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**The XXIIIth Congress
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ABSTRACTS

SESSION VII

Combating parasitic infections

How may we control the densities of the marsh tick *Dermacentor reticulatus*? The effect of agricultural practices on tick abundance.

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The importance of *Dermacentor reticulatus* stems from its main role in the transmission of *Babesia canis*, the agent of canine babesiosis. The geographical range of this tick species and consequently, of canine babesiosis, has increased alarmingly in Central Europe during the last decade. Among the many suspected reasons, changes in agricultural practices and the development of habitats favouring ticks (i.e. fallow lands, re-cultivated river valleys) are likely to have played a crucial role in the expansion of the range of *D. reticulatus*. The old Polish farming custom of burning old grasses/stubble in early spring is now strictly prohibited and almost eradicated. As the first peak of occurrence of marsh ticks is in early spring, this custom was likely to have played an important role in controlling tick populations in the past. Therefore, simple agricultural practices, such as the cutting and removal of grass, may constitute an easy, safe and relatively cheap measures for the control of marsh tick populations.

The aim of the present study was to compare tick densities in different habitats (pasture, fallow land, meadow (grass cut and removed 1–2 times a year), post-fire areas) to assess the efficiency of different agricultural practices for the control of tick densities in vicinities close to human habitation. Between September 2011 and June 2013, marsh ticks were collected by dragging in endemic areas of the Mazovia and Warmia-Mazuria regions. In each region, 3 study sites were selected situated near to a surface water source (i.e. pond or canal). At each site, three neighboring habitats of surface 300–600 m² were dragged: one on cattle/ horse pasture; the second on a meadow; the third on fallow land, at least twice during each spring and autumn (2 regions×3 sites×3 habitats =18). Additionally, in spring 2013, one post-fire area was discovered in the Mazovia province and flagging was conducted there twice, including – ‘a control area’– intact fallow land situated on the opposite side of a small river. Tick densities were calculated per 100m² and compared by a multifactorial ANOVA test. The highest tick densities were recorded on the fallow lands, and the lowest – on the meadows and pastures. Tick densities were considerably higher on the control site in comparison to the post-fire meadow. Some agricultural practices may help to control the tick densities close to human habitation.

The study was funded by the National Science Centre (NCN) grant OPUS 2011/03/B/NZ802212.

Biological interactions between soil saprotrophic fungi isolated from children's recreation areas and *Ascaris suum* eggs

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The biological control of parasitic nematodes by fungi is a promising field of application on a large scale. Soil saprotrophic fungi can exert ovicidal and ovistatic effects on geohelminth eggs.

The *in vitro* effect of six saprotrophic soil fungi (*Aspergillus fumigatus*, *A. terreus*, *Fusarium oxysporum*, *Penicillium citrinum*, *P. expansum* and *Trichothecium roseum*) on the embryonic development of *Ascaris suum* was evaluated. In another trial, the ovicidal activity of eight fungal strains (*Acremonium alabamense*, *Alternaria chlamydospora*, *Cladosporium herbarum*, *Fusarium solani*, *Penicillium verruculosum*, *Paecilomyces viridis*, *P. variotii* and *Trichophyton schoenleinii*) was determined. Fungal strains were isolated from children's recreation areas in the city of Lodz. The fungal mycelium was co-cultured with *A. suum* eggs, either in sterile water solution containing 0.05% formalin and antibiotics for 60 days or on plates with 2% agar-water for 28 days. Exposed eggs and unexposed (control) samples were observed weekly by light microscopy and the percentage of eggs with dead and malformed embryos or larvae in each sample were determined. The eggs were classified according to effects as a type: 1. biochemical and physiological effect without morphological damage to the egg shell; 2. a lytic effect with morphological alteration of the egg shell and embryo; and 3. a lytic effect with morphological alteration of egg shell and embryo with hyphal penetration and internal egg colonization.

