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Comparison of three immunodiagnostic tests for experimental *Heterophyes heterophyes* infection in dogs

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Abstract

The aim of this study was to compare the performance of three in-house diagnostic tests, i.e. counter current immunoelectrophoresis (CCIE), intradermal (ID) and indirect fluorescent immunoassay (IFI), for the diagnosis of *Heterophyes* infection. One hundred and twenty puppies were randomly divided into eight groups ($n = 15$/group). *Heterophyes heterophyes* infections were established in these puppies by administering a dose of 50 *H. heterophyes* encysted metacercariae/puppy by gavage. Forty puppies of similar age and sex were divided into eight groups, of five puppies each and were used as negative controls. Sera pooled from separate infected and uninfected groups were tested against *H. heterophyes* antigens, weekly for 8 weeks post-infection (PI). The ID assay detected infected puppies sooner than any of the serological tests. Sero-conversion was first detected 2 weeks PI by ID assay and 1 week later by CCIE and IFI assays. ID test performed well till the end of the experiment (sensitivity and specificity: 100% and 90%, respectively). Both IFI and CCIE assays had a sensitivity of 40% and 20%, respectively for early detection of antibody, which was less sensitive than ID but both assays were more specific (100%) than the ID assay. The lowest agreement was between ID and IFI tests (40.3%), whilst the highest was observed between CCIE and IFI tests (67.2%). Of the three evaluated methods, the ID test could be recommended for scientific and laboratory diagnosis of heterophyosis in naturally infected animals. However, since none of the investigated method are optimal (i.e, offers 100% specificity and sensitivity), the choice of test employed must depend on the aim of the study.

Keywords: Heterophyosis; Dogs; Diagnosis; Serology; Experimental

1. Introduction

The Heterophyidae (Trematoda: Digenea) have a three-host life cycle. As adults they live in a vertebrate definitive host (humans and other fish-eating mammals), the larvae emerging from their eggs has to develop and propagate in a first intermediate host (usually a snail), and the cercarial stages produced in the snail must then encyst as metacercariae in or on a second intermediate host (fish). The life cycle is completed when infected fish is ingested by the definitive host. Transmission of these heterophyids takes place where all the hosts co-occur. Fish infection occurs via skin penetration by infective cercariae. Infection of humans with the intestinal fluke *Heterophyes heterophyes* is acquired by ingestion of

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infective metacercariae encysted in a fish eaten raw or undercooked (Hamed and Elias, 1969).

H. heterophyes is endemic in the Far East, Southeast Asia, and Nile Delta of Egypt (Chai et al., 1984; Chai and Lee, 1990; CDC, 1999; Belizario et al., 2001; Kim et al., 2003). In heterophyosis, diarrhea and colicky abdominal pain are the main clinical symptoms and signs. However, migration of the eggs to the extra-intestinal sites such as heart, liver and brain can occur resulting in potentially fatal consequences (Belizario et al., 2001; Elsheikha, 2007).

The current *H. heterophyes* diagnosis is based on the microscopic identification of eggs in the stool, which is not specific because their eggs are indistinguishable from those of *Metagonimus yokogawai* and resemble those of *Clonorchis* and *Opisthorchis* (Parija et al., 2003). Additionally, heterophyosis is greatly under-diagnosed due to its similarity to common ailments such as acid peptic disease and peptic ulcer disease (Belizario et al., 2001). Blood eosinophilia is indicative for stool examination if other risk factors (travel to an endemic region and/or eating raw fish) are evident.

Based on the widespread use of counter current immunoelectrophoresis (CCIE) for the serodiagnosis of parasitic diseases (Despommier et al., 1974; Krupp, 1974; Hillyer and Capron, 1976; Cruickshank and Mackenzie, 1981; Vullo et al., 1984; Yen and Chen, 1989), the present work was performed to evaluate the applicability and efficacy of a CCIE test for use in diagnosis of experimental *H. heterophyes* infection, compared to intradermal (ID) and indirect fluorescent immunoassay (IFI) tests.

2. Materials and methods

2.1. Parasite sampling

Mullet (*Mugil cephalus*) fish samples ($n = 250$) collected from Manzala Lake in Egypt were screened for the presence of encysted metacercariae by compression method in which snips were taken from different parts of the fish mainly from the head, dorsal and tail regions and viscera. Each piece was compressed between two microscopic glass slides and examined for the presence of encysted metacercariae of heterophyid parasites. Artificial digestion method (Yokogawa and Sano, 1968) using acidified pepsin solution (pepsin–HCl) was then used in case of infected fish in order to separate metacercariae. The digested materials were filtered through a sieve and washed several times with 0.85% physiological saline. Metacercariae were recovered from the sediment using a binocular stereomicroscope with 20× and 40× ocular objectives. Metacercariae of *H. heterophyes* were identified based on their characteristic location between the muscle fibers, thick cyst wall, relatively large size, and heavy pigmentation (Witenberg, 1929). Only metacercariae with size and morphological characteristics compatible with those of *H. heterophyes* were used for infection experiment.

2.2. Study animals

Animals enrolled in the present study were 70-day-old male local domestic dogs ($n = 120$), weighing about 3 kg, clinically healthy and parasite negative by corporological examination. They were obtained in the nearby city of Cairo, where there is no heterophyosis and were confirmed to be serologically (IFI) negative at the start of the experiment. All animals were maintained in our animal facilities for the duration of the experiment. They were kept under strict hygiene conditions and proper management. The puppies were experimentally inoculated orally with 1 ml of phosphate buffered saline (PBS, pH 7.6) solution containing 50 *H. heterophyes* encysted metacercariae. Infected puppies were randomly divided into eight groups of 15 puppies each. In addition, 40 puppies with similar age and sex were randomly allocated into eight groups of five puppies each. These were mock inoculated with 1 ml of PBS only and were used as negative controls. Five animals from the controls and 15 animals from one infection group were sacrificed at weekly intervals post-infection (PI) over a period of 7 weeks PI. Five millilitres of blood samples were taken from each sacrificed animals. Sera were obtained and stored frozen at −20 °C until use. Serum samples were examined for the presence of anti-*H. heterophyes* antibodies using the diagnostic assays described below. Intestines of dogs were examined for the presence of adult *H. heterophyes* worms. Portions of the intestines were trimmed, frozen and used for IFI test. All protocols used in the study were approved by the Institutional Review Board Committee of Mansoura University, Egypt.

2.3. Immunodiagnostic tests performed

2.3.1. Counter current immunoelectrophoresis (CCIE) test

The CCIE technique of Dorff et al. (1971) was adopted in performing this test using undiluted sera obtained from experimentally infected and uninfected puppies. Serum samples were inactivated at 56 °C for 30 min, prior to use, in order to avoid any non-specific