WIADOMOŚCI PARAZYTOLOGICZNE
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XIV WROCŁAWSKA KONFERENCJA PARAZYTOLOGICZNA pt. „Parazytologia na przełomie XX/XXI wieku"

XIV Wrocławska sesja naukowa, poświęcona starym i nowym kierunkom badań w systematyce, biologii i zwalczaniu pasożytów na przełomie wieków, została zorganizowana 18 października 2002 roku, z podwójnej okazji: Jubileuszu 300-lecia Uniwersytetu we Wrocławiu oraz 40. rocznicy powołania Zakładu Parazytologii Ogólnej w Uniwersytecie Wrocławskim (1962-2002).

W sali im. Czekanowskiego zabytkowego Collegium Antropologicum (miejscu comiesięcznych posiedzeń Rady Wydziału Nauk Przyrodniczych UWr) prof. Elżbieta Lonc przywitała serdecznie wszystkich uczestników w imieniu organizatorów – pracowników Zakładu Parazytologii – oraz własnym. Szczególne słowa podziękowania za przyjęcie zaproszenia i obecność skierowała pod adresem wieloletnich wiernych i niezawodnych przyjaciół wrocławskiej parazytologii, stałych uczestników konferencji wrocławskich – em. profesorów Katarzyny Niewiadomskiej i Teresy Pojmańskiej z Instytutu Parazytologii im. W. Stefańskiego PAN w Warszawie oraz prof. em. Zbigniewa Jary z Wrocławia. Obie Panie są „złotymi filarami", które zainicjowały ten cykl konferencyjny, przez wszystkie lata wspierały pomysły organizatorów, pomagały je urzeczywistniać swoją nieocenioną radą i pomocą, a przede wszystkim obecnością i zaangażowaniem, podobnie jak wielce zasłużony dla parazytologii prof. em. Zbigniew Jara, który jak zwykle, przypomniał nam prof. Z. Kabatę nie tylko uczonego, ale także autora wspomnień wojennych pt. „Żniwa na głębinie" i urokliwych wierszy, które dzięki hojności firmy wydawniczej (Biuro Richter Polska we Wrocławiu) wzbogaciły nasze Konferencyjne Materiały. Zawsze obecny na konferencjach wrocławskich Instytut Parazytologii PAN, był także reprezentowany przez prof. Halinę Wędrychowicz – prezesa Polskiego Towarzystwa Parazytologicznego i przez doc.doc Władysława Cabaja – Zastępcę Dyrektora tego Instytutu, i Bożenę Moskwę – Sekretarza Komitetu Parazytologii PAN. Drugim filarem wspierającym konferencyjną aktywność od samego początku był i jest poznański ośrodek parazytologiczny, tym razem reprezentowany przez prof. Annę C. Majewską – kierownika Katedry Biologii i Parazytologii Lekarskiej AM w Poznaniu wraz ze współpracownikami – prof. Krystyną Boczoń i dr Izabelą Andrzejewską, oraz przez prof. Hannę Mizgajską – kierownika Zakładu Biologii i Ochrony Środowiska, w bieżącej kadencji także dziekana Wydziału Wychowania Fizycznego AWF w Poznaniu.

Uczestnicy Konferencji, jednocześnie prelegenci i autorzy posterów, reprezentowali różne ośrodki parazytologiczne w Polsce: Zakład Zoologii Systematycznej

oraz Zakład Taksonomii i Ekologii Uniwersytetu im. Adama Mickiewicza w Poznaniu, Zakład Parazytologii Uniwersytetu Warszawskiego, Zakład Zoologii Bezkręgowców Uniwersytetu Gdańskiego, Katedrę i Zakład Biologii i Parazytologii Medycznej AM w Szczecinie, Instytut Medycyny Morskiej i Tropikalnej w Gdyni, Katedrę Biologii i Parazytologii Lekarskiej AM w Lublinie, Zakład Biologii i Parazytologii Lekarskiej AM w Łodzi, Katedrę i Zakład Ogólnej Biologii Lekarskiej ŚAM w Zabrzu.

Wśród licznych reprezentantów ośrodka wrocławskiego byli, między innymi, prof. Ryszard Haitlinger – przewodniczący Wrocławskiego Oddziału PTP, prof. Alina Wieliczko z zaprzyjaźnionej Katedry Epizootiologii i Administracji Wydziału Medycyny Weterynaryjnej, prof. Stanisław Jankowski – kierownik Zakładu Biologii i Parazytologii Lekarskiej AM we Wrocławiu, a także prof. em. Andrzej Wiktor, wieloletni dyrektor Muzeum Przyrodniczego UWr i prof. Andrzej Witkowski (obecnie dziekan WNP, który w imieniu władz Uczelni również witał uczestników Konferencji).

Z żalem przyjęto informację o nieobecności prof. Armina Geusa z Uniwersytetu w Marburgu (Klinikum der Philipps Uniwersitat, Geschichte der Medicin), współautora dziejów wrocławskiej parazytologii (1811-2002), który z powodu wypadku nie mógł uczestniczyć w Konferencji.

Chwilą ciszy uczczono pamięć zmarłych: we wrześniu dra Ryszarda Wójcika (z ZHW w Toruniu), który jako pierwszy w maju br. zgłosił swój udział w konferencji, oraz prof. em. Jadwigi Złotorzyckiej-Kalisz, która zmarła nagle 2 października br. „Nasza Pani Profesor" była nie tylko wielką uczoną, ale także – a może przede wszystkim – wspaniałym człowiekiem. Od czasów tuż powojennych współtworzyła intelektualny klimat Wrocławia i naukowy potencjał Uniwersytetu Wrocławskiego. Wykształciła wielu magistrów i doktorów; umiała być mistrzem i nauczycielem. Dla nas – swoich uczniów i współpracowników – była nie tylko autorytetem naukowym, lecz także życzliwym i pomocnym przyjacielem, osobą niezwykle bezpośrednią i serdeczną, zawsze gotową wysłuchać drugiego człowieka, pełną zrozumienia dla ludzkich słabości. Świat nauki stracił wielkiego uczonego, a my kogoś bliskiego, bez kogo parazytologia z pewnością będzie uboższa. Sylwetkę i dorobek naukowy prof. Złotorzyckiej przedstawiła prof. Anna Okulewicz (obecny kierownik Zakładu Parazytologii).

Adresy podkreślające dotychczasowe osiągnięcia Zakładu Parazytologii UWr wraz z okolicznościowymi życzeniami składali w trakcie kolejnych wystąpień: prof. prof. Halina Wędrychowicz, Anna Majewska, Hanna Mizgajska, Bożena Moskwa, która odczytała list gratulacyjny Dyrekcji Instytutu Parazytologii PAN, i koledzy z innych, zaprzyjaźnionych ośrodków.

Nastepnie Przewodnicząca PTP, prof. Halina Wędrychowicz dokonała oficjalnego otwarcia Konferencji.

W pierwszej części, prowadzonej przez prof. Katarzynę Niewiadomską i prof. Krystynę Boczoń odczyty wygłosili:

– dr Andrzej Kotłowski, który w referacie pt. „ Unia Europejska – epidemiologiczne wyzwania dla polskiej parazytologii lekarskiej" przedstawił dotychczasowe postanowienia Unii Europejskiej, odnoszące się bezpośrednio do problemów epidemiologii polskiej parazytologii;

– prof. Hanna Mizgajska – w pięknej prezentacji medialnej – obok omówienia problematyki X Międzynarodowego Kongresu Parazytologii przybliżyła niepowtarzalny klimat, jaki stworzyły malownicze krajobrazy Kanady oraz przyjaźni Kanadyjczycy. Z dużą przyjemnością powtórzyła usłyszane w Kanadzie słowa pochwały na temat perfekcyjnej organizacji Europejskiego Multikolokwium Parazytologicznego, które odbyło się w Poznaniu w 2000 r;

– prof. Beata Pokryszko, która swoim zajmującym wystąpieniem nt. współczesnego pojęcia gatunku i zdolności rozdzielczych procedur taksonomicznych przypomniała nam jak piękny i interesujący może być klasyczny – bez audiowizualnych środków – uniwersytecki wykład, a także mówiąc o zawiłościach i meandrach sztuki filogenetycznej uzmysłowiła nam wagę i znaczenie nauk taksonomicznych w parazytologii – nauce o relacjach w układzie pasożyt-żywiciel, gdzie znajomość systematyki obu partnerów jest nieodzowna.

