgA could also be observed.

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