Cite this article: *Hajiyeva N.A. Association of Balantidium coli and intestinal coccidia (Eimeria, Cryptosporidium, Isospora) in domestic pigs in Azerbaijan. The Journal of V. N. Karazin Kharkiv National University, Series "Biology", 2021, 36, 41–47.*

••• ЗООЛОГІЯ ТА ЕКОЛОГІЯ ••• ZOOLOGY AND ECOLOGY •••

UDC: 586.893.16

Association of *Balantidium coli* and intestinal coccidia (*Eimeria, Cryptosporidium, Isospora*) in domestic pigs in Azerbaijan

N.A. Hajiyeva

The purpose of our work is to determine the prevalence of associative infections of domestic pigs in the farms of Azerbaijan. The research was conducted in 2009–2015. The faeces samples were taken from 2272 domestic pigs of different ages kept in private farms in the districts of Balaken, Zagatala, Shamakhi, Ismayilli, Guba, Khachmaz, Khudat, Salyan and Absheron peninsula in Azerbaijan, in the region of the Great Caucasus. Species composition, infection intensity, and ecological features of the pig parasitic protozoans had not been studied prior to our research. In the course of our study, the associative invasions in domestic pigs were as follows: *Eimeria* and *Isospora* oocysts were found in 582 pigs (25.6 % out of the total animals examined), *Eimeria* and *Cryptosporidium* oocysts in 622 pigs (27.4 %), *Eimeria* oocysts with *Balantidium* cysts in 273 pigs (12.0 %), *Isospora* and *Balantidium* oocysts in 18 pigs (0.8 %), *Eimeria, Isospora* and *Cryptosporidium* oocysts were detected in mixed form from one pig only (0.04 %). None of the analysed faecal samples contained a four-component mixed parasitic invasion. The two-component mixed infestations, viz. Eimeriosis + Cryptosporidiosis and Eimeriosis + Isosporiasis, were recorded during the whole year, while the prevalence of Eimeriosis + Balantidiosis and Isosporiasis + Balantidiosis mixed infestations decreased in summer. Keeping pigs of different ages in one pen at the farms increases the risk of associative invasion. In the pig farms equipped with various technological devices, the epizootic situation is different. If the animals kept traditionally, the infestation with associative invasions is much higher. Among the animals that live in unventilated pens with a wooden floor, the intensity of mixed infestation is the highest that results in the extremely high economic loss in such farms. In the animals kept in warm pens with high humidity, the invasion intensity is high even in winter. The primary reasons of this phenomenon are heat and humidity that create favourable conditions for the exogenous development of coccidian oocysts.

Key words: *Eimeria, Isospora, Cryptosporidium, Balantidium, domestic pigs, monoinvasion, associative invasion.*

About the author:
N.A. Hajiyeva – Institute of Zoology, Azerbaijan NAS, A. Abbaszadeh Str., passage 1128, block 504, Baku, Azerbaijan, AZ1073, nuruhaciyeva@gmail.com, https://orcid.org/0000-0002-2500-5073

Introduction

One of the factors hindering the increase in livestock and productivity is the association of various parasitic diseases. Thousands of specimens of a number of parasitic species can invade simultaneously the host animal and then reside in its organs and tissues, which results in severe degenerative changes, development delay, and, finally, death (Anisimova, 2013; Kulikova, 2010; Ponomarev et al., 2011). Parasitoses rarely act as monoinvasions. Mostly, they are caused by a combination of various species, which form an associative invasion. The course of associative invasions is more severe than that of monoinvasions (Gavrilova, Petrova, 2015; Maikai et al., 2009; Safiullin, Basynin, 2008; Ponomarev et al., 2011). Investigation of associative invasions has scientific and practical significance for animal husbandry. In particular, the study of separate and mixed infestations of balantidium and intestinal and tissue coccids in domestic pigs is always relevant. It gives grounds for taking preventive measures and/or measures of pathogen eradication. Species composition, infection intensity, and ecological features of the pig parasitic protozoans in the region of Great Caucasus of Azerbaijan has not been studied prior to our research.