Niestety, ze względu na brak kompatybilności między „poznańskim" a „wrocławskim" sprzętem multimedialnym prof. Anna Majewska nie mogła przedstawić swojego referatu: „*Mikrosporydioza u ludzi – nowe wyzwanie w parazytologii*".

W przerwie spowodowanej próbami (nieudanymi) usunięcia usterki medialnego rzutnika, prof. Pojmańska podsumowała sesję posterową. Zaprezentowano 17 posterów, bardzo zróżnicowanych tak pod względem treści jak i formy, ale które odzwierciedlają bądź działalność określonego ośrodka naukowego, bądź grupy badaczy, specjalizujących się w określonej tematyce badawczej. Z przedstawionych plakatów wynika, że w wielu ośrodkach rozwijane są badania, zapoczątkowane w późnej połowie XX wieku, a które z pewnością będą się dynamicznie rozwijać w wieku XXI. Należą tu, przede wszystkim, badania molekularne o charakterze biochemicznym i immunologicznym, zmierzające w kierunku zwalczania chorób pasożytniczych nękających ludzi i zwierzęta użytkowe (Instytut Parazytologii PAN, Zakład Parazytologii UW, Zakład Parazytologii SGGW, Zakład Parazytologii UWr). Drugą ważną grupę stanowią, posługujące się zupełnie innymi metodami, badania o charakterze ekologicznym, prowadzące do lepszego zrozumienia mechanizmów regulujących występowanie pasożytów u zwierząt wolno żyjących, a tym samym pozwalające prognozować przebieg niektórych zjawisk demograficznych we względnie naturalnych środowiskach (badania nad kleszczami i innymi przenosicielami patogenów, prowadzone w kilku ośrodkach, w tym na szeroką skalę w Akademii Medycznej w Lublinie i na Uniwersytecie Wrocławskim, badanie zjawiska forezy – Uniwersytet im Adama Mickiewicza w Poznaniu, badania nad pasożytami ryb – Uniwersytet Gdański i inne). Tych badań, podobnie jak badań faunistycznych, jest zdaniem prof. Pojmańskiej w Polsce za mało i niekiedy są prowa-

dzone w niewłaściwy sposób, bo opierają się na przypadkowo zebranym materiale. Badania nad funkcjonowaniem zgrupowań pasożytniczych muszą mieć jasno wytyczony cel i muszą być dobrze zaplanowane, w oparciu o znajomość metod, stosowanych przez ekologów. Słabo były także reprezentowane studia morfologiczne, które, choć niekiedy uważane za XIX-wieczne, powinny być rozwijane przy stosowaniu różnych, w tym najbardziej nowoczesnych metod; właściwe rozróżnianie gatunków ma nie tylko teoretyczne znaczenie dla systematyków, ekologów czy ewolucjonistów, ale także dla lekarzy, mających do czynienia z chorobami ludzi i zwierząt. Nawiązując do hasła Konferencji prof. Pojmańska stwierdziła, że przedstawione w posterach badania wskazują, iż w polskich ośrodkach naukowych rozwijają się wielokierunkowe badania parazytologiczne, a polscy parazytolodzy są dobrze przygotowani do sprostania wyzwaniom XXI wieku.

W drugiej części, prowadzonej przez prof. Alicję Buczek i prof. Jerzego Rokickiego, wystąpiło czterech prelegentów:

– doc. Bożena Moskwa omówiła nowoczesne metody diagnozowania chorób wywoływanych przez *Neospora caninum*;

– dr Brygida Adamek opisała kliniczny przypadek bezobjawowej bąblowicy wątroby u pacjentki, która dzięki prawidłowej, dokładnej diagnozie i udanej operacji powróciła do całkowitego zdrowia;

– mgr Jolanta Kucińska (doktorantka prof. E. Lonc) przedstawiła blaski i cienie transgenicznych preparatów (zawierających wszczepione, od kryształotwórczych szczepów *Bacillus thuringiensis*, geny kodujące toksyczne dla larw owadów delta-endotoksyny) wykorzystywanych w biologicznym zwalczaniu wektorów chorób infekcyjnych i inwazyjnych, głównie komarów i meszek;

– dr Andrzej Gaweł przedstawił kokcydiozę jako wciąż aktualny, a nawet narastający problem na przełomie XX i XXI wieku.

Streszczenia referatów i prezentowanych posterów są znajdują się w dalszej części materiałów konferencyjnych.

W trakcie konferencji zawiązywały się liczne i ciekawe dyskusje inspirowane przez przewodniczących, którzy służyli fachowym komentarzem, rozszerzali je i wzbogacali wystąpienia. Zabrakło czasu na dokładne zwiedzenie interesującej ekspozycji wystawionej w nowopowstałym Muzeum Człowieka w Collegium Anthropologicum. Ostatnie przedjubileuszowe prace renowacyjne Głównego Gmachu utrudniły wgląd w historię i kontemplację piękna barokowej Auli Leopoldina, sali muzycznej Oratorium Marianum i Muzeum Uniwersytetu Wrocławskiego w Wieży Matematycznej.

Konferencję zakończyło owocne spotkanie autorów Monografii „Dzieje Parazytologii Polskiej w latach 1945–2000" z redaktorami opracowania. Dr hab. Bożena Płonka, profesor historii medycyny w Instytucie Historycznym UWr, fachowymi argumentami przekonała nas o potrzebie ujednolicenia układu wszystkich dotychczas nadesłanych (przez poszczególnych autorów) rozdziałów książki, tak aby by-

ły porównywalne pod względem merytorycznym i redakcyjnym. Kilkustronicowe „Uwagi redaktora" wraz ze „wzorcowym" opracowaniem placówki poznańskiej są rozsyłane do wszystkich autorów z nadzieją na rychłe poprawki i ukończenie dzieła w 2003 roku.

Elżbieta Lonc

STRESZCZENIA REFERATÓW I POSTERÓW

SPECIES CONCEPT AND THE RESOLUTION OF TAXONOMIC PROCEDURES

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According to the rules of phylogenetic [=cladistic] systematics, which is commonly applied currently, taxa should be defined by apomorphies – evolutionary novelties found only in the members of a given taxon. A logical consequence of strict application of cladistic procedures is that each species can be characterised by its own, unique apomorphy. Though in cladograms all species are in terminal positions, and thus no ancestor-descendant relationship is specified, in evolutionary reality some species are ancestors of other species. This implies that an ancestral species will have no apomorphy of its own and, though preserving its species identity, cannot be defined according to cladistic procedures. Various species concepts and definitions are discussed in the context of this problem.

NEOSPORA CANINUM: ADVANCES IN DIAGNOSTIC

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Neospora caninum, an apicomplexan protozoan, has a worldwide distribution. It was first isolated from a paralyzed dog, and has recently gained considerable attention, due largely to its impact on the dairy and beef industry where it caused economic losses due to reproductive failure associated with abortion. Serological and molecular evidence of neosporosis has been found in aborted dairy caws and calves in Poland too.

The life cycle of *N. caninum* is not fully described. The dog is a definitive host and following oral administration of oocysts shed in the faeces, a wide range of animal species are infected. Due to localisation (especially brain and spinal cord), as well as to nonspecific and various clinical signs, parasite identification is very difficult.