All examined fungi extended embryogenesis, but the retardation of embryonic development was varied. Species: *A. terreus*, *P. expansum* and *F. oxysporum* exhibited very high inhibitory activity on *A. suum* egg development. Embryopathies and non-viable embryos/larvae were observed significantly more frequently in the eggs co-cultured with fungal species than in control cultures. The fungus-exposed eggs revealed morphological alternations in the early zygotic cleavage, blastula, gastrula and larval stages. *P. expansum*, *P. viridis*, *P. variotii*, *F. oxysporum* and *F. solani* showed hyphal penetration and internal egg colonization of *A. suum* eggs (type 3); *A. fumigatus*, *A. terreus*, *A. chlamydospora*, *A. alabamense*, *C. herbarum*, *T. schoenleinii*, *T. roseum* and *P. verruculosum* demonstrated morphological alternation eggs with destruction of *A. suum* egg shell (type 2). The examined fungi may be considered as potential limiting factors of parasitic geohelminth populations in the soil ecosystem.

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Plant extracts as amoebicidal drugs in acanthamebiasis

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The free-living amoebae from the genus *Acanthamoeba* are the causative agents of granulomatous amoebic encephalitis (GAE), a chronic progressive disease of the central nervous system; amoebic keratitis (AK), a chronic eye infection; amoebic pneumitis (AP), a chronic lung infection, and skin infection. Chemotherapy of *Acanthamoeba* infection is problematic. The majority of infections have been fatal. Only a few cases are reported to have been treated successfully with very highly toxic drugs. The therapy might be succeed, if the diagnosis and therapy is made at a very early stage of infection.

The aim of our study was to examine the ethanol plant extracts as potential therapeutical substances in the treatment of acanthamebiasis. Effect of medicinal substances (extracts) from plants were tested *in vitro* on *Acanthamoeba castellanii* – strain 309. In our experiments, the following plant extracts were used: *Centaurea bella*, *Centaurea dagestonica*, *Rhaponticum pulchrum* and *Tanacetum vulgare*. These therapeutic agents and plants extracts have been tested *in vitro* for amoebicidal or amoebostatic activity against pathogenic *Acanthamoeba* spp. The amoebae strains were axenically cultured. The plant extracts were dissolved in ethyl alcohol. Plant extracts were added to the axenic culture of amoebae containing 5×10^4 cells/ml at the following concentrations: 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml and 5 mg/ml. The increase or decrease in the number of amoebae was checked at 24 hour intervals during three days in a Thoma haemocytometer chamber. The control group was a culture of amoebae without extracts. Our results showed that methanol extracts obtained from *Centaurea bella*, *Centaurea dagestonica* and *Tanacetum vulgare* are active against axenic trophozoites of *Acanthamoeba* spp. *in vitro* in all tested concentrations. The extract of *Rhaponticum pulchrum* did not show amoebicidal or amoebostatic activity. Based on our results, we may confirm that the three of the tested ethanol extracts have therapeutic properties against pathogenic strains of *Acanthamoeba* spp.

Plant extracts can be used in combination with antibiotics in the therapy of acanthamebiasis.

In-vitro* effect of ivermectin on the expression of the trehalose metabolism genes of adult female *Ascaris suum

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Trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is a non-reducing disaccharide which plays a role in a variety of key functions in nematodes. In trehalose synthesis two enzymes are involved: trehalose 6-phosphate synthase (TPS: EC2.4.1.15) and trehalose 6-phosphate phosphatase (TPP: EC3.1.3.12). In *A. suum* the former is encoded by two genes *tps1* (JF412033.2) and *tps2* (JF412034.2), the latter by gene *tp* (JI169681.1).

It seems very interesting to determine how a well-known anti-parasitic drug ivermectin affects the metabolism of trehalose in helminths. Therefore, the aim of study was to develop a real-time quantitative PCR (qPCR) method to determine the effect of ivermectin on the expression of genes encoding enzymes involved in trehalose synthesis in muscles of parasites. In addition, the effect of ivermectin on the activity of T6P synthase and phosphatase was examined. In the present study adult females of *A. suum* were cultured in the ARS medium (control group) and in the medium supplemented with 11.44 μM ivermectin (study group). Samples were taken after 4, 8 and 20 hours of incubation.