The aim of our study was to determine prevalence and composition of associative infections of domestic pigs in the farms of Azerbaijan.

Material and methods

The material was collected in the districts of Absheron, Salyan (lowland), Khudat, Khachmaz, Guba

DOI: 10.26565/2075-5457-2021-36-5

(hills), Shamakhi, Ismayilli, Zagatala, and Balakan (mountains) in 2009–2015 (Fig. 1). To identify parasitic protozoans, we sampled faecal of 2272 pigs of different ages and then examined the collected material in the Laboratory of Protistology, Institute of Zoology of National Academy of Sciences of Azerbaijan. Each faecal sample was kept in 2.5 % potassium dichromate solution. The samples were processed by Flülleborn's flotation with the use of Darling's solution.

After centrifugation, the upper layer of supernatant was screened under a light microscope AMPLIVAL with magnification ×100, 400, and 1000 depending on the objects, viz. *Isospora* and *Cryptocporidium* oocycts, *Balantidium* cycts and trophozoits. After the ethanol fixation, the slides were stained with carbol fuchsin and methyl green by Ziehl–Nielsen with a modification adopted to look for *Cryptosporidium* oocysts (Henriksen, Pohlenz, 1981). Safaraliyev's preservative was used to maintain viability of the balantidium cysts. Fresh faecal samples were placed on a microscope slide and mixed with an equal amount of warm isotonic sodium chloride solution. Then the sample was covered with a cover glass and examined at a low magnification (×100). Detected balantidium vegetative forms and cysts, as well as coccidian oocysts were measured on the eyepiece micrometer scale ×7.

Fig. 1. Map of collecting localities in Azerbaijan

Sample proportion (percentage ratio of the number of animals excreting oocysts to the total number of examined animals – the disease prevalence (Pr)) and its standard error were calculated (Lakin, 1990) for each variant of associative invasion.

Results and discussion

Out of 2272 domestic pigs examined in the farms of Azerbaijan, *Eimeria* and *Isospora* oocysts were found in 582 pigs (25.6 % of all surveyed animals), *Eimeria* and *Cryptosporidium* oocysts in 622 pigs (27.4 %), *Eimeria* oocysts with *Balantidium* cysts in 273 pigs (12.0 %), *Isospora* oocysts in 18 pigs (0.8 %), and a mixture of *Eimeria*, *Isospora* and *Cryptosporidium* oocysts in one pig only (0.04 %).

Table 1. Associative invasions recorded in domestic pigs in Azerbaijan

Collecting locality	Number of examined animals	Diseases											
		Eimeriosis + Isosporiasis		Eimeriosis + Cryptosporidiosis		Eimeriosis + Balantidiosis		Cryptosporidiosis + Balantidiosis		Isosporiasis + Balantidiosis		Eimeriosis + Isosporiasis + Cryptosporidiosis	
		n	Pr ± mp%	*n*	Pr ± mp%	*n*	Pr ± mp%	*n*	Pr ± mp%	*n*	Pr ± mp%	*n*	Pr ± mp%
Absheron	380	71	18,7 ± 2,00	74	19,5 ± 2,03	68	17,9 ± 1,97	11	2,3 ± 0,77	2	0,5 ± 0,36	-	-
Salyan	97	41	42,3 ± 5,02	52	53,6 ± 5,06	22	22,7 ± 4,25	-	-	-	-	-	-
Khudat	224	88	39,3 ± 3,26	80	35,7 ± 3,20	27	12,1 ± 2,18	16	7,1 ± 1,72	1	0,4 ± 0,42	-	-
Khachmaz	274	73	26,6 ± 2,67	117	42,7 ± 2,99	28	10,2 ± 1,83	11	4,0 ± 1,18	5	1,8 ± 0,8	-	-
Guba	222	54	24,3 ± 2,88	68	30,6 ± 3,09	17	7,6 ± 1,78	7	3,2 ± 1,18	3	1,4 ± 0,79	-	-
Shamakhi	152	53	34,9 ± 3,87	53	34,9 ± 3,87	18	11,8 ± 2,62	9	5,9 ± 1,91	3	1,9 ± 1,11	-	-
Ismayilli	714	153	21,4 ± 1,53	138	19,3 ± 1,48	75	10,5 ± 1,15	21	2,9 ± 0,63	1	0,1 ± 0,12	1	0,1 ± 0,12
Balakan	127	25	19,7 ± 3,53	12	9,4 ± 2,59	12	9,4 ± 2,59	7	5,5 ± 2,02	-	-	-	-
Zagatala	82	24	29,3 ± 5,03	28	34,1 ± 5,23	6	7,3 ± 2,87	3	3,7 ± 2,08	3	3,7 ± 2,08	-	-
Total	2272	582	25,6 ± 0,92	622	27,4 ± 0,94	273	12,0 ± 0,68	86	3,7 ± 0,40	18	0,81 ± 0,57	1	0,04 ± 0,04