The main route of diagnosis of *N. caninum* infection are clinical signs, observed mainly in young calves and pups. In these animals, protozoan cause severe encephalomyelitis and muscular disordses. Moreover, in young animals, neosporosis could lead to death. In adult animals, the most frequently observed clinical signs are disturbances during reproduction and abortion.

The diagnosis of infection is primarily based on the histochemical identification of the parasite. Due to the low numbers of parasite in infected tissues, the results of these tests could be miscelouses.

Ultrastructure of several organelles of *N. caninum* is not visible by standard light microscopy. In these cases, more ultractructural differences exist between each parasite stages as well as between closely related species and could be studied by electron microscopy.

Demonstration of *N. caninum* in tissues or anti-*N. caninum* antibodies in serum, milk and foetal fluid, using different serological techniques as IFAT and different ELISAs is routinely used to monitore infection. According to manufacturers, using commercial tests (KITs) it is possible to detect IgG only. The level of the other immunoglobulines could be detect in-house ELISA based on the own obtained antigen.

Molecular biological techniques such as PCR or RAPD offer a high sensitive and specific alternative to histochemical and immunological approaches for diagnosis. Because *N. caninum* is normal-ly present in tissues in very low numbers and some clinical samples may be degraded due to processes such as autolysis, molecular tests are more recently used for the detection of parasite basing on a highly repeated DNA sequence in *Neospora* genome.

The standard tests used for diagnosis of neosporosis comprise histological, immunohistochemical, immunological and molecular biological techniques to detect and measure relative level of parasites in the tissues of infected tissues. There is no one test, which result is enough for positive diagnosis. It is necessary to use some complementary tests for *N. caninum* detection.

ASYMPTOMATIC LIVER HYDATIOSIS. A CASE REPORT

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Echinococcus infection remain silent for years before the enlarging cysts cause symptoms in the affected organs – the liver, lungs, and less commonly the brain and other organs. If left untreated, infection can be fatal. A case of a woman (born in 1958) with asymptomatic hydatic disease is presented. In 1996 during hospitalization because of hypertension an ultrasound imaging revealed a solitary, 32 mm diameter cyst in the right liver lobe. It was considered as a cyst of unknown origin. There were any clinical or laboratory signs of liver disease. In 1998 the cyst shown 65 x 53 mm of dimension. That time the woman was directed to Infectious Diseases Outpatient Clinic, still without any symptoms. Anamnesis did not revealed any environmental circumstances suggesting the possibility of parasitic disease. The events of routine laboratory tests remained within normal range. Only the passive hemagglutination assay (PHA) for echinococcosis was positive in titre 1:200. A mebendazol (Vermox) and subsequently albendazol (Zentel) treatment was administered. After two months the PHA titre remain unchanged. A radical surgery was conducted in February 1999. Postoperative period was complicated with moderate ALT elevation lasting about three months. In July 2000 patient started to complain of a headache. Computed tomography of a head did not revealed any pathological changes. The same time control PHA examination was negative.

The patient has been staying in a good condition for now.

Conclusion. Although human cases of hydatic disease are rare, it should be taken into account in the differential diagnostics of hepatic cysts. Especially because of asymptomatic course does not exclude diagnosis like that.

COCCIDIOSIS – A DISEASE OF THE XXTH AND XXIST CENTURY

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Coccidiosis is a parasitic disease caused by numerous species of Protozoa of *Eimeria genus*. The disease occurs mainly in birds and mammals. Considering economic losses, coccidiosis in chickens and turkeys has become of great importance. It is not easy to assess the losses due to coccidiosis. It is estimated that annual losses suffered only in Great Britain in the chicken rearing amount to approx. £ 23–38 million whereas annual losses all over the world, excluding expenses on prevention and treatment, reach about U$ 0.8–1 billion.

The control of coccidiosis in turkeys and chickens is practically based on chemoprophylaxis. However, nowadays, chemoprophylaxis encounters several problems, of which the most important is a gradual increase of drug-resistance in coccidia. High costs of placing new preparations on the markets and more and more common customers' reluctance to chemical additives in food products prompt the immunoprophylaxis to be applied on a large scale. Development of commercial live vaccines against chicken coccidiosis in Europe, such as Paracox (Pitman-Moore, UK), Livacox D, Livacox T and Livacox Q (Biopharm, Czech Republic) are considered to be a significant progress in the immunoprophylaxis of coccidiosis. Coccidia strains used in vaccines Paracox and Livacox are attenuated. They have been obtained by way of selection – shortened period of development or by passages on chicken embryos (*E. tenella* in Livacox).

Immunity to coccidiosis depends on cell mechanisms of the digestive tract immunity system GALT. At present a uniform view is presented that the role of antibodies in the development of immunity to *Eimeria* infection is relatively low and may be limited to certain stages of a parasite development or periods of immune response. Discussing the issues of coccidiosis immunoprophylaxis we should keep in mind that an immune response in chickens is affected by many factors: birds' health status, feed quality, rearing conditions. Pathologic factors involve stress, virus infections (Marek's disease, Gumboro disease, infectious anemia, reoviruses) and mycotoxins. Environmental conditions include NH3 which has an immunosuppressive effect on chickens and deficiencies of vitamins A, E, and C, and selenium which belong to feeding factors.

HUMAN MICROSPORIDIOSIS: A NEW CHALLENGE IN PARASITOLOGY

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Microsporidia are obligate intracellular parasites. This is a unique group of organisms because they belong to unicellular eukaryotes but they posses also many molecular and cytologic features of prokaryotes. Until now more than 1200 species belonging to 143 genera have been described, and taxonomic revisions are still performed.

For many years microsporidia were known as pathogens of silkworms, honey bees, fish and mammals which have been responsible for significant economic losses. In medicine the interest in

microsporidia grew when they were recognised as lifethreathening pathogens during the AIDS pandemic and since then several new genera and species have been identified in humans. In man infections of several species of microsporidia belonging to the genera of *Entercytozoon*, *Encephalitozoon*, *Pleistophora*, *Trachipleistophora*, *Brachiola*, *Nosema* and *Vittaforma* have been described. Moreover, microsporidia of unknown taxonomic status have been described and they referred to as *Microsporidium* in literature. Microsporidia have been found in every tissue and in most systems; therefore symptoms of infection vary and depend on microsporidium species, site and intensity of infection as well as on immunologic status of infected host.

Since microsporidia are omnipresent in external environment human exposure to infection is unavoidable. The most common routes of infection are faecal-oral and urine-oral transmissions of the parasite spores. Acquisition of infection by inhalation, ingestion of raw or poorly cooked fish and bites of mosquitoes are also possible. Waterborne outbrek of intestinal microsporidiosis is also known. Recently more and more evidences of zoonotic cases of human microsporidiosis have been identified.

The first case of human microsporidiosis was described in 1959. Until now about 2000 cases of microsporidial infections in man have been documented and majority of them was found in HIV-infected patients. Most probably, the frequency of human microsporidiosis is higher but methods used in diagnosis are unreliable.

Several methods have been used in diagnosis of microsporidiosis but only electron microscopy and molecular techniques, especially PCR, are useful for species differentiation of microsporidia. Identification of microsporidium species is requisite since they differ in drug susceptibility and in predilection of causing disseminated infection. Unfortunately, electron microscopy and molecular techniques are rarely used in routine diagnostics and this is the most probable reason why microsporidial infection in humans are seldom recognized.

The data of the last decade indicate that human microsporidiosis is identified as a lifethreathining opportunistic infection associated with immunocompromised cases (HIV-infected patients and organ transplant recipients). It has also been reported in HIV-negative immunodeficient patients and immunocompetent persons. In spite of their medical importance microsporidia are often neglected by physicans. It is caused either by sparse identification of the infection in humans conected with imperfect diagnostic methods or, which happens more often, because few physicans are aware of the existance of these dangerous pathogens.

