POLISH PARASITOLOGICAL SOCIETY

ANNALS OF PARASITOLOGY

volume 59 · supplement · 2013

PL ISSN 2299-0631

The XXIIIth Congress of PPS is organized by Executive Board of the Polish Parasitological Society and Wrocław Branch of the Society, and Wrocław University, Wrocław University of Environmental and Life Sciences and Wrocław Medical University

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The Organizing Committee for the XXIIIth Congress of the Polish Parasitological Society acknowledges with gratitude the generous support received from the following sponsors:

Print of the Congress materials is supported by Ministry of Science and Higher Education of Poland.

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The XXIIIth Congress of the Polish Parasitological Society

4-7 September 2013, Szklarska Poręba-Piechowice

ABSTRACTS

SESSION IV

Recent advances in parasitological diagnostics

The genetic diversity of protozoan *Hepatozoon* in local populations of bank vole in the Mazury lake district

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The first *Hepatozoon* parasite was identified in a cat in India in 1908. To date, the genus *Hepatozoon* comprises almost 300 species of blood parasites. Distinguishing different species of *Hepatozoon* by microscopical observation is difficult because of the similar morphology of trophozoites, so molecular techniques like PCR are recommended for the identification of the species.

The aim of our study was to compare the prevalence and genetic diversity of *Hepatozoon* infections in three separated populations of bank vole (*Myodes glareolus*) (n=294) in the Mazury lake district.

The voles were live-trapped in August and September 2010. Three forest sites were selected near three towns: Mikołajki, Ryn and Pisz and were separated one from the other by a distance of ca. 10–20 kilometers. These sites were separated either by water (canals and lakes) or by agricultural open areas. *Hepatozoon* infections were detected on the basis of microscopical observation of Giemsa-stained blood smears and by the amplification of the *Babesia/Theileria* 18S rRNA gene fragment using PCR. For comparison, sequences of *Hepatozoon* strains obtained from GenBank (www.ncbi.nlm.nih.gov) were implemented in the sequence alignment.

The overall prevalence of the *Hepatozoon* invasion was 31% but varied between trapping sites and host local populations. The highest prevalence was detected in voles from the Ryn area (37.9%), lower in Urwitałt (34%) and the lowest near Pisz (14.3%). Preliminary molecular characterization revealed the occurrence of two genetic variants of *Hepatozoon:* BV1 and BV2, which were previously reported in bank voles from Spain. A genetic variant closely related to BV1 was the dominant variant observed in three local bank vole populations (71–86%). Further studies on genetic diversity of these isolates are needed using more variable genetic targets (genes).

The project was partly funded by the Ministry of Science and Higher Education research for the development of young researchers and PhD students funded by internal competition procedure for the Faculty of Biology, DSM no. 140000/501-86/104917.

Trichinella spiralis: recognition of antigens of three different stages during experimental infection of swine

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Trichinellosis is a typical food-borne zoonotic disease and the nematode *Trichinella spiralis* is its main pathogen. The *T. spiralis* life cycle is completed within a single host species, and the three major life stages are muscle larvae (ML), adult (Ad) worms and new born larvae (NBL). An important aspect in the diagnosis of trichinellosis is the identification of antigens which are parasite specific and highly immunogenic for the infected host. It has been demonstrated that following *T. spiralis* infection, this nematode expresses many immunodominant antigens and stimulates the host immune system. Detection of the antigenic proteins produced by *T. spiralis* which may elicit an immune response in infected animals (i.e. pigs), and thus hold promise as potential target proteins for specific diagnostics, is required. To identify *T. spiralis* antigens that are specifically recognized by host antibodies, the crude extract and excretory-secretory (E/S) antigens from ML and Ad worms, as well as the crude extract from the NBL of *T. spiralis* were subjected to immunoblotting with antisera derived from pigs experimentally infected with *T. spiralis*.

Our results show that the immunoblotting of *T. spiralis* crude extracts and E/S proteins with specific antisera resulted in variability in immunoreactive protein patterns, including both specifically and commonly recognized antigens. Interestingly, all *T. spiralis* antisera regardless of infective dose recognized a common protein for each of the examined life stages of *T. spiralis* with a molecular weight around 41 kDa. Other commonly recognized proteins were located in the area around 20–27 kDa, and between 105 and 195 kDa. Based on our results we suspect that in parallel to the stage-specific antigens, the *T. spiralis* nematode may produce some species-specific antigens. However, to confirm our hypothesis, it is necessary to use other techniques such as fluorescence two-dimensional difference gel electrophoresis (2-D DIGE) to establish statistically valid thresholds for assigning qualitative and quantitative differences between antigens of the three different life stages of *T. spiralis*.

Molecular identification of human cystic echinococcosis in central Poland

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Cystic echinococcosis (CE) is an extremely important parasitic infection in livestock worldwide caused by a small tapeworm of the *Echinococcus* sp. complex belonging to the family Taeniidae. In humans, the cysts develop as unilocular fluid-filled bladders mainly in the liver (70%), lungs (20%) and in other organs like the brain, heart and bones. Genetic studies of *Echinococcus* based on sequence data from mitochondrial and nuclear genes has led to the description of 10 genotypes (G1-10). Echinococcosis is not considered a frequent disease in Poland. According to the Polish National Institute of Public Health-National Institute of Hygiene the number of registered human cases is between 20 and 60 annually.