Notes: n – number of infected animals; Pr – prevalence of parasitosis; mp% – standard error.

In the course of our study, *Eimeria* (*E. debliecki* Douwes, 1921*, E. polita* Pellerdy, 1949*, E. scabra* Henry, 1931*, E. perminuta* Henry, 1931), *Isospora* (*I. suis Biester,* Murray, 1934), *Cryptosporidium* (*C. parvum* Tyzzer, 1912), and *Balantidium* (*B. suis (sp. nov.)*) were constantly detected at the pig farms. Most mixed infections were caused by the protists of two genera that formed two-component parasitic infection: Eimeriosis + Isosporiasis, Eimeriosis + Cryptosporidiosis, Eimeriosis + Balantidiosis, Cryptosporidiosis + Balantidiosis, and Isosporiasis + Balantidiosis. Only one case of a three-component infestation (Eimeriosis + Isosporiasis + Cryptosporidiosis) was registered. The most frequent forms were Eimeriosis + Isosporidiosis, Eimeriosis + Cryptosporidiosis and Eimeriosis + Balantidiosis. The prevalance of *Eimeria* + *Isospora* infestation ranged from 18.7 % to 42.3 %, and that of *Eimera* + *Cryptosporia* from 9.4 % to 42.7 %. In case of Cryptosporidiosis + Balantidiosis and Isosporiasis + Balantidiosis, the prevalence was much lower, 3.7 % and 0.81 %, respectively. Eimeriosis +

Isosporidiosis + Cryptosporidiosis association was registered in one out of 714 animals examined in the District of Ismayilli (Table 1).

Two-component invasions, viz. Eimeriosis + Isosporidiosis, Eimeriosis + Cryptosporidiosis, and Eimeriosis + Balantidiosis, were observed in suckling pigs and in 2–5 year-old pigs. Associations of *Eimeria* + *Isospora* (Pr 25.6 %) and *Isospora* + *Cryptosporidia* (Pr 27.4 %) were detected in pigs of 6–9 months. Cryptosporidiosis + Balantidiosis and Isosporiasis + Balantidiosis affect pigs of 1–2 and 3–4 years. Due to higher immunity against coccidiosis, the disease prevalence in the animals of aged group was lower than that in young animals.

We consider that high prevalence of mixed infestations in animals of six days and from one to nine months is associated with high animal density in pigsties, failure to comply with veterinary standards and lack of immunity against these parasitoses. Epizootic situation in the collective farm named after Nikitin in Ivanovka village of Ismayilli district differs from the situation in the individual farm economies. Keeping pigs clean in small groups, temporary cleaning and washing the pig flop and place, periodic disinfection of pens, and automation of technological processes results in low prevalence of infestation. *Balantidium* cysts and coccid oocysts were one by one found as a form of mixed infestation during faecal examination of the materials taken from the animals in this farm. An average prevalence of infestation in Ismayilli District averaged 0.1–2.14 % (Table 1).

The study of seasonal dynamics of various mixed invasions revealed two peaks, in spring and autumn.