We can presume that in the nearest future molecular diagnosis will become more common and this will make possible the detection of a larger number of microsporidiosis cases both in immunodeficient and immunocompetent patients. The molecular techniques will be very useful for the identification of the source of infection and discovery of new species occurring in humans. It should not be surprising if we take into account the existence of huge number of species in *Microspora* phylum.

HELIGMOSOMOIDES POLYGYRUS: INHIBITION OF TH1-, TH2- RELATED CYTOKINES AND IGA AND IG1 IN THE INTESTINE DURING PRIMARY INFECTION OF BALB/C MICE

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The immune response against *H. polygyrus* is characterized as typical Th2 related response. The host protective mechanisms are suppressed by adult worms during a primary infection but are very effective in protection after challenge (Urban et al., 1991). The nematode fourth stage larvae are high-

ly antigenic and can stimulate protection. However, during a primary response the larvae escape from their tissue niche into the lumen of the intestine, where they mature into adults. This stage is believed to release an immunosuppressive factor that protects worms against effective host immune responses (Telford et al. 1998).

The pattern of the immune response induced during nematode infection is dependent on cytokine activity secreted by different populations of cells. Functionally Th1 cells participate in cell-mediated inflammatory reactions and are characterized by an increase in IL-12 and IFN-γ. Th2 cells regulate the production of IgG1, IgA, IgE and stimulate eosinophilia and mastocytosis. This response is characterized by the production of IL-4, IL-5, and IL-13. (Artis and Grencis 2000). The immunosuppressive activity of the worms could be mediated through the action of so called Th3 cells (Chen et al 1994) although this has not been formally proven. An alternative is that the network of cytokine responses may be regulated by the parasites themselves (Grencis 2001).

We have investigated the importance of the local intestinal responses in the maintenance of infection and which particular cytokines operate *in vivo* at different points of *H. polygyrus* infection in mice, by measuring cytokine production *ex vivo*. BALB/c mice were given a primary infection with *H. polygyrus*. Immunoglobulins IgG1 and IgA as well as cytokines – IFN-γ, IL-12 and IL-5, IL-6, IL-4 and IL-10 were measured by enzyme-linked immunosorbent assay. Leukocytosis and eosinophilia were also assessed.

The production of IFN-γ, IL-12 and IL-4, IL-5, IL-6, and IL-10 were mostly depressed locally in the intestine 28 days post primary infection, in spite of elevated concentrations in the blood and peritoneal fluid. Co-incident with the suppressed secretion of cytokines mucosal IgA and IgG1 levels were also reduced in comparison to those seen in the peritoneal fluid. The changes in the level of immunoglobulins were correlated with parasite rejection, and the pattern of IgA secretion was different from IgG1, both in the intestine and peritoneal fluid.

PRODUCTION OF *HAEMONCHUS CONTORTUS* ES ANTIGENS USING RACE-PCR AND PGEX-4T EXPRESSION VECTOR

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Haemonchus contortus is a highly pathogenic blood-feeding nematode, which resides in the abomasum of sheep, goats and other small ruminants world-wide. The nematode releases a variety of molecules into the host environment, which are referred to as excretory-secretory products (ESP). It has been shown that the vaccines based on 15 and 24 kDa native proteins from ESP protect adult sheep against infection. However, there have been problems with isolating the necessary amount of the 15 and 24 kDa proteins from the parasites. The aim of the present study was to clone cDNAs of 15 and 24 kDa proteins and to express the recombinant antigens in bacterial expression system.

Adult *H. contortus* worms were isolated from the abomasum of infected sheep, cleaned carefully and homogenized in liquid nitrogen. The mRNA was isolated directly from the tissue using Qiagen kit according to the manufacture's instructions and next transcribed to cDNA with omniscript reverse transcriptase kit (Qiagen).

On the basis of nucleotide sequences of 15 kDa and 24 kDa proteins (Gen Bank) the following two pairs primers were designed: 15 kDa: L (5'- ATG TTC TTC GCT TTT GCA GTG ; R (5'- TTA TTG AGA AAA TCT GAA TTG CAT TG); 24 kDa: L (5'- ATG TTT TCA CTT GCC ACT GTC); R (5'- TAT GAA GGT TTA TTG TCA GTG CTT GT) and used in RACE-PCR.

Amplicons of 500 and 730 bp were obtained. Sequences of the PCR products were identical with those predicted from amino acid sequences of the native 15 kDa and 24 kDa ESP of *H. contortus*.

The PCR products were ligated into pGEX-4T vector (Amersham). For expression of recombinant proteins *Escherichia coli* XL1-blue cells were transformed with the recombinated vector by electroporation method using BTX Electro Cell Manipulator 600.

Over night culture of *E. coli* XL1-Blue containing pGEX-4T-3 vectors with 15 and 24 kDa was transferred into 1 litter of LB medium. Bacterial culture was grown up to OD_{600}=0.6 and next IPTG was added up to the concentration of 100 μM. Bacteria were cultivated for 2 hours in RT. Next the culture was centrifuged at 3000 x g for 10 min. The pellet was washing two times with cold PBS buffer and suspended in 20 ml of PBS. 100 μl 1 M DTT, lysozyme up to 0.1 mg/ml, DNAse up to 0.01 mg/ml, 10 μl 10 mM PMSF were added. This mixture was incubated in 37°C. Lysate was centrifuged at 15 000 x g for 15 min in 4°C.

Purification of the fusion proteins was accomplished by affinity chromatography method on glutathione-sepharose column. Supernatants were collected and analysed by Western blotting.

The results of the present study showed that RACE-PCR may be useful for cloning of ESP antigens of *H. contortus* using cDNA obtained from adult worms as an template. pGEX-4T expression vector allows a rapid purification of the recombinated antigens from bacterial proteins.

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THE INFLUENCE OF THE VACCINATION PROCEDURE ON THE ANTIBODY RESPONSE OF RATS IMMUNISED WITH CDNA AND PROTEIN FORM OF GLUTATHIONE S-TRANSFERASE OF *FASCIOLA HEPATICA*

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Fasciolosis, the disease caused by the trematode *Fasciola hepatica*, is very important in both human and veterinary medicine. Although many attempts have been made, no commercially available vaccine against fasciolosis exists. Among the antigens recognised as potential candidates for anti-fluke vaccine antigens, glutathione S-transferases (GSTs) seem to be of great importance. GSTs are present as several isoenzymes in *F. hepatica*, and are important for the detoxification of endogenous or exogenous toxic compounds. As an adaptive response, the GST are induced through the regulatory gene elements by the chemical stress of electrophiles and by the reactive oxygen species capable of generating free radicals *in vivo*. Several experiments have been conducted with native GST proteins isolated from the flukes. For example, sheep which received multiple vaccinations with native *F. hepatica* GST in Freund's complete adjuvant showed a 57 % reduction in worm burden. However, the isolation of these antigens from the flukes is very expensive and time consuming. In our research we focused on investigation of protectiviy and immunogenicity of a cloned GST administered to the vaccinated hosts in cDNA or protein forms. Experiments conducted so far have shown that rats vaccinated with either recombinant GST or cDNA of the enzyme developed 48% and 54% percent of protection respectively, against *F. hepatica* cercarial challenge infection. Rats vaccinated with cDNA of GST showed very low antibody response. There have been some reports from studies concerning development of anti-

malaria vaccine that priming with cDNA of the vaccine antigen then boosting with protein form of the antigen may increase the effectiveness of vaccination. In the present experiment we designed to investigate this vaccination procedure.

Three groups of rats were used in the experiment. Rats of group 1 were initially injected with 50μg of GST cDNA containing vector and three weeks later received injection of 50μg of GST protein. Rats of group 2 received 50μg of GST protein as the first immunisation and three weeks later were injected with 50μg of GST cDNA containing vector. Rats of group 3 were injected with an empty plasmid and served as a control. Three weeks after the second immunisation, all rats were challenged orally with metacercariae of *F. hepatica*. On the day of challenge, and then every three weeks, the rats were bled *via* the tail vein in order to obtain serum samples. All rats were 9 weeks after the infection and their livers were examined carefully to estimate fluke burdens.