As a result of the experiment complete lack of movement in the group supplemented with ivermectin was observed after 20 hours of incubation, while the nematodes in the control group retained full mobility. This confirmed the influence of ivermectin on the neuromuscular system of the parasite. It has been shown that ivermectin has an effect on reducing the activity of both examined enzymes after 4 hours of incubation. Subsequently, the enzymes activity increased (especially for TPS). After 20 hours of incubation it persisted at the same level as in the control group. We concluded that ivermectin had the greatest negative impact on the activity of enzymes of trehalose synthesis in the first 4 hours after dosing. A more pronounced effect of ivermectin was observed in the case of expression of trehalose metabolism genes. A gradual decrease in expression of all three genes in the group supplemented with ivermectin was demonstrated. We may conclude that, although ivermectin affects the synthesis of trehalose, it does not block it entirely.

Polyinosinic-polycytidylic acid (Poly I:C) as an effective adjuvant enhancing the immunogenic and protective efficacy of trivalent subunit rROP2+rROP4+rSAG1 anti-*Toxoplasma* vaccine

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Utilization of adjuvants, the immune modulators that boost the potency and longevity of specific immune responses to delivered antigens, is a fundamental strategy for optimal vaccine development. The discovery of Toll-like receptors (TLRs), a family of evolutionary conserved pattern recognition receptors, led to the identification of TLR agonists, a new group of molecules with adjuvant properties that stimulate innate and adoptive immunity by triggering TLRs. Polyinosinic-polycytidylic acid (Poly I:C), a member of TLR agonists family, is a synthetic analog of viral double-stranded RNA that is recognized by two distinct pattern recognition receptors, endosomal TLR3 and cytoplasmic MDA-5 (Melanoma Differentiation – Associated Protein 5), and is considered a potent stimulator of Th1 biased immunity with proved adjuvant activity.

In the presented studies, we estimated the immunogenic and protective efficacy of recombinant, subunit rROP2+rROP4+rSAG1 anti-*Toxoplasma* vaccine combined with Poly I:C as an adjuvant. Immunization of BALB/c mice with the tested antigen cocktail revealed that replacing the Freund's adjuvant with Poly I:C provided a strong increase of cellular immune response manifested as a significantly higher level of the antigen-specific *in vitro* production of Th1-type cytokines, IFN- γ and IL-2. The performed immunization strategy also led to the development of humoral immunity displayed as a significant synthesis of the specific, systemic IgG antibodies and *in vitro* production of IL-10, the Th2-type cytokine. Additionally, vaccination of BALB/c mice with rROP2+rROP4+rSAG1 trivalent vaccine combined with Poly I:C partially protected the laboratory animals against a lethal challenge with the tachyzoites of *T. gondii* RH strain by prolonging the survival time of the antigens plus adjuvant-immunized animals.

Since the research on the tested vaccine is still ongoing, detailed final results and conclusions will be presented at the conference.

The protective and immunogenic activity of recombinant *Toxoplasma gondii* antigens co-administered with MPL and alhydrogel in murine experimental toxoplasmosis

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Toxoplasmosis is one of the most common parasitoses worldwide, affecting not only humans but also a wide range of warm-blooded animals. A *T. gondii* invasion may prove life threatening for immunocompromised individuals, however, a body of literature data suggests that it might also be associated with some serious disorders of the central nervous system in immunocompetent hosts. Moreover, parasite infection is of great economic importance due to livestock losses. In that respect the development of effective immunoprophylactic measures is a necessity and recombinant antigens co-administered with safe and efficient adjuvants offer a useful solution.

Hence, using a murine experimental toxoplasmosis model, we tested the protective and immunogenic activity of selected *T. gondii* antigens ROP2, ROP4, SAG1 and MAG1 combined with MPL and alhydrogel as adjuvants. The first step was to select the vaccine composition providing the highest protection against cyst formation in the brain. Tests performed on C3H/HeJ males showed that of two proposed antigenic combinations, ROP2+ROP4+SAG1 and ROP2+ROP4+SAG1+MAG1, the latter proved more effective (72% compared to 65%) and was chosen for further evaluation. Consecutive studies revealed the protective activity of the vaccine to be 58% and 65% on C3H/HeJ and BALB/c females, respectively.

The results of further research on immunoproliferative response to vaccination will be presented and discussed during the conference.