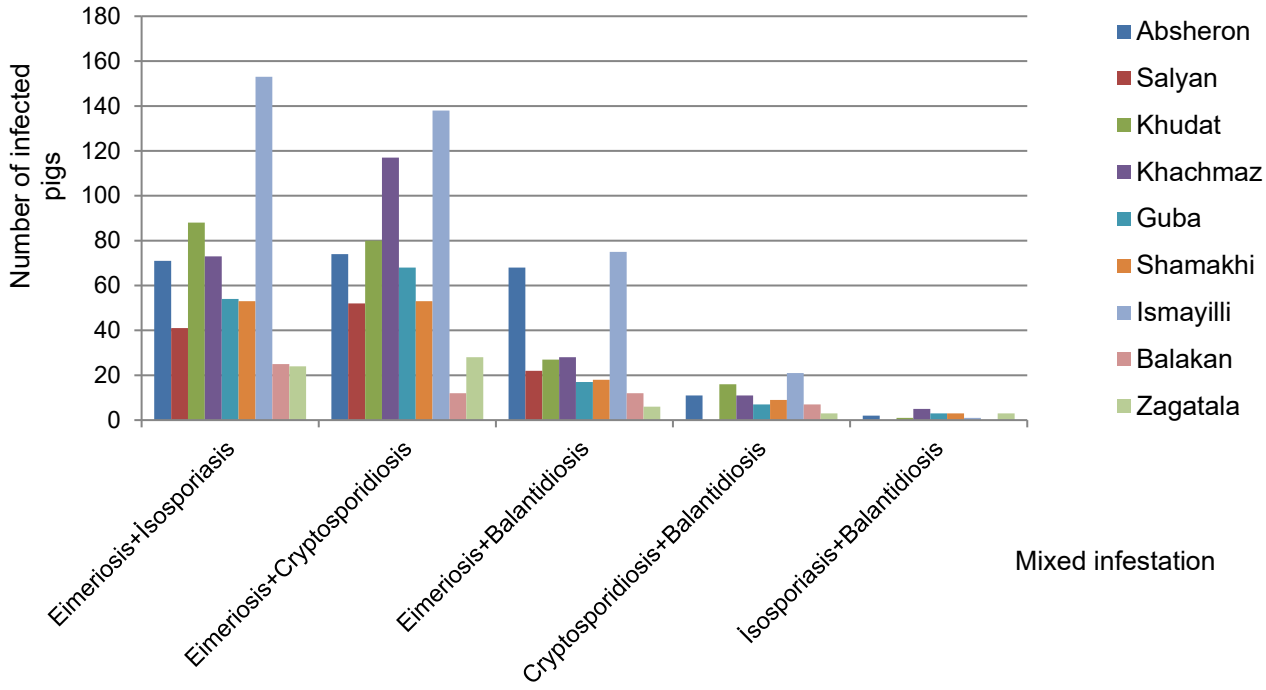

Fig. 2. Occurrence of domestic pig infections in the studied districts of Azerbaijan

A complete analysis of faecal samples taken all year round showed that the two-component mixed infestations (Eimeriosis + Cryptosporidiosis and Eimeriosis + Isosporiasis) were recorded during the whole year, while the prevalance of Eimeriosis + Balantidiosis and Isosporiasis + Balantidiosis was lower in summer. *Eimeria* + *Isospora* and *Eimeria* + *Cryptosporidium* oocysts were found in mixed form during the whole year. The highest prevalence of Eimeriosis + Balantidiosis was registered in Salyan District (22.7 %) and on the Absheron peninsula (17.9 %), the lowest ones were recorded from the districts of Guba (7.6 %) and Zagatala (7.3 %). Invasion of *Cryptosporidium* + *Balantidium* was not registered in Salyan District; the highest prevalence of this invasion was recorded from Khudat District (7.1 %), and the lowest one, from Ismayilli District (2.9 %) (Fig. 2).

A three-component mixed infestation (Eimeriosis + Isosporiasis + Cryptosporidiosis) was found in spring in Ismayilli district. A four-component mixed infestation (*Eimeria* + *Isospora* + *Cryptosporidium* + *Balantidium*) was not detected in the examined pigs. In addition to intestinal protozoan parasites, we found eggs of *Ascarissuum spp*. (Nematoda) in aged (geriatric) mother pigs.