The protection level observed in group 1 was 71% and in group 2–62%. Rats of group 1 showed a very prominent IgG1, IgG2a, IgG2b and IgA anti-GST antibody responses. After the challenge infection only a slight increase of anti-GST IgA was observed. Rats of group 2 developed a very poor antibody response following vaccination, after the challenge infection an increase of IgG2a and IgG2b antibody levels was observed.

The results of the present experiment suggest that injection of cDNA of GST as the first vaccination and GST protein as a second vaccination induces higher protection level and higher antibody response than priming with the GST protein and boosting with cDNA of the antigen.

THE INFLUENCE OF ANNNEALING TEMPERATURE AND MAGNESIUM CONCENTRATION ON THE SPECIFICITY AND SENSITIVITY OF A PCR REACTION FOR THE DETECTION OF *FASCIOLA HEPATICA* IN THE SNAIL INTERMEDIATE HOST

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PCR – the polymerase chain reaction is a widely used and developing diagnostic tool in parasitology. The method has many advantages such as high specificity, sensitivity, efficiency and speed. However to achieve the best possible performance of PCR, it is necessary to determine the optimal reaction parameters, both reagent concentrations and cycling conditions. Among these parameters annealing temperature and magnesium concentration are ones of the most crucial for the specificity and sensitivity of the reaction.

Annealing is the process in which primers attach to complementary places on the template DNA. It is important that no mispriming occurs because this leads to unspecific products. The temperature of annealing is a critical parameter for this process. It depends on the length and base content of the primers. If the temperature is too low primers will anneal to sequences other than the target sequence. Too high annealing temperature will decrease product yield.

Magnesium ions act as cofactors to the polymerase which catalyses the elongation of products. Their concentration determines the activity and fidelity of the enzyme. Too high magnesium concentrations decrease fidelity, too little of magnesium ions decreases enzyme efficiency and therefore reaction yield. Moreover, the amount of available ions also depends on the concentration of primers, template DNA and nucleotides because all sequester magnesium.

In this research the influence of various annealing temperatures and magnesium concentrations was investigated while elaborating a PCR-based test for the detection of *Fasciola hepatica* in the snail host. The target sequence was a 124 bp fragment, repeated in about 300000 copies in the parasite genome. There were also attempts to use the cysteine proteinase (cathepsine) sequence but it was not species specific. Primers were designed with the PRIMER 3 programme. The left and right primers were 22 and 20 bp in length, had a melting temperature (Tm) of 57.9 and 59.9°C, GC content of 36.7 and 55% respectively. The optimal annealing temperature was determined to be 54°C – 3.9° lower than the Tm of the left primer; the magnesium concentration 3 mM, with mean template DNA concentration being 1.99 µg per reaction, nucleotide concentration – 0.4 mM and primer concentration – 0.4µM each primer, in the presence of 2 UI of Taq polymerase. Generally the recommended PCR reaction conditions include an annealing temperature about 5 degrees below the lower Tm of a set of primers and a magnesium concentration of 1.5 mM or higher. In this study the correct annealing temperature was only 3.9° lower than the corresponding Tm, whereas the correct magnesium concentration was twice the initial recommended value. Results show that empirical optimization of these two parameters is essential for satisfactory and correct product yield, which determines the specificity and sensitivity of the PCR reaction.

SOMATIC AND SURFACE PROTEINS FROM *SYPHACIA OBVELATA* (OXYURIDAE) INFECTED MICE (BALB/C STRAIN)

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Syphacia obvelata, common oxyurid affecting laboratory mice, is an intestinal nematode belonging to the family *Oxyuridae*. The life cycle is simple and mice become infected by ingesting eggs directly from the perianal region of an infected animal or directly through contaminated food, water and bedding. According to the literature data, their infection prevalence in *Mus musculus* might reach 91.5% and the intensity ranged from 13 to 67 specimens in host.

The pinworms in mice have been thought to affect weight gain, growth rate and some various disorders of the intestine. Natural infection with this parasite can modulate immune response of the host and influence experimental results.

There have been limited data on the antigens of *Syphacia obvelata* thus present work provides molecular weights of somatic and surface proteins extracted from this parasite.

Both somatic (AS) and surface extracts (AP1, AP2, AP3) were separated by SDS-PAGE electrophoresis showing the protein profile as follows: AS extract showed 23 protein bands (90, 87, 82, 80, 79, 72, 69, 66, 65, 63, 54, 50, 48, 47, 44, 41, 39, 37, 35, 34, 31, 28, 27 kDa); surface AP1 extract showed 22 protein bands with MW of 126, 117, 115, 110, 108, 102, 95, 89, 84, 70, 65, 60, 58, 50, 48, 45, 44, 39, 35, 32, 30, 27 kDa, AP2 extract showed 14 bands with MW of 126, 119, 110, 106, 91, 75, 70, 61, 57, 55, 52, 48, 46, 30 kDa and AP3 fraction revealed 11 clear protein bands with MW of 124, 117, 108, 97, 91, 82, 75, 70, 58, 52, 49 kDa respectively.

CO-FEEDING OF *DERMACENTOR RETICULATUS* (FABR.) AND *RHIPICEPHALUS APPENDICULATUS* NEUMANN ADULTS (ACARI: IXODIDAE) ON RABBITS SKIN

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Co-feeding of ticks is an object of increasing interest of epidemiologists because pathogen transmission, both of viruses and bacteria, may occur between infected and uninfected ticks in the absence of systemic infection (Gern and Rais 1996, Labuda et al. 1997). In the present study simultaneous feeding of the brown ear tick, *Rhipicephalus appendiculatus* Neumann, 1901 and *Dermacentor reticulatus* (Fabricius, 1794) ticks was investigated. Both tick species belong to the subfamily Amblyomminae.

Materials and methods. Unfed adult ticks of *D. reticulatus* used in the study were collected by flagging in their habitat near Lublin in 2000 and then held under laboratory conditions at a temperature of 25˚C and 85% RH. A laboratory colony of *R. appendiculatus* was maintained by feeding all three stages on New Zealand rabbits. The ticks were fed on tick naive, 6 months old New Zealand female rabbits. Three groups of rabbits, two rabbits each, were infected with the same number of ticks. The first group was infected with 8 females and 4 males of *D. reticulatus* and simultaneously with 7 females and 3 males of *R. appendiculatus*. In the second group 15 female and 7 male of *R. appendiculatus* were used, in the third group equivalent numbers of *D. reticulatus* were applied. The process of attachment was observed every hour during the first day of invasion, and then every 24 h.

Results and discussion. The attachment of adult ticks on rabbits over simultaneous infection was significantly different than in the groups in which specimens of the same tick species occurred. While slow and incomplete attachment was observed in the group of *Rhipicephalus* feeding alone, there was a significant change in the mixed infection, as the *R. appendciculatus* in the presence of *D. reticulatus* was attaching faster. Female's of brown ear tick began to attach after 24 h of infestation when fed simultaneously with *D. reticulatus*, whereas when fed alone began attachment on the fourth day of invasion. While maximum 66% of applied females of *R. appendiculatus* were attached in the homogenous group, in the presence of *D. reticulatus* 100% of applied ticks were attached. In contrast, *D. reticulatus* female in the mixed infection were attaching slower in comparison to the homogenous group. The results of the study indicate that co-feeding ticks modify the duration of attachment period. It can be caused by the production of a substance with pheromone characteristics that affects both species. This phenomenon can be also connected with immunomodulation at the tick-feeding site that was reported by recent studies on other tick species.

THE SURVIVAL OF *ARGAS REFLEXUS* (FABR.) LARVAE (ACARI: ARGASIDAE) FROM POLISH POPULATION UNDER DIFFERENT HUMIDITY CONDITIONS

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Argas reflexus (Fabricius, 1794) is a tick species that occupies facades and attics of buildings and towers of churches. Hungry ticks invade human flats when the number of pigeons is strongly reduced or when these birds are expelled during the restorations of buildings.