The study was supported by the Polish Ministry of Science and Higher Education / National Science Centre (grant N N302 636340).

***Toxoplasma gondii* recombinant ROP18 proteins – cloning and expression**

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Toxoplasma gondii is an important opportunistic parasite that infects almost all warm-blooded animals and humans. The high prevalence of toxoplasmosis, as well as the severe congenital, neurological or ocular disease occurring in immunocompromised individuals, clearly indicate the need for the development of a vaccine. The pivotal event in the host cell invasion by *T. gondii* is a discharge of apical organelles – micronemes and rhoptries. Some rhoptry proteins secreted by the parasite are targeted into the host cell nucleus, while the others associate with membrane of the parasitophorous vacuole (PV). Rhoptry protein 18, a member of ROP2-like family, is currently recognized as one of the key factors of *T. gondii*. Following the parasite invasion, the ROP18 protein is secreted to the host cytoplasm, localizes to the membrane of PV and functions as catalytically active. It has been revealed that ROP18 could downregulate the innate and adaptive immune responses, for instance by targeting the host's endoplasmic reticulum-bound transcription factor ATF6 β .

In this study we cloned and expressed three recombinant forms of ROP18 protein to be used as candidates for vaccine development. The virulent RH strain of *T. gondii* was used for genomic DNA isolation. The PCR-amplified gene fragments corresponding to whole ROP18 protein (1-554 aa), N-terminal ROP18 protein fragment without predicted signal sequence (98-335 aa) and C-terminal ROP18 protein (316-554 aa) were introduced into the pHis.Parallel1 vector and expressed in *E. coli* BL21(DE3) cells in the presence of IPTG as an inductor. The recombinant proteins were found in inclusion bodies, which were dissolved under denaturing conditions using urea and guanidine hydrochloride. The proteins were then purified by affinity chromatography and analyzed by SDS-PAGE and Western blot. In spite of differences in expression level, the recombinant forms of ROP18 protein have been successfully biosynthesized and will be used in further animal studies. This will shed light on the immunogenic potency of the ROP18 antigen that is considered as a promising vaccine candidate.

The effect of saprotrophic fungi on the development and hatching of *Fasciola hepatica* eggs

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Abiotic factors in the ecosystem determine the occurrence of mutually-interacting organisms. Such a dynamic system includes a number of inter-species interactions which determine the number and vitality of species. The populations of organisms, including helminth parasites of humans and animals, may be reduced via various forms of antagonisms, for example those involving micro-fungi.

The aim of this study was to determine the effect of common species of soil fungi, *Alternaria alternata* (Fr.) Keissl., *Aspergillus candidus* Link, *Penicillium chrysogenum* Thom, *P. commune* Thom, *Trichothecium roseum* (Pers.) Link and *Ulocladium* sp., on the hatching of miracidia – the free-living larvae of the liver fluke (*Fasciola hepatica*). To this end, the eggs of *F. hepatica* were incubated in water in the presence of one of the aforementioned fungus species and in tap water (control) at a temperature of 26°C. On the 15th day of incubation, the number of nonembryonated, embryonated and hatched eggs was determined. In order to determine the enzymatic activity of the fungi strains of the *Penicillium* genus, the most common in the environment were selected according to our own studies: *P. chrysogenum* (R-3; R-73) and *P. commune* (R-5). The API-ZYM[®] test (bioMérieux, Lyon, France) was used to determine semi-quantitatively the activity of 19 hydrolytic enzymes.

The tested fungal strains were observed to have varying degrees of antagonistic influence on the development of *F. hepatica* eggs. Among the examined fungi, the strongest ovistatic effects were exhibited by *Trichothecium roseum*, *Penicillium chrysogenum* (R-3) and *P. commune* (R-5). The results indicate that the most common *Penicillium* species are capable of synthesizing hydrolytic enzymes. The research did not reveal any morphological damage to the egg shells of *F. hepatica*, which may suggest a biochemical background of the observed antagonistic effect.