According to literature data, parasitic diseases are detected in all regions where people keep domestic pigs. The most spread are parasitic protozoans, helminths and mixed infestations. For instance, the economies of 14 cities and suburbs were investigated in Kenya (Eastern Africa). In the cities, the disease prevalence was as follows: Strongylid nematodi (11.7 %), *Ascarissuum* spp. (1.8 %), *Entamoeba* ssp (27.2 %), *Balantidium coli* (3.8 %) and Coccidia spp. (3.3 %); in the suburbs, it was rather different: Strongylid nematodi (9.7 %), *Ascarissuum* spp. (12.9 %), *Entamoeba* spp. (51.6 %), *Balantidium coli* (20.6 %), and *Coccidia* spp. (5.5 %) (Mhoma et al., 2011). In Russia (the City of Ryazan, 2013), the prevalance of Ascaridosis + Balantidiosis was 41 %, Ascaridosis + Oesophagostomosis – 21.5 %, Oesophagostomosis + Balantidiosis – 23.5 %, Nematodiasis + Balantidiosis + Isosporiasis – 9–12 % (Yengashev et al., 2014).

In Azerbaijan, mixed invasions were studied in various domestic animals (Musaev et al., 1985; Iskenderova, 2007). For example, in the poultry (goose, duck, quail and turkey) investigated in several districts in 2002–2010, the highest prevalence was registered in quails and the lowest in turkey (Gasanova, 2012). The prevalence of *Eimeriosis + Cryptosporidiosis* in cattle in the Balakan-Shaki region in 2009–2011 was 14.3 % (Gaibova et al., 2011).

Epizootic situations of parasitic diseases in the pig breeding farms vary depending on the technologies used. The prevalence of various parasitoses is higher in the animals under traditional treatment if they are kept in pens with wooden floor not cleaned in a proper way. Monoinfections complicated with mixed invasions thrive in such farm economies that causes severe economic losses.

We consider that keeping old (geriatric) and young pigs together in farm economies promotes occurrence of associative invasions. Being the source of infection in latent period, these animals play an important role in spreading pathogens of various diseases. Untimely cleaning and disinfection results in activation the pathogenic forms (appearance of infective stages of parasites) that, in turn, increases the prevalence and infection intensity of both mono and mixed invasions. Therefore, keeping conditions impacts dramatically on the epizootic state of protozooses.

Conclusion

Our research has showed that mixed infestation of domestic pigs at private farms and pig breeding complexes in the study area of Azerbaijan is high. Eimeriosis + Cryptosporidiosis (27.4 % of the total cases studied) is prevalent; the disease peaks in spring and autumn.

References

Anisimova M.A. (2013). Mixed infestation of pigs (features of epizootology, complex treatment and prophylactic measures). Materials of the scientific conference "*Theory and Practice of the Control of Parasitic Diseases*". Moscow. Issue 14. P. 23–26. (in Russian)

Gaibova G.D., Iskenderova N.G., Hajiyeva N.A. et al. (2011). Intestinal coccidians of cattle in the Balakan-Sheki region of Azerbaijan. *Actual problems of parasitology in Georgia*. Tbilisi. P. 84–88. (in Russian)

Gasanova J.V. (2012). Parasitic protozoa (Protozoa, Apicomplexa) of poultry in Absheron. Thesis for the Degree of Candidate of Biological Sciences. Baku. P. 78–80. (in Russian)

Gavrilova N.A., Petrova M.S. (2015). Mixed infestation of pigs in industrial farms. Materials of the II International Veterinary Congress “International VET Istanbul Group Congress”. St. Petersburg. P. 333–334. (in Russian)

Henriksen S.A., Pohlenz J.F. (1981). Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta veterinaria Scandinavica*, *22*(3–4), 594–596.