In this study unfed individuals of *A. reflexus* collected from the walls and crevices of attics in the vicinity of pigeons' nests in Upper Silesia were used. In the laboratory these ticks were fed on pigeons. Engorged individuals were held under optimal conditions, i.e. at 25°C and relative humidity (RH) of 30% attained by using $CaCl_2$ solution. Eggs laid in each egg batch were transferred into incubation chambers. A single egg deposit constituted the material for one experiment. The embryonic development of *A. reflexus* took place under optimal conditions for this tick species (Buczek 1988). Just after the hatching larvae were either transferred to RH 90% attained by using KNO_3 solution and 25°C or they remained at RH 30% and 25°C. 124 larvae of *A. reflexus* were observed in this study. The duration of survival period was described on the basis of everyday observations.

The results were statistically worked out by using Man-Whitney`s test. The differences in results between two groups are statistically significant (the value of a function $z = 4.151$ at $p = 0.000033$). Differences between two groups were compared by using a χ^2 test, as well ($\chi^2 = 48.28$; $p < 0.001$).

Larvae of *A. reflexus* lived approximately 45.6 days under optimal conditions, whereas 37 days at RH 90%. As many as 80.7% larvae lived from 3 to 42 days at 90% RH. Only some specimens showed longer survival time under these humidity conditions. At 30% RH 34.3% of larvae lived from 1 to 42 days. The group of larvae at 30% RH that survived more than 42 days was more numerous; 58.2 % individuals survived 69 days after hatching and 7.5% even more than 70 days (together 65.7% of ticks kept at 30% RH).

The results of our study show that *A. reflexus* is adapted to conditions of its natural habitat that is characterised by relatively low humidity. The development of this tick species at high humidity (90 RH) is disturbed (Buczek 1991, 1993).

TICKS (*IXODES RICINUS*) AS VECTORS OF DISEASES IN ŚLĘŻA MASSIF (LOWER SILESIA)

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Ixodes ricinus, being the most common tick in Europe, is immensely important in human and veterinary medicine. Faunistic survey of ticks, performed using the flag method, in the Ślęża Massif in years 1998-2001 showed the wide spread occurrence only *I. ricinus* specimens. A total of 3843 individuals were found (8.7% males, 8.6% females, 72.5% nymphs, 10.2% larvae). The samples with the highest number of ticks were collected close forest trails, where humidity was over 70%. The peak of seasonal activity in each year appeared in spring and fall.

The most frequently reported tick-borne infection in Poland are TBE and Lyme diseases and *I. ricinus* is the main vector of the tick-borne encephalitis virus and the spirochete *Borrelia burgdorferi*.

The aim of the present study was the estimation of quantify infection of spirochetes in the population of *I. ricinus* collected from vegetation in forest habitat of Ślęża Massif (Lower Silesia), during the study periods: August-November 2001 and April-June 2002. The area sampled has been considered as recreational and it is visited by a large number of people.

A total of 567 ticks (27 adults, 396 nymphs and 144 larvae) collected by dragging a flag over the vegetation (grass and shrubs in forest) were examined, individually or pooled in groups of 2-3 specimens each, using dark field microscopy for the presence of spirochetes. Spirochetes were found in 26 samples of *I. ricinus* – the infection rate among ticks was 4.58%; among 27 adults 11.1% were positive; among 396 nymphs 5.3% were positive; among 144 larvae 1.4% were positive. The intensity of infection in individual ticks was low, which means up to 10 spirochetes were found in one tick.

MOSQUITO LARVAE (DIPTERA: CULICINAE) COLLECTED FROM WATER BODIES IN THE WROCŁAW AREA

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A constant increase in eutrophication of inland urban and suburban natural and man-made aquatic and semi-aquatic ecosystems, resulting from natural and anthropogenic factors, recorded in recent decade in whole Poland, has been conducive to growth of the population of aquatic insect larvae, mostly those of mosquitoes.

A total of 13214 larvae specimens were collected from twelve sampling sites in 1999-2000. There represented eight hemathophagous and antropophilic species: *Culex pipiens* (Linnaeus), *Culiseta annulata* (Schrank), *Aedes cantans* (Meigen), *A. communis* (de Geer), *A. excrucians* (Walker), *A. sticticus* (Meigen), *A. vexans* (Meigen), *Anopheles maculipennis* (Meigen).

Only one species (*Culex pipiens*) can be recognised as constant (S = 80.1–100%), two (*Aedes vexans* i *Culiseta annulata*) were not frequent (S = 20.1–40%) and the remaining five (*Anopheles maculipennis*, *Aedes excrucians*, *A. cantans*, *A. communis*, *A. sticticus*) were sporadic (accidental) species (S = 0-20%). According to the dominance criterion four species (*Cs. annulata*, *Cx. pipiens*, *An. maculipennis*, *Ae. vexans*) were within the dominant class; one of them, cosmopolitan *Cx. pipiens*, showed parallelly the highest constancy during three seasons. Three species (*Ae. cantans*, *Ae. excrucians*, *Ae. sticticus*) were included in the subdominant class, and only *Ae. communis* in satellite species. Holarctic species (*An. maculipennis*, *Ae. excrucians*, *Ae. communis*, *Ae. sticticus*, *Ae. vexans*) formed the most abundant group (56%), two palaearctic species (*Cs. annulata* i *Ae. cantans*) and one cosmopolitan species (*Cx. pipiens*) occurred in equal proportions (22%).

Mosquito larvae developed in habitats where fluctuations of average water temperature was between 16.7°C and 21.6°C in 1999-2000. The lowest temperature (below 20°C) were noted in wooded areas (Zakrzowski Forest) located on the edge of north-eastern part of the city (mean 17.1°C) and at Sołtysowicki Forest (19.5°C), as well as at Irrigation Fields (19.7°C). Only in Strachociński Forest (similar to Szczytnicki Park, Leśnicki Park and Złotnicki Park) the mean water temperature was slightly above 20°C.

In our own investigations, the triggering role of temperature in mosquito development was confirmed. In most sampling stations the temperature determined also the development rate of mosquitoes. Correlation between the abundance of particular species and the water temperature varied from r = 0.33 to r = 0.76; there was no weak correlation ($0.1 \leq r_{xy} < 0.3$).

Two dominant species (*Cx. pipiens* and *Cs. annulata*) were found in the polluted samples from the stations of the highest mosquito abundance (Irrigation Fields, Strachociński Forest, Bystrzyca Settlement, Kozanów Settlement, Złotnicki Park). The NH_4 concentration exceeding the standards was noted in all the sampling sites (except for Złotnicki Park), NO_2^- – at Irrigation Fields, CHOD – at Irrigation Fields and Bystrzyca settlement and BOD Bystrzyca settlement and Złotnicki Park. Abundance of synanthropic mosquito species at breeding sites (e.g. Irrigation Fields and Bystrzyca Settlement) was directly proportional to the concentration of nitrogen compounds and chloride ions (correlation coefficients r = 0.89 and 0.98, respectively). There were no significant correlation between the mosquito larvae abundance and the number of bacteria. The native *An. maculipennis* which, like the American species of the genus *Anopheles* (*An. darlingi*, *An. oswaldoi*, *An. triannulatus*) occurring in the Orinoco valley (Venezuela), preferred insulated parts of water bodies located in Zakrzowski Forest and Złotnicki Park at Wrocław area.

THE BODY OF KNOWLEDGE ABOUT BAT FLIES NYCTERIBIIDAE (DIPTERA: PUPIPARA) IN POLAND

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In the systematics of true flies, the family Nycteribiidae is placed together with Streblidae and Hippoboscidae in the group Pupipara. About 260 Nycteribiidae species are known to date. They are distributed throughout the world. In the Palaearctic 34 species have been recorded, of which 15 in Europe and 9 in Poland (with the occurrence of three being doubtful and requiring confirmation). The state of research into Nycteribiidae varies in Europe from country to country, but is always closely connected with the level of knowledge about the bat fauna of the given country.