The aim of this study was to identify the genotypes responsible for cases of cystic echinococcosis in Poland by conducting molecular analysis of larvae isolated from Polish patients. Samples of larva fragments were obtained from 47 patients after hepatectomy carried out between 2000 and 2010 in the Department of General, Transplant and Liver Surgery, Medical University of Warsaw. The cysts were removed from the liver (44 cases), spleen and liver (2 cases) and kidney (1 case). DNA was isolated using a DNA extraction NucleoSpin Kit (Macherey-Nagel). The PCR-amplified region was the *nad1* and *cox1* gene fragments. The PCR products of the *nad1* fragment were sequenced. Thirty samples (64%) were diagnosed positive by amplification of both gene fragments. The *nad1* sequences were compared with sequences of *Echinococcus* genotypes available in the NCBI GenBank. All isolates were highly similar to that of the pig strain G7, designated *Echinococcus canadensis*. The sequences from three isolates differed by a few substitutions and a polymorphism. All *nad1* sequences were deposited in GenBank with accession numbers JX266793-JX266824. Out of 47 examined samples from patients who were suspected of having cystic echinococcosis, we were able to confirm 30 cases as CE by PCR. Our study identified *E. canadensis* G7 in all isolates from humans. Although it has been postulated that the *E. canadensis* G7 genotype is poorly infective to humans, our research demonstrates its distribution in humans, suggesting that it plays a significant role as the aetiological agent of human cystic echinococcosis in Poland.

The usefulness of *Toxoplasma gondii* MIC1-MAG1-SAG1 chimeric antigen in the serodiagnosis of ovine toxoplasmosis

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Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*, which infects humans and most warm-blooded animals throughout the world. Although human toxoplasmosis in healthy adults is usually asymptomatic, a serious disease can occur in the case of congenital infection and immunocompromised individuals. Among food animals, sheep, along with goats and pigs, possess the highest incidence of *T. gondii* cysts in meat, and play a major role as a source of human infection. Moreover, prevention and control of *T. gondii* infection is of economic importance in sheep production, because it also causes abortions and neonatal deaths of sheep. The diagnosis of toxoplasmosis in sheep is usually based on the detection of specific antibodies. Most commercial kits use prepared tachyzoites grown in mice or tissue culture to prepare *Toxoplasma* lysate antigen (TLA) for antibody detection. These antigens may contain varying amounts of host material and therefore affect the specificity and reproducibility of the test results. The use of recombinant antigens could avoid these drawbacks and permit the development of an improved diagnostic test.

This study is the first evaluation of the use of *T. gondii* MIC1-MAG1-SAG1 recombinant chimeric antigen for the serodiagnosis of ovine toxoplasmosis. Previously, this recombinant protein has been successfully used for detection of *T. gondii* infection in humans (1). The diagnostic efficiency of MIC1-MAG1-SAG1 antigen was assessed in IgG ELISA test with the use of 150 reference sheep sera (100 seropositive and 50 seronegative) previously tested using a commercial agglutination test (Toxo-ScreenDA, bioMérieux). The results obtained for chimeric antigen were compared with those of IgG ELISAs using a TLA and a combination of three recombinant antigens (MIC1+MAG1+SAG1). The sensitivity of the IgG ELISA calculated from all of the positive serum samples was the same for the chimeric antigen and the TLA (100%), whereas the sensitivity of the mixture of recombinant proteins used were definitely lower (78%). Therefore, the present study shows that the MIC1-MAG1-SAG1 chimeric antigen is a very useful tool for the detection of anti-*T. gondii* IgG antibodies in sheep sera, giving far better results than a mixture of three antigens.

1.Holec-Gąsior, L., Ferra, B., Drapała, D. 2012. MIC1-MAG1-SAG1 chimeric protein, a most effective antigen for detection of human toxoplasmosis. *Clinical Vaccine Immunology*, 19(12):1977-1979.

The first detection of *Echinococcus multilocularis* DNA in water – an environmental survey conducted in the Warmia-Masuria province, north-eastern Poland

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Echinococcus multilocularis is an ethiological agent of alveolar echinococcosis (AE), a severe disease occurring in the northern hemisphere, including central Europe. It's main definitive host in Europe is red fox, but infected domestic dogs and cats have also been described. Intermediate hosts of *E. multilocularis* are different species of rodents. Humans can become accidental intermediate hosts after ingestion of tapeworm eggs originating from infected animal faeces and present in the environment. The size of foxes population, their expansion from forest and rural to urban areas, and the number of human AE cases in Poland have increased during last three decades. This work is a part of large surveillance of environment conducted in endemic regions of Poland to establish the exposure of people living in these areas to risk of infection with *E. multilocularis*. Nineteen water samples from the Warmia-Masuria province were analyzed. The DNA of *E. multilocularis* was detected in one sample coming from the barnyard well. It proves that water can pose direct threat to human health. The investigations have been continued.

Work supported by research grant No. N N402 587140 from the State Committee for Scientific Research, Poland.