***In vitro* effect of antiparasitic drugs on the expression of the trehalose metabolism genes of L3 larvae of *Anisakis simplex* (Nematoda)**

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Anisakis simplex is a gastrointestinal parasitic nematode with a complex life cycle; the definitive hosts are marine mammals as well as humans. As anisakiasis is a serious condition that can be fatal to humans and may cause allergic reaction in patients sensitive to *A. simplex* allergens, it is necessary to intensify molecular biology research on the parasite's larvae. Trehalose acts as energy reserve and a protectant under stress conditions as an important saccharide in the metabolism of parasites. The synthesis of trehalose in nematodes follows a two-step pathway. The first step is catalyzed by trehalose 6-phosphate synthase – TPS (EC 2.4.1.15), the second by trehalose 6-phosphate phosphatase – TPP (EC3.1.3.12). Trehalose synthesis gene expression was examined basing on previously obtained partial mRNA sequence of L3 larvae of *A. simplex*, *tps-1* gene with the length of 268 bp and 194 bp of *tpp* gene.

In the present research we decided to determine *in vitro* (Iglesias et al., 1997) the effect of ivermectin, levamisole and combination of these drugs (10; 100; 1000; 5000 µg/ml) on the expression of mRNA and activity of enzymes TPS and TPP involved in trehalose synthesis in invasive L3 larvae of *A. simplex* after 48 and 96 hours. After 48h nematodes were placed in media containing no medication or with drugs. Isolation of total RNA was performed using total RNA mini kit according to the manufacturer's instructions (A&A Biotechnology), RT PCR (cDNA synthesis or Reverse Transcription PCR) by TranScriba kit (A&A Biotechnology). Quantitative real-time PCR was performed using the SYBRGreen PCR-MIX™ Taq (A&A Biotechnology). All samples were tested in triplicate on LightCycler (Applied Biosystems, FAST 7500). Melting curves were constructed after amplification. The data were analyzed and normalized relative to *Ryb* (an endogenous control gene) at transcript level by an AB analysis software (7500v2.0). The mean value ± SD was used for analysis of relative transcript levels for each time point using the $2^{-\Delta\Delta C_t}$ method. Activity of TPS was determined using the method by Giaever et al. (1988), the activity of TPP by Kaasen et al. (1992). The reaction product – trehalose was determined using HPLC. A reduction in mRNA expression of *tpp* gene of L3 larvae during incubation with ivermectin and levamisole was observed with increasing concentration of drug, while the expression of *tps* only in control group after 96h. The expression of *tpp* after 96h in the media with both drugs increased relative to the starting sample except concentration 10 ivermectin and 5000 levamisole. The effect of combination of drugs (10ng/ml) induced a reduction in *tps* expression after 48 hours. The activity of TPP decreased with increasing concentration of drugs after 48 hours, while the activity of TPS only with the addition of ivermectin.

A case of *trichostrongylosis* and *capillariosis* in a geese farm

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Among the roundworm infections in water and digging birds a common nematode is *Trichostrongylus tenuis* of the genus *Trichostrongylus*. It locates itself in the blind gut and sometimes also in the small intestine. The females reach the length of 6.5–11 mm. The clinical symptoms are similar to amidostomosis.

In the flock of White Koluda oat geese (W 33 and W 11) in late November–December the signs of weakness, lethargy, loss of appetite, staggering gait and drooping wings, diarrhea and so called “rowing wings” were observed, as well as the increase in the number of death falls. Total, 1000 one-year-old geese (reared for laying) were held at closed space on litter, and 700 older laying geese were kept at a separate facility with free access to the pond. Samples of birds droppings were examined by Fülleborn’s flotation technique with saturated NaCl solution and by decantation method. Dead gees were necropsied.

Double-repeated (in the interval of 7 days) coproscopic examination showed single eggs of *Capillaria* spp. In necropsy erythema and congestion of caecal and small intestinal mucosa were found. Mucosa was swollen, covered with a large amount of mucus, under which a number of nematodes was found. The mesentery was hemorrhagic. On the basis of observed symptoms, levamisole solution (Levamisole 10%) was administered in the drinking water at a dose of 30 mg/kg. Due to the lack of improvement in the health of birds and re-detection of single eggs of *Capillaria* spp. after 14 days the treatment was repeated using fenbendazole (Fenbenat 4%) at a dose of 5 mg/kg mixed with food. The morphological studies obtained at necropsy showed *Trichostrongylus tenuis* infection. The geese were treated with albendazole (Vermidan 10%), at a dose of 13.5 mg/kg in drinking water, after which the symptoms disappeared. Another double-up examination of fecal samples did not reveal the presence of *T. tenuis* eggs. Death falls disappeared, and the health of other birds has improved. Necropsy findings confirm the diagnosis in mixed nematode infections in geese and allow to determine the parasite species. The application of albendazole (benzimidazole carbamate) is effective in the control of *T. tenuis*.