The most abundant representative of Nycteribiidae in Poland is *Nycteribia (N.) kolenatii*, whose principal host is Daubenton's bat (*Myotis daubentonii*) and which it infects in great numbers as a rule. This fly species has so far been recorded in 37 localities in various parts of Poland. The remaining species are few in numbers and uncommon. They include: *Basilia nana* (parasitizing mainly on Bechstein's bat, *M. bechsteinii*), *Penicillida monoceros* (a boreal species, usually caught in late autumn and winter, parasitizing on Daubenton's bat, *M. daubentonii*), *Nycteribia (N.) latreillii*, and a West Palaearctic species *Nycteribia (A.) vexata* (whose chief hosts are the large mouse-eared bat, *M. myotis*, and a species alien to Poland, the lesser mouse-eared bat, *M. blythii oxygnathus*). The rarest species among indigenous bat flies is *Basilia italica* (the main parasite of the whiskered bat, *M. mystacinus* and Brandt's bat, *M. brandtii*).

The species whose occurrence in Poland is doubtful include: *N. (N.) schmidlii* (a Mediterranean species parasitizing mainly on the common Schreiberg's bat *Miniopterus schreibersii*, which does not occur in Poland), *B. nattereri* (whose principal host is probably Natterer's bat, *M. nattereri)*, and *P. dufourii* (a Palaearctic species whose principal hosts are the large mouse-eared bat, *M. myotis*, and the lesser mouse-eared bat, *M. b. oxygnathus*).

The Nycteribiidae fauna in Poland is known rather well, but our knowledge about the bat flies in the particular regions varies. They have been described in greatest detail in western and south-western Poland (especially in Lower Silesia), and this is where the most abundant materials come from. There are no data on the Nycteribiidae from southern Poland, especially the mountain areas (the Bieszczady, Pieniny and Tatras), where one can expect to find species of bat flies (and perhaps also of bats) that will be new records for Poland.

As follows from the studies carried out to date, the most infected bat species in our country is Daubenton's bat. The prevalence of its infection amounts to about 81%, and the maximum number of flies on a single specimen can reach 38. One can conclude, therefore, that the occurrence of *N. (N.) kolenatii* is controlled by the presence of Daubenton's bat, and the role of the other bat species in the spread of this fly is altogether negligible. Among frequently infected species are Natterer's bat (11%) and the whiskered bat (8%), followed probably by Bechstein's bat (42%). The last species is comparatively uncommon in our fauna, but nearly half of the specimens examined had bat flies. The remaining bat species recorded from Poland were infected extremely rarely, including the large mouse-eared bat, on which higher fly infections have been reported much more frequently from the more southern parts of Europe. The large mouse-eared bat is considered the principal host of three bat fly species: *N. latreillii*, *N. vexat*a, and *P. dufourii.*

PREFERENCES OF PARTICULAR COCKCHAFERS' BODY PARTS WITHIN PHORETIC FORMS OF *UROPODA ORBICULARIS* (MÜLLER, 1776)

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Authors present the results of observations on the preferences of phoretic deutonymphs of *Uropoda orbicularis* for particular parts of body of the cockchafer from the genus *Aphodius*. Scientific research of this kind was conducted for the first time.

When analyzing the location of mites, selected parts of hosts were taken into account, i.e. head, pronotum, elytrae and ventral side. Ventral side was separated into legs, abdomen's sterna and the rest of ventral side.

Researches showed that deutonymphs of *U. orbicularis* get attached to various parts of the host's body; however, they have significant preferences for some parts of the body. The largest number of phorents was found on the ventral side of insects. Most of the mites (49%) were found on cockchafers' third pair of legs, and the number was decreasing towards the first pair of legs. The second largest number of mites were attached to elytrae (29%) and it was recorded that deutonymphs were concentrated on its apical part. The mites significantly avoided the rest of ventral side of the host's body, besides legs, on which they were located on abdomen's sterna and pronotum. No mite was found on heads of analyzed cockchafers.

Type and intensity of phorents' location can partly reveal how deutonymphs get fixed onto the insect.

PHENOMENON OF HYPERPHORESY AMONG MITES

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The phenomenon of hyperphoresy is based on the situation where an organism being phoretically transported carries other organism. Some authors additionally distinguish the phenomenon of overphoresy (double phoresy), for instance in the case of the relationship mite-flea-bird. Regarding the insufficient knowledge and documentation of those phenomena, authors present results of their observations concerning hyperphoresy. The results are illustrated by means of scanned pictures showing hyperphoresy and overphoresy among various organisms. Several cases were observed:

(1) deutonymphs of *Uropoda orbicularis* carrying other deutonymphs of the same species,

(2) female of *Macrocheles glaber* transporting deutonymphs of *Uropoda orbicularis*,

(3) flea from mole's nest (*Talpa europea*) transporting deutonymphs of *Phaulodiaspis borealis* (overphoresy).

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ANGUILLICOLA CRASSUS (NEMATODA: DRACUNCULOIDEA) STAGE III LARVAE IN GASTEROSTEID (GASTEROSTEIDAE) FISH OF THE GULF OF GDAŃSK (BALTIC SEA)

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Anguillicola crassus is a Far East native and parasitizes the swim bladder of eel. Since its introduction to Europe, in the early 1980s, along with the imported eel, the nematode has been playing an economically important role. In a short time following its introduction the nematode attacked native populations of the European eel (*Anguilla anguilla*) and is at present found almost in the entire Europe, including Poland (Kennedy and Fitch 1990, Własow 1991, Rolbiecki et al. 1996). The eel (definitive host) become infected either *via* intermediate hosts (primarily copepods) or *via* paratenic hosts (small fish) containing the invasive stage 3 larva (De Charleroy et al. 1990, Haenen and Banning 1990).

The study described was carried out from May until December 2001. The fish were caught in the Gulf of Gdańsk (off Hel, Chałupy, and Gdynia) and in the Death Vistula (Górki Wschodnie). The parasites were looked for in temporary mounts of the digestive tract walls, liver, spleen, swim bladder, and gonads. A total of 1854 gasterosteids (1523 sticklebacks *Gasterosteus aculeatus*, 330 three-spined sticklebacks *Pungitius pungitius*, and 1 sea stickleback *Spinachia spinachia*) were examined. The sticklebacks were divided into three length classes: 2.5–4.5cm, 4.51–6.5cm, and longer than 6.51cm.

The nematode was found in 8.1% of the sticklebacks (1.2 ind. mean infection intensity) and in 3 three-spined stickleback (5.2-, 6.4-, and 7.3- cm long; 4 nematodes were found in June and one in August).

The stickleback infection was season-dependent. The infection level was correlated with fish length and capture site. The heaviest infection occurred in August (off Hel) and in September (Gdynia, Chałupy, and Górki Wschodnie); the fish smaller than 6.51 cm were more heavily infected. The heaviest infection was recorded in the stickleback caught off Chałupy.

The data demonstrate that stickleback may act in the Gulf of Gdańsk as a paratenic host of *Anguillicola crassus*. This is particularly important for larger eel which, due to their size, feed predominantly on fish.

ECTOPARASITE AND ENDOPARASITE OF GOSHAWK CHICKS (*ACCIPITER GENTILIS*) NESTING IN WROCŁAW VICINITY

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This study was an evaluation of the health status of goshawk chicks (*Accipiter gentilis*) from the nests in the vicinity of Wrocław. The research was conducted in spring 2000. Each nest was inspected three times at different phases of the reproduction period and the protocol included: the counting of eggs in each nest, clinical examination of chicks, collecting swabs from the crop, blood and litter samples for parasitological examinations.