Iskenderova N.G. (2007). Emeriid coccidians (Sporozoa, Apicomplexa) of farm animals in Azerbaijan. *Proceedings of Azerbaijan National Academy of Sciences (Biological and Medical Sciences), 2*, 81–91. (in Russian)

Kulikova O.L. (2010). *Mono- and mixed parasitoses of animals in the Middle and Lower Volga region and their biological hazard (epizootological monitoring and control measures).* Abstract of the thesis for the Degree of the Doctor of Vet. Sciences. Nizhny Novgorod. 51 p. (in Russian)
Lakin G.F. (1990). Biometry. Moscow: Vysshaya Shkola. P. 178–182. (in Russian)
Maikai B.V., Umoh J.U., Kwaga J.K. et al. (2009). Prevalence and risk factors associated with faecal shedding of *Cryptosporidium* oocysts in piglets, Kaduna. *Nigeria Journal of Parasitology and Vector Biology, 1*(1), 1–4.
Mhoma J.R.L., Kanyari P.W.N., Kagira J.M. (2011). The prevalence of gastro-intestinal parasites in goats in urban and periurban areas of Mwanza City, Tanzania. *Sci. Parazitol., 12*(4), 191–196.
Musaev M.A., Alieva F.K., Abidova H.S., Borovskikh D.R. (1985). Coccidia (Apicomplexa: Eimeridae, Sarcocystidae) of pigs in Azerbaijan. *Proceedings of the Transcaucasian Conference on Parasitology* (12–14 June 1984). Tbilisi, Metzniereba. P. 69–70. (in Russian)
Ponomarev N.M., Tikhaya N.V., Ponomarev A.N. (2011). Epizootology of mixed infestation of pigs in the farms of the Altai Territory. *Bulletin of the Altai State Agrarian University, 7*(81), 71–75. (in Russian)
Safiullin R.T., Basynin S.E. (2008). Monitoring of the epizootic situation of the most common pig parasitic diseases in farms of various types by zones of the country. Materials of reports of the scientific conference *"Theory and Practice of the Control of Parasitic Diseases"*, Moscow. P. 411–415. (in Russian)
Yengashev S.V., Daugalieva E.H., Novak M.D., Anisimova M.A. (2014). Methodical guidelines for treatment and prevention of mixed infections in pigs in commercial, individual and husbandry farms. *Russian Journal of Parasitology, 2*, 121–125. (in Russian)

Асоціація *Balantidium coli* і кишкових кокцидій (*Eimeria, Cryptosporidium, Isospora*) у свійських свиней в Азербайджані

Н.А. Гаджиєва

Мета дослідження – визначення картини поширення асоціативних інвазій у свійських свиней у фермерських господарствах. Дослідження проводилися в 2009–2015 рр. Були зібрані і проаналізовані зразки калу свійських свиней (2272 голів) різного віку в приватних фермерських господарствах у Балакененському, Загатальському, Шамахінському, Ісмаїлінському, Губинському, Хачмазькому, Худатському, Сальянському районах і на Апшеронському півострові, що входять до складу Азербайджану. Видовий склад, ступінь поширення у свиней та екологічні особливості паразитичних найпростіших на цій території раніше вивчені були. Виявлено, що асоціація інвазивності у свійських свиней у зазначених районах така: ооцисти *Eimeria* і *Isospora* виявлені у 582 свиней (25,6 %), ооцисти *Eimeria* і *Cryptosporidium* у 622 свиней (27,4 %), ооцисти *Eimeria* і цисти *Balantidium* у 273 свиней (12,0 %), ооцисти *Isospora* і *Balantidium* у 18 свиней (0,8 %), ооцисти *Eimeria*, *Isospora* і *Cryptosporidium* у 0,04 % свиней. З проаналізованих зразків калу жоден не містив 4-компонентну мікст-інвазію. Двокомпонентні мікст-інвазії Eimeriosis + Cryptosporidiosis і Eimeriosis + Isosporiasis відзначалися протягом усього року, рівень асоціативних інвазій Eimeriosis + Balantidiosis і Isosporiasis + Balantidiosis був нижче влітку. Утримання свиней різного віку в одному приміщенні на фермах сприяє їх зараженню різними видами паразитів, що призводить до появи асоціативних інвазій. У свинарських фермах, які оснащені різними технологічними пристосуваннями, епізоотичні ситуації паразитарних захворювань будуть різні. Таким чином, серед тварин, які утримуються традиційним чином, зараженість набагато вище. У тварин, які утримуються в непровітрюваних приміщеннях з дерев'яною підлогою, екстенсивність мікст-інвазій найбільш висока, і економічний збиток на таких фермах великий. У тварин, які утримуються в теплих приміщеннях з високою вологістю, навіть взимку екстенсивність інвазії буває високою, оскільки тепло і вологість створюють сприятливі умови для екзогенного розвитку ооцист кокцидій.