The impact of mineral licks containing herbal extracts with antiparasitic properties on the level of infection in sheep during grazing season

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The occurrence of coccidia (in herds from confinement systems), and gastrointestinal helminths (while raised on pasture) decreases the productivity of sheep, also in the case of subclinical course of parasitoses. Presently, owing mainly to the occurrence of drug resistance in parasites and observed rapid development of organic farms, alternative methods of parasite control are being developed. One of these methods is the use of plants with antiparasitic properties.

The aim of this field study was to determine the effect of application of commercially available licks with herbal extracts acting against parasites on the level and dynamics of infection in sheep. The study was conducted during the 2012 grazing season in the Experimental Station of the Department of Swine and Small Ruminants Breeding of the University of Agriculture in Cracow. Grazing was carried out in a quartered system from May to October, and lambs grazed together with their mothers. Prior to grazing mothers were dewormed using Ivomec (ivermectin) and lambs received Systamex (oxfendasole). Subsequently, the animals were randomly assigned to two separately kept groups - experimental and control ones, with 25 mothers and lambs in each. The animals of the experimental group received the Star Bloc Phyto Vers lick produced by Guyokrma Ltd. containing extracts of 10 plants with antiparasitic activity, while the control animals got mineral lick without the addition of herbs. The two groups of sheep were not given any antiparasitic drugs during the experiment. Faecal samples were monthly collected *per rectum* from mothers and lambs for McMaster analyses, and at the end of the grazing season diagnostic sections of digestive tracts of three sheep were performed. Coproscopically, differences in the infection level with gastrointestinal nematodes, coccidia of the genus *Eimeria*, or tapeworms *Moniezia* sp. demonstrated between the two groups of animals were not clear. However, post-mortem, a highly pathogenic *Haemonchus contortus* was present in much greater number in the sheep from the control group compared to two sheep from the experimental one.

Alveolar echinococcosis in Poland. What next?

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In January 2013, a final report on alveolar echinococcosis (AE) in Poland in the years 1990–2011 was published (Nahorski et al., 2013). Parts of it have already been reported at the XXII Congress of the Polish Parasitological Society (Knap et al., 2010). Now the question arises about the future of AE control and medical care of AE cases in Poland. It may be expected that in the coming years, the number of AE cases will increase in Poland. Recently an intensified spread of human AE has been observed in France and in Austria.

In November 2010 in Zurich a meeting was organized by Swiss parasitologists on the control, diagnosis and treatment of AE in Poland, Lithuania and Switzerland. One of the conclusions of this meeting was that human cases of AE in Poland are diagnosed too late, causing a high proportion of severe cases (Deplazes et al. 2010, unpublished report).

In March 2011 a meeting was organized in Warsaw about the future of AE control in Poland. The major conclusions were as follows. The essential part of control is through continued studies on the spread of AE in animals and in humans, as well as by some control measures in highly endemic foci already identified in the north-east of Poland. In human populations in the other endemic areas, the early detection of human AE by ultrasound (US) and molecular techniques has to be promoted. The final clinical diagnoses of AE should be concentrated in two existing reference clinical centers in Gdynia and Poznan, the surgical management of the cases should remain in the surgical centers in Warsaw. This concentration of care seems to be necessary to maintain a high level of clinical expertise. Follow-up care has to be organized closer to the patient's place of residence, under the supervision of a reference center. In order to facilitate diagnoses, a US atlas has to be prepared and widely popularized. The problems associated with a payment for the anthelmintics used for prolonged courses of chemotherapy, and the problem of the availability of expensive relevant molecular tests, should be solved.