Parasitological examination. The type of ectoparasite and intensity of the infection was determined during the clinical examination. Crops swabs were inoculated at 37°C on a *Trichomonas* Medium (Biomed, Kraków, Poland) to isolate *Trichomonas* spp. They were incubated for 48 h at the same temperature and than examined on the slide. Growth of *Trichomonas* spp. was examined by light microscope. The flagellates could be seen due to its characteristic movement. Positive samples were confirmed with Giemsa stained smears.

The blood smears were stained following the Giemsa method and examined under magnification of 1000x (Oil immersion).

Out of the 40 eggs that were found in 11 goshawk nests, 33 chicks hatched and 28 nestlings survived at least until day 35-40.

The presence of *Trichomonas gallinae* was confirmed in 35% of the crops of chicks at the second visit and in all crops at the third visit. In 22.2% of the infected birds there were visible pathological changes with yellow wheatgrain-sized nodule formations on the oral cavity.

During the second nest control, the fly *Carnus hemapterus* (Diptera) were noticed on chicks in 5 nests. The intensity of invasion varied from 6 to 24 parasites on one bird. At the third visit this parasite was not found.

In the blood smears of 53.6% of the goshawk chicks *Leucocytozoon* spp. was found.

NEW DATA ON PARASITES OF MOLE *TALPA EUROPAEA* (MAMMALIA: INSECTIVORA) IN NORTHERN POLAND

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The knowledge of parasitic fauna of *Talpa europaea* is very incomplete. Although studies on various parasites of small mammals involved also a few moles, they were indeed very few because of their inaccessibility resulting from their mode of life and because of legal protection extended on those animals. Best known among mole parasites are fleas, of which 14 species were identified; most data were collected during studies on mole nests (e.g., Niewiadomska 1953; Skuratowicz 1954, 1966, 1972; Zwolski 1960; Bartkowska 1973, 1981; Haitlinger 1978). In addition, one record of a louse *Hoplopleura acanthopus*, most probably an accidental presence (Wegner 1956) and a number of Acari taxa, including *Ixodes ricinus*, *I. trianguliceps*, *Hirstionyssus talpae*, *H. carnifex*, *Eulaelaps stabularis*, *Eadiea brevihamata, Neotrombicula talmiensis*, *Labidophorus talpae* are known (Micherdzińska 1959; Zwolski 1960; Haitlinger 1976, 1981, 1983, 1988, 1989; Siuda 1993). Among the helminths, the trematode *Ityogonimus talpae*, the cestodes *Choanotaenia filametosa* and *Staphylocystis bacillaris* as well as 4 nematode species have been recorded (Serafiński 1928, Łukasiak 1939, Żarnowski 1955, Furmaga 1959).

The present study involved 11 moles collected from northern Poland: 7 from the Tricity area and single specimens from Pszczółki (near Tczew) and the vicinity of Zelewo (near Reda), Wyskok (near Kętrzyn), and Olsztyn-Kortowo. The materials, dead mole specimens found during field drips, were collected in 2000–2002.

The moles examined showed the presence of a total of 10 parasitic species. Their list includes insects belonging to the Aphaniptera – *Histrichopsylla talpae*, *Ctenophthalmus bisoctodentatus*, *Paleopsylla kohauti,* as well as the Acari – *Ixodes trianguliceps*, *I. ricinus*, *Hirstionyssus carnifex*, *Eadiea brevihamata*, *Demodex talpae*. Internal parasites were represented by the nematode *Spirura talpae* and an eimerid protozoan.

Demodex talpae was recorded in Poland for the first time. This is a specific mole parasite, described in 1921 by Hirst and basically not mentioned later on, except in reviews (Lombardini 1941; Bukva 1993, 1995). This is most probably related to a poor knowledge both on the mole parasitic fauna and on follicle mites in wild mammals. It seems that the species should be redescribed according to the modern standards used for demodecid taxonomic descriptions. In the present study, the parasite was found in skin sections of 4 hosts and averaged 7 individuals per host. *H. carnifex* (*Echinonyssus carnifex*) was found to be abundant (76 individuals) on a single host; *E. brevihamata* was recorded in two specimens (2 and 3 individuals). Of ticks, an *I. triangulíceps* nymph and an *I. ricinus* larva were found. Relatively few fleas were found, due to the collection method (examination of dead moles) that was likely to result in their underrepresentation. *H. talpae* was found in two specimens (1 and 2 individuals); this largest European flea, although common, is never very abundant on a host. *C. bisoctodentatus* was represented by 5 individuals found in 3 hosts, while *P. kohauti* (1 individuals) occurred in only one.

Stomachs of 2 hosts were found to house 43 nematodes belonging to *Spirura talpae*, a species occurring primarily in the Insectivora; one host harboured 38, while the other 5 individuals. In addition, the Eimeridae protozoans, not recorded earlier in Poland, were found in the liver of a single host.

THE PRELIMINARY REPORT CONCERNING OCCURRENCE OF *ENTAMOEBA GINGIVALIS* IN PATIENTS WITH PERIODONTAL DISEASES HOSPITALIZED IN DEPARTMENT OF MAXILLO-FACIAL SURGERY MEDICAL UNIVERSITY OF WROCŁAW

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The aim of this study was to assess the frequency of occurrence of *E. gingivalis* in patients with periodontal diseases. The pathogenicity of this protozoan has not been examined thoroughly as not many researchers studied this problem yet. The opinions on pathogenicity of *E. gingivalis* vary. On one hand this protozoan is considered as a harmless commensal, on the other hand it is rated among pathogenes, which cause periodontitis and gingivitis.

Until now (from May to July 2002) we examined 38 patients hospitalized in Department of Maxillo-Facial Surgery Medical University of Wrocław. The presence of *E. gingivalis* was acknowledged in 24 individuals, which amounts to 63%.

The way of detecting of *E. gingivalis* was based on making microscopic preparations using a special liquid, which extends the vitality of Protozoa. Next these specimens were examined in a contrast-phase microscope. *E. gingivalis* was identified by characteristic movement by pseudopodia. The protozoans present in diagnostic material (swabs taken from gums and dental pockets) were photographed (lens x 40, eyepiece x 10).

The research which is going to follow will be based on the correlation between occurrence of *E. gingivalis* and types of periodontal diseases, age, sex and treatment.

CRYPTOSPORIDIOSIS IN PATIENTS WITH COLON CANCER

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Cryptosporidium sp. is an intracellular parasitic protozoan infecting a wide range of mammals, birds, reptiles, and fishes. Coccidians of this genus have been considered the aetiological factor of diarrhoeas of humans and animals worldwide. The source of infection is either drinking water or an infected person or animal. Among numerous species of the genus *Cryptosporidium* the highest epidemiological importance for humans is demonstrated by *C. parvum*. The course of cryptosporidiosis is dependent on the state of the immunological system of the host. Therefore this pathogen is highly infective for immunodeficient people (e.g. AIDS patients), immunosupressed people, children, and elderly people.

Within the first half of 2002 faecal samples from 28 patients (15 men, 13 women) were examined. The patients were earlier diagnosed with colon cancer and they were hospitalised in the Clinic of Surgery, Clinic of Nephrology, Transplantology, and Internal Diseases, and the Clinic of Gastroenterology of the Pomeranian Medical University in Szczecin. Control group consisted of 12 farm workers, employed in a farm identified as a focus of calf cryptosporidiosis. An immunoenzymatic test (ProSpecT®Cryptosporidium Microplate Assay) produced by Alexon Trend was used in the study. Positive reactions to coproantigen of *Cryptosporidium* were found in 10 patients with colon cancer (6 women and 4 men) which constituted 35.7% of patients studied. People of control group showed no infection with *Cryptosporidium*.

The results of this pilot study indicate a relatively high percentage of *Cryptosporidium* infection in patients suffering from colon cancer compared to control group. The present results also do encourage a wider study on a larger group of patients to be carried out.