Ключові слова: *еймерії, ізоспоридії, криптоспоридії, балантидії, свійські свині, моноінвазія, асоціативна інвазія, Азербайджан.*

Про автора:
Н.А. Гаджиєва – Інститут зоології НАН Азербайджану, вул. А. Аббасзаде, проїзд 1128, квартал 504, Баку, Азербайджан, AZ1073, nuruhaciyeva@gmail.com, https://orcid.org/0000-0002-2500-5073

Ассоциация *Balantidium coli* и кишечных кокцидий (*Eimeria, Cryptosporidium, Isospora*) у домашних свиней в Азербайджане

Н.А. Гаджиева

Цель исследования – определение картины распространения ассоциативных инвазий у домашних свиней в фермерских хозяйствах. Исследования проводились в 2009–2015 гг. Были собраны и проанализированы образцы кала домашних свиней (2272 голов) разного возраста в частных фермерских хозяйствах в Балакененском, Загатальском, Шамахинском, Исмаиллинском, Губинском, Хачмазском, Худатском, Сальянском районах и на Апшеронском полуострове, входящих в состав Азербайджана. Видовой состав, степень распространения у свиней и экологические особенности паразитических простейших на этой территории ранее изучены не были. Обнаружено, что ассоциация инвазивности у домашних свиней в указанных районах такова: ооцисты *Eimeria* и *Isospora* обнаружены у 582 свиней (25,6 %), ооцисты *Eimeria* и *Cryptosporidium* у 622 свиней (27,4 %), ооцисты *Eimeria* и цисты *Balantidium* у 273 свиней (12,0 %), ооцисты *Isospora* и *Balantidium* у 18 свиней (0,8 %), ооцисты *Eimeria*, *Isospora* и *Cryptosporidium* у 0,04 % свиней. Из проанализированных образцов кала ни один не содержал 4-компонентную микст-инвазию. Двухкомпонентные микст-инвазии Eimeriosis + Cryptosporidiosis и Eimeriosis + Isosporiasis отмечались на протяжении всего года, уровень ассоциативных инвазий Eimeriosis + Balantidiosis и Isosporiasis + Balantidiosis был ниже летом. Содержание свиней разного возраста в одном помещении на фермах способствует их заражению различными видами паразитов, что приводит к появлению ассоциативных инвазий. В свиноводческих фермах, которые оснащены различными технологическими приспособлениями, эпизоотические ситуации паразитарных заболеваний будут разные. Таким образом, среди животных, которые содержатся традиционным образом, зараженность намного выше. У животных, которые содержатся в непроветриваемых помещениях с деревянным полом, экстенсивность микст-инвазий наиболее высокая, и экономический ущерб на таких фермах велик. У животных, которые содержатся в теплых помещениях с высокой влажностью, даже зимой экстенсивность инвазии бывает высокая, так как тепло и влажность создают благоприятные условия для экзогенного развития ооцист кокцидий.

Ключевые слова*: эймерии, изоспоридии, криптоспоридии, балантидии, домашние свиньи, моноинвазия, ассоциативная инвазия, Азербайджан.*

Об авторе:
Н.А. Гаджиева – Институт зоологии НАН Азербайджана, ул. А. Аббасзаде, проезд 1128, квартал 504, Баку, Азербайджан, AZ1073, nuruhaciyeva@gmail.com, https://orcid.org/0000-0002-2500-5073

Подано до редакції / Received: 21.12.2020
Прорецензовано / Revised: 09.04.2021
Прийнято до друку / Accepted: 11.05.2021