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collected from the experimentally infected cattle and naturally infected cattle and cervids showed cross reactivity on IFA and a commercial C-ELISA for bovine anaplasmosis. Although the clinical significance for animals infected with the novel *Ehrlichia* genotype appears to be minimal, the zoonotic potential is unknown, and the implications of the serological cross reactivity are significant for the diagnosis and control of bovine anaplasmosis. Further research is needed to elucidate the biology and transmission of this novel *Ehrlichia* genotype, and to develop reliable and specific diagnostic tools for rickettsial pathogens.

**SY19.P.03**
DETECTION OF THE MOST IMPORTANT SPECIES OF CRYPTOSPORIDIUM FOR HUMAN HEALTH IN RIVER WATER OF IRAN BY GP60 PRIMER

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Human cryptosporidiosis is mainly caused by *Cryptosporidium parvum* and *Cryptosporidium hominis*. *C. hominis* is found almost exclusively in humans, whereas *C. parvum* is found in domestic livestock, wild animals, and humans. These important species have been identified in cases of cryptosporidiosis outbreaks and these species are represented as a potential risk of cryptosporidiosis from water for humans and livestock. Totally 25 water samples collected, 20 samples from river in north of Iran and 5 samples from 2 water treatment plant in Tehran. 5 liter of each River water sample filtrated by membrane filter then purified by sucrose flotation method and 50 liter of each water treatment plant sample filtrated by Filta-Max xpress filters and purified by IMS method. Genomic DNA was isolated from concentrated oocysts by a QIAamp DNA mini kit protocol (Qiagen GmbH, Hilden, Germany) as recommended Jiang et al. (2005). As previously described *C. hominis* and *C. parvum* were determined by nested PCR of the GP60 gene as described by Abe et al 2006 (Abe et al., 2006). PCR products were visualized by electrophoresis in 1.5% agarose gels stained with ethidium bromide. 15 out of 20 river water samples and 2 out of 5 water treatment plant were positive by Gp60 primer. As this primer only detected *C. parvum* and *C. hominis*, so a positive PCR result indicated to presence of important species of *Cryptosporidium* for human health. These results, although limited by the small number of isolates studied, but suggest that the occurrence of the *C. parvum* and *C. hominis* are frequently in water samples study area, so humans could potentially infected by using this water during entertainments activity or drinking unfiltered water.

**SY19.P.04**
RANDOM PRELIMINARY SCREENING OF AN EXPRESSED SEQUENCE TAG LIBRARY OF DICROCOELIUM DENDRITICUM

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Dicrocoeliosis, caused by *Dicrocoelium dendriticum*, is an important hepatic trematodosis which affects a wide range of mammals, mainly ruminants, and occasionally infects humans. However, in spite of the financial and health significant of Dicrocoeliosis its immunological diagnosis is still highly unsatisfactory, as well as its control. A search on NCBI databases for the family Dicrocoeliidae only retrieves 3 proteins and no expressed sequence tag (EST). These findings indicate the need for further research on both, new diagnostic methods and parasite molecular
biology. Our main goal was to clone diagnosis *D. dendriticum* genes to be used as recombinant antigens in the specific immunological diagnosis of the disease. A cDNA library was constructed, with mRNA extracted from *D. dendriticum* adults, using the cDNA synthesis system ZAP-cDNA® (Stratagene). A random screening was performed to identify characteristic ESTs of the trematode by PCR (T3 and T7 primers). The generated amplicons were sequenced and compared with those in GenBank. The selected cDNAs were subcloned into the pGEMT vector (Promega), and competent XL1-Blue cells were transformed with the ligation mixture. Plasmid DNA was extracted with the Qiaprep (QIAGEN) kit, and sequenced using D and SP6 universal primers.

The recombinant percentage of the amplified library was 90% and the size of the inserts ranged from 486 bp to 2000 pb, with most over 700 bp. A preliminary screening of 250 phage plaques from the library resulted in the identification of 113 different cDNAs. According to the literature some of these proteins have been described as possible vaccine targets in other trematodes, and/or as relevant diagnosis antigens: (a) *D. dendriticum* myoglobin (GI: 122064715), proven to be reactive against sera from infected sheep with high specificity against other trematodes; (b) *Clonorchis sinensis* 7KD protein (GI: 21489590) which a potential diagnostic role in this parasitosis, and (c) *Fasciola hepatica* homologous cystatin (GI: 55978577). These cDNAs were subcog in expression vectors using specific primers with the restriction enzyme sites. PCR assay was carried out and the purified products were cloned into both the Glutation-S-Transferase (GST) pGEX6P vector (Health Care), and the His6-tag pRSET vector (Life Technologies), with the recombinant proteins as diagnosis antigens.

This is the first study conducted for identification and characterization of *D. dendriticum* ESTs. A total of 103 different proteins were identified, and three of them subcloned in expression vectors to be tested as potential diagnostic targets.

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SY19.P.05

**STAGES OF INTERSPECIFIC AND INTRASPECIFIC INTERACTIONS BETWEEN HELMINTHES**

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The using of measurement analysis is correct and universal method for valuation of interspecific and intraspecific interactions between helminthes. The objects of our exploration were nematodes *Syphacia obvelata, Aspiculuris tetraperta* from wood and house mice, *Ivaschkinonema alticola, Citellina alatau* from silver vole (*Alticola argenteus*), *Heterakis gallinarum, Ascaridia galli* from home hens, *Ganguleterakis dispar* from home aquatic birds, nematodes *Oswaldocruzia filiformis, Rhabdias bufonis* and trematodes *Opisthioglyphe ranae, Hahlometa cylindracea, Pleurogenes intermedius* from moo frog (*Rana arvalis*). Measured exemplars of every worm species we united dependently on the number of parasites specimens in the host's organism (for the studying of intraspecific relationships) and on the presence of other worm species (for the research of interspecific interactions). The results of investigations showed that helminthes of own and other species may be both synergists and antagonists proceeding from the possibilities of host and parasites. Incidentally we may classify the next stages of interactions. Neutralism, when individual specimens of small and low-pathogenic helminthes are not detected by the host's organism, don't cause the damage by their feeding and living activity, don't compete with each other when the sources of organism is sufficient; they usually have the large sizes. Opposition to the host's organism with the mutual synergism between parasites of one or different species – when the sources of the host's organism are sufficient, but difficulty accessible, and the parasites' synergism is directed to the overcoming the immune barriers and making the accessible to the source. Parasites usually are not numerous, and their sizes are small. Stage of optimal balance – when