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## Actualities in animal parasitosis research: aspects of nanology and genomics

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**Abstract.** The report presents interdisciplinary aspects of nanology and genomics of interest in the knowledge of parasites in humans and animals. In nanology, electron microscopic technologies have succeeded in recent decades in discovering new species of protozoa parasites. At the same time by fine techniques the microstructures of some parasites were revealed, especially in sporozoa and flagelates such as *Toxoplasma*, *Sarcocystis*, *Babesia*, *Trichomonas* and others. Genomics research in parasitology favored the knowledge of nucleic acid sequences (DNA and RNA) and the establishment of genotypes of: *Giardia*, *Trichomonas*, *Cryptosporidium* and others. From some protozoa, such as *Babesia*, surface proteins of interest in the immunoprophylaxis of animals have been obtained. Interesting results have also been obtained in apoptotic processes, some parasites are activating or inactivating some effects in the host cells.

**Keywords:** Parasitosis; Electron microscopic structures; Bionic; Genomic; Protozoa; Apoptosis.

### Actualități în cercetarea parazitozelor la animale: aspecte de nanologie și genomică

**Rezumat.** Sunt prezentate aspecte interdisciplinare – de nanologie și genomică – de interes în cunoașterea parazitozelor la om și animale. În nanologie, prin utilizarea tehnologiilor electronomicroscopice s-a reușit – în ultimele decenii – descoperirea de noi specii de protozoare parazite. Totodată – prin tehnici de finețe – s-au evidențiat microstructurile celulare la unii paraziți, în special la sporozoa și flagelate precum *Toxoplasma*, *Sarcocystis*, *Babesia*, *Trichomonas* ș.a. Cercetările de genomică în parazitologie au favorizat cunoașterea secvențelor acizilor nucleici (ADN și ARN) și stabilirea unor genotipuri de: *Giardia*, *Trichomonas*, *Cryptosporidium* ș.a. De la unele protozoare, precum *Babesia*, s-au obținut proteine de suprafață, de interes în imunoprofilaxia animalelor. Rezultate interesante s-au obținut și în procesul apoptozei, unii paraziți având efecte activante sau inactivante ale celulelor gazdă.

**Cuvinte cheie:** Parazitoze și structuri electronomicroscopice; Bionica, genomics și apoptoza la protozoare parazite.

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## Introduction

Scientific research over the last three decades used electron microscopy and immunoenzymatic techniques to identify different species of parasites. These techniques finally lead to the discovery of new parasitic species and to future advances in the knowledge of ultra microscopic structures (Constantin, 2014; Cosoroabă et al., 2002; Dărăbuș et al., 2006; Mitrea, 2002; Niculescu, 1998).

## Microtechnologies

The use of fine microtechnologies in nanology has facilitated the identification of new parasite (protozoa) species. Therefore, Dubey et al. (2003) diagnosed a dermatitis produced by a *Toxoplasma*-like sporozoite in dogs, which was named "*Toxoplasma gondii*-like" organism. In the same year, Haves et al. identified in preputial secretion from bulls different *Trichomonas* species than *Trichomonas foetus* by using molecular biology. Moreover, a recent study on hemosporidiosis identified a *Babesia* spp. different than *Babesia divergens* which is considered to have a zoonotic character in Europe (Herwaldt et al., 2003). The development of nanology and the use of high-tech methods in parasitology had led to exceptional results in the field of electron microscopic structures. Electron microscopic modifications induced by parasites helped to differentiate parasitic ecotypes (Dărăbuș et al., 2006; Dubey et al., 1988; Herwaldt et al., 2003; Șuteu, 2017). Dubey et al. (2003) revealed that "*Toxoplasma*-like" parasite differs structurally from *T. gondii* and *Neospora canis*. Electron microscopically, the merozoites of this new species exhibit a larger number of spikes in the apical pole. Merozoites have colloidal rings and their mitochondria are different as well. Dubey et al. (1998) have established, experimentally, the ultrastructures of the genus *Sarcocystis* and then established the differences between *Sarcocystis hirsuta* and *S. hominis*. In our country, the research conducted by Turcu et al. (1992) established the microstructures of *Sarcocystis capracanis*. They have revealed that merozoites have elongated rhizomes up to the middle third of the cell and some posterior vesicles were also observable. Cozma et al. (1992) established in lambs with eimeriosis, the microstructures of merozoites