The proposed ways of controlling *E. multilocularis* infections and the standards of the clinical management of AE are open for discussion at the XXIII Congress of Polish Parasitological Society.

The efficacy of Ivermectin in the treatment of gastro-intestinal nematodes in red deer, mouflon and fallow deer in farm breeding

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A coproscopic examination was conducted in a red deer, mouflon and fallow deer breeding farm in West Pomerania. The herds are kept in an extensive system and reside in the pasture throughout the year. The aim of this study was to estimate the efficacy of Ivermectin deworming treatment on the farm-bred red deer, mouflon and fallow deer. In total, 72 faecal samples were examined from 40 individuals of red deer, 17 from mouflons and 15 from fallow deer. Subcutaneous injections of 1% Ivermectin were administered at a concentration of 1 ml/50 kg body weight. Drug resistance was evaluated with a Fecal Egg Count Reduction Test (FECRT). This method is recommended by the World Association for the Advancement of Veterinary Parasitology, and involves a comparison between the average amount of eggs in 1 g faecal sample before and after treatment. The extent and intensity of infection was estimated using the Willis-Schlaf and McMaster methods (Ziomko and Cenczek 1995). The efficacy of deworming with Ivermectin is shown in Table 1.

Table 1. Infection in red deer, mouflon and fallow deer

Species	Infection extensity E. I. (%)		Amount of eggs in 1 g faecal sample EPG		Deworming efficacy
	Before deworming	After deworming	Before deworming	After deworming	
Red deer	90	47.5	1050	150	85.71
Fallow deer	93.33	13.33	1000	50	95
Mouflon	100	5.88	2000	50	97.5

Diagnosis and treatment of *Tritrichomonas foetus* infections in cats from South-West Poland (2006–2013)

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The first case of invasion of *Tritrichomonas foetus* in cats was found in the Division of Parasitology of the Wrocław University of Environmental and Life Sciences in December 2006, the last was found in January of this year. In total, tritrichomoniasis cases were diagnosed in 9 cats, five Maine Coon, two Norwegian Forest Cats, and one from the breeds Russian Blue and Sphinx. The age of infected animals ranged from 8 weeks to 2 years.

Clinical signs. Chronic diarrhea was found in all animals. The consistency of fecal excretion was changed from that of the characteristic “cow pat” to more watery. Strong pain was felt around the anus during measurement of rectal temperature. In all cats, body temperature was normal, appetite remained, and there were no features of dehydration.

Diagnostics. In all animals, the live *Trichomonas* were observed in direct fecal smears. Feces were stored and delivered for testing at room temperature. For differentiation of *Tritrichomonas foetus* and *Pentatrichomonas hominis*, the direct smear method was used with the addition of Lugol’s solution. In the first of the infected cats, species of *Trichomonas* was also identified using genetic methods (genotyping). The feces of all animals were also tested for the presence of the coproantigen of *Giardia duodenalis* with the SNAP Giardia fast immunoenzymatic test (IDEXX). In five cats, simultaneous invasion of *T. foetus* and *G. duodenalis* was observed.

Treatment. Exclusive application of metronidazole in the Flagyl (Aventis) preparation at a dose of 50 mg/kg of body weight for 7 or even 14 days, resulted in a only a periodic improvement of their health (diarrhea abated). An effective and durable treatment effect was achieved, when metronidazole was used for 7 days at dose of 50 mg/kg body weight together with probiotic, which was applied at the recommended dose for 30 days. In six cases, the probiotic preparation Pro-Kolin+ (Protexin) was used, in three cases – Promax (VetPlus). No recurrence of disease was noted, nor any undesirable side effects after application of these preparations.

Efficacy of Baycox® for control of coccidiosis in Japanese quails *Coturnix coturnix japonica*

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Coccidiosis in Japanese quail (*Coturnix coturnix japonica*) belongs to the parasitic diseases that occur frequently but are rarely diagnosed. It affects birds health and productivity. Several species of genus *Eimeria* were described in quails: *Eimeria uzura*, *E. bateri* and *E. taldykurganica* located in small intestine, and *Eimeria tsunodai* in caecum. For the control of coccidiosis in domestic birds many drug preparations are used: amprolium, clopidol, diclazuril, decoquinate, lasalocid, monensin, narsin/nicarbazin, robenidine, roxasone, sulfadimethoxine/ormetoprin, salinomycine, semduramicin, zoalen.

The aim of the study was to evaluate the efficacy of toltrazuril (Baycox) for coccidiosis control in Japanese quails. The study was performed on 80 Japanese quails. Birds were reared in commercial quail flock (10,000 bird in the farm). Birds were kept on litter. Parasitological examination of their droppings shown a number of oocysts in the 1 g of feces. Selected quails were kept in separated cages with perforated floor to the daily collection of feces. Quails were divided into three experimental groups for 20 birds each, and one control (untreated) group. Group I received Baycox 2.5% at the dose recommended for laying hens – 1.5 mg/kg/ per day (1 ml/ 1 l H₂O) in drinking water available 24 h. Group II received 3 ml/1 l H₂O, and group III – 5 ml/1l H₂O) available for 8 h. During 14 days after drug administration all feces were collected from each group. The daily aggregated sample were mixed and then 10 g sample were collected for parasitological examination. Fecal samples were tested by Fülleborns' flotation method with Darlings' solution in 10× repetition (10×1 g of feces). From every centrifugated sample three drops were placed on the microscopic slide and viewed under the light microscope at the magnification 400×. Oocysts were counted in five fields of view and the mean count of oocysts in 1 g of feces was calculated, as well as the percentage of sporulated, non- sporulated and damaged oocysts.

Baycox application resulted in a statistically significant reduction in the number of oocysts in the third day after treatment regardless of the dose. In analysed period the number of oocyst remained at the very low level (0–0.4 oocyst per 1 g of feces) and in the control group was on the constant level (6.4–13.1 oocyst per 1 g of feces). On 4th day after treatment in group II and on 5th day in group I, 100% of oocysts were damaged. The percentage of sporulated oocysts in the control group was 20–25%, unsporulated 60–70% and damaged 5–20% depending on the day of examination. Toltrazuril application is effective in reducing the coccidia invasion in Japanese quails.

Efficacy of ivermectin against parasites in horses

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The occurrence of parasites in horses may be frequently observed, despite regular deworming. Nematode parasites from Strongylidae family (the subfamily Strongylinae and Cyathostominae) are most frequently found in horses and, additionally, *Parascaris equorum*, *Strongyloides westerii* and *Eimeria leucarti* in young horses. In horses staying in pastures infestations with tapeworm of Anoplocephalidae family are often diagnosed. Numerous reports indicate the occurrence of drug resistance to antiparasitic agents used, such as fenbendazole, pyrantel and ivermectin.

The aim of this study is to evaluate the efficacy of ivermectin in eliminating nematodes in horses and the demonstration of any existing drug resistance.

The study included 243 horses from 11 stables. They were of different races, males and females, aged from 1 to 25 years. Faecal samples were analyzed with the application of two methods: McMaster and flotation. The samples were taken at the following times: before the treatment and after 14, 21 and 28 days of therapy. The horses were treated with ivermectin.

Prior to treatment 180 horses from all stables were infected (74%). 6% of the foals were infected with *P. equorum*, 0.5% with *E. leucarti* and 0.5% with *S. westerii*. The eggs of Strongylidae were found in 74% of the horses with the application of McMaster method. All horses diagnosed as infected were infested with Strongylidae family, with the mean number of eggs in 1g of faeces equal to 665 EPG (50–6400). *P. equorum*, found in only 5 stables was represented by the mean number of eggs in 1g of faeces equal to 1622 (50–850). After treatment, the parasites were found only in 11 horses (6%): *P. equorum* in 2 horses, Strongylidae family in 9 horses. On the basis of FECR (faecal egg count reduction test), in 4 stables isolated cases of horses likely to develop drug resistance to ivermectin (FECR < 90%) were found. The efficacy of ivermectin in the elimination of nematodes proved to be high (75–100%). The research will be continued. It was also found that McMaster method seems to be insufficient for diagnosis of the tapeworm of the Anoplocephalidae family. Examining the same sample with flotation allowed us to identify the presence of the tapeworm eggs, which had not been confirmed with the application of McMaster method.