and microgametocytes. In gastric eimeriosis in sheep, merozoites have only a few rhizomes. These rhizomes have in the middle several electron dense vesicles (Șuteu et al., 1992). Herwaldt et al. (2003) found molecular characteristics of *Babesia divergens* in other species of *Babesia*. The trophozoites are equipped with several anterior polar rings but also with two posterior rings. Research on parasitic protozoa revealed that *Trichomonas foetus* has flagella that contains two central fibers, that are surrounded by double peripheral fibers. The previous structures are wrapped in an external layer. The proximal part of the undulating membrane is cytoplasmic, and the distal part contains the axonema. These microstructures differentiate *Trichomonas foetus* from other *Trichomonas* species in cattle (Haves et al., 2003). Advanced techniques (ELISA, PCR) in nanology has opened up new ways of identifying parasites, especially protozoa. Thus, new parasitic species have been identified, based on microcellular aspects (Dubey et al., 2003).

## Parasitic genomics

Research in parasitic genomics regarding diagnostic technologies and genetic issues have also been addressed since 1994 by Dobson and Garnham. More recently, Dominguez et al. (2011) identified various histones in *Babesia bovis* and predicted their role in genetic regulation. Applied micro technologies helped to establish genes and nucleic acid sequences in different parasite species. Several species of parasites because of their genes can influence the internal processes of neighbouring cells. In parasitology we are interested in genes that are involved in pathogenesis, adaptability and resistance of the parasite. These results helped to discover new methods to prevent parasitic infestations (Olteanu, 1999; Cosoroabă, 2000; Cosoroabă et al., 2003). In the study of Giardiasis in humans and animals, Abe et al. (2003) identified genotypes of *Giardia intestinalis* which were isolated from dogs by PCR methods. Papini et al. (2007) have genetically managed to identify *Giardia* spp. isolates from canines. The majority of the animals were asymptomatic but a few of them presented different symptoms of the disease. Regarding trichomoniasis in cattle, Haves et al. (2003) had distinguished different types of

Trichomonas with intestinal location which were different from those of *Trichomonas foetus*. Regarding sporozooses, Enemark et al. (2003) were able to establish genotypes of *Cryptosporidium parvum* in pigs. Elis et al. (2017) have shown the presence of *Hammondia heydorni* by oocyst PCR analysis. In immunology, Wilkowski et al. (2003) used MSA genes (which were obtained from *Babesia bovis* merozoites) to obtain a highly immunogenic (-2C) surface protein, from which a vaccine was produced. The administration of this vaccine in cattle caused the production of specific antibodies. The PCR method also determined that bovine *Cryptosporidium andersoni* had DNA sequences identical to those of *C. parvum* (Enemark et al., 2003). Experimentally a vaccine against *C. parvum* was produced (Hoong et al., 2017). This vaccine protected several pregnant goats from *C. parvum* infestation. Angus et al. (2000) used SAG1 protein from *Toxoplasma gondii* to immunize laboratory rodents. Afterwards these animals became resistant to *Toxoplasma gondii* infestations. In the case of ocular microsporiosis, Devis et al. (1980) managed to establish the ultrastructure of this sporozoa.

### Apoptosis

Several studies in the last few years focused on apoptosis, which is a programmed cell death, that can also be regulated by parasites, particularly protozoa. In human and animal infestations, parasites can inhibit or stimulate the apoptotic processes (Kroemer et al., 1997; Heussier et al., 2001). Luder et al. (2001), by following the complex mechanisms that appear in intracellular parasitism, have found that they can influence the host's immune mechanisms by either favoring their intracellular survival or by favoring their delivery through the host organism. In *Cryptosporidium parvum* infections, the modulation of apoptosis is varied, the parasite can inhibit intestinal cell apoptosis, favoring the survival of trophozoites. Non-infected mucosal cells are also affected by this process (Kroemer et al., 1997; Heussier et al., 2001; Enemark et al., 2003). *Trypanosoma brucei* infections sometimes can induce apoptosis in the cerebellum (Welburn et al., 1997; Stiles et al., 2001). Clinical, epidemiological and

molecular investigations in leishmaniosis helped the molecular genotyping of *Leishmania* species (Osman et al., 2011). Spickett and Malen (1978) found that there is a genetic incompatibility between *Boophilus decoloratus* from Australia and *Boophilus microplus* from Africa, and the hybrids of these become sterile in terms of fecundity. Stear et al. (1994) found that there is a genetic component regarding the hosts resistance to some parasitic infestations. Their observations revealed that some ruminants are resistant against some gastrointestinal nematodes.

We consider in this paper – that the progress in these interdisciplinary branches: nanology and genomics can generate access to scientific research projects of high relevance in our country.

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