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COMPARISON OF COUNTERIMMUNO-ELECTROPHORESIS WITH INDIRECT HAEMAGGLUTINATION TEST IN THE DETECTION OF ANTIBODIES IN RABBITS EXPERIMENTALLY INFECTED WITH VARIOUS SPECIES OF ASCARIDS

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Abstract. Counterimmunoelectrophoresis (CIEP) and indirect haemagglutination (IHA) were used for the detection of antibodies in 437 sera of rabbits experimentally infected with *Ascaris suum*, *Toxocara canis* and *Toxascaris leonina*. CIEP showed 44.0—52.3 % positivity in *A. suum*, 25.5 % in *T. canis* and 19.7—29.2 % in *T. leonina* infection, whereas the positivity detected by IHA was 59.8—90.2 % in *A. suum*, 44.8 % in *T. canis* and 59.6 % in *T. leonina* infection. A comparison of the two tests reveals that CIEP is a suitable, rapid and simple method for orientation examinations in ascarid infections.

The immunological techniques used for the diagnosis of parasitoses become still more important, particularly the serological tests in tissue helminthoses, e. g., larval toxocarosis. The most common methods used in the last time are indirect immunofluorescent antibody test (Viens et al. 1975) and indirect haemagglutination test (Aljeboori and Ivey 1970). Nevertheless, none of the tests used is sufficiently sensitive and specific.

In our country, Benková and Borošková (1976, 1978) compared the effectiveness of complement fixation, latex-agglutination and indirect haemagglutination tests, Borošková (1981) compared indirect haemagglutination test and indirect immunofluorescent antibody test in experimental ascaridosis of rabbits. Enayat and Pezeshki (1977) used counterimmunoelectrophoresis for the diagnosis of experimental toxocarosis. Lukeš (1982) pointed out the advantages of this method, which is rapid and simple. In the present paper, the effectiveness of counterimmunoelectrophoresis (CIEP) is compared with indirect haemagglutination (IHA) test in the detection of antibodies in rabbits experimentally infected with several ascarid species.

MATERIAL AND METHODS

Sixty chinchilla rabbits (obtained from VELAZ) weighing 2.5 kg on the average (sex ratio 1 : 1) were divided into 7 groups. Each group consisted of 9 animals, except for the control group (group 7), which included 6 rabbits. The groups were infected as follows: group 1 with 5 000 eggs of *A. suum*, group 2 with 10 000 eggs of *A. suum*, group 3 with 20 000 eggs of *A. suum*, group 4 with 5 000 eggs of *T. canis*, group 5 with 5 000 eggs of *T. leonina* and group 6 with 10 000 eggs of *T. leonina*.

On day 35 p. i., 6 rabbits from groups 1, 2, 3 and 5 were reinfected with the same species and same dose of eggs.

On day 65 after the first infection, 3 rabbits from groups 1, 2, 3 were reinfected once more. Animals from groups 4 and 6 were not reinfected.

A total of 437 sera from infected rabbits and 40 sera from control rabbits were tested. All rabbits were previously serologically examined for a control.

Counterimmunoelectrophoresis was performed as described by Krupp (1974) using 90 × 60 mm plates covered with 1 % agarose in 0.1 M veronal-acetate buffer, pH 8.6. Two parallel rows of wells 3 mm in diameter were cut 5 mm apart in agar. On each plate 11 pairs of wells were cut out and the distance between the rows was 25 mm. Electrophoresis was carried out in a IEP-2 tank at 15 milliampere per one plate for 60 min. The plates were washed with saline for 1 h, then with distilled water and dried using a modified method after Laurell (1972). They were stained with Ponceau S stain and

Table 1. Detection of positive sera by means of IHA in rabbits experimentally infected with *Ascaris suum*, *Toxocara canis* and *Toxascaris leonina* larvae

Parasite species Dosis	1st infestion (days 12—82) positive/ examined	2nd infection (days 35—82)	3rd infection (days 65—82)	Total
Ascaris suum 5 000 eggs	34/50 68.0 %	14/33 42.4 %	7/9 77.8 %	55/92 59.8 %
Ascaris suum 10 000 eggs	24/42 57.1 %	22/27 81.5 %	7/9 77.8 %	53/78 67.9 %
Ascaris suum 20 000 eggs	34/36 94.4 %	14/17 82.4 %	7/9 87.5 %	55/61 90.2 %
Toxocara canis 5 000 eggs	26/58 44.8 %	not tested		26/58 44.8 %
Toxascaris leonina 5 000 eggs	31/60 51.7 %	23/31 74.2 %	not tested	54/91 59.3 %
Toxascaris leonina 10 000 eggs	34/57 59.6 %	not tested		34/57 59.6 %

Table 2. Detection of positive sera by means of CIEP in rabbits experimentally infected with *Ascaris suum*, *Toxocara canis* and *Toxascaris leonina* larvae

Parasite species Dosis	1st infection (days 12—82) positive/ examined	2nd infection (days 35—82)	3rd infection (days 65—82)	Total
Ascaris suum 5 000 eggs	22/50 44.0 %	19/31 61.0 %	5/7 71.4 %	46/88 52.3 %
Ascaris suum 10 000 eggs	9/37 24.3 %	15/28 53.6 %	9/10 90.0 %	33/75 44.0 %
Ascaris suum 20 000 eggs	13/34 38.2 %	11/16 68.8 %	4/7 57.1 %	28/57 49.1 %
Toxocara canis 5 000 eggs	14/55 25.5 %	not tested		14/55 25.5 %
Toxascaris leonina 5 000 eggs	14/58 24.1 %	12/31 38.7 %	not tested	26/89 29.2 %
Toxascaris leonina 10 000 eggs	12/61 19.7 %	not tested		12/61 19.7 %

de-stained with acetic acid. The antigen concentration was 2 mg protein/ml (same as in IHA). Homologous antigens of *T. canis* and *T. leonina* in the same concentrations were used for CIEP.

Indirect haemagglutination test was performed after Boyden (1951) using sheep erythrocytes modified with tannin in 1 : 20 000 dilution. Optimal concentration of antigen was determined by titration. The test was performed as group-specific with antigen from adult *A. suum* extracted in saline at pH 7.2. Microplates with Dynatech microtitrator were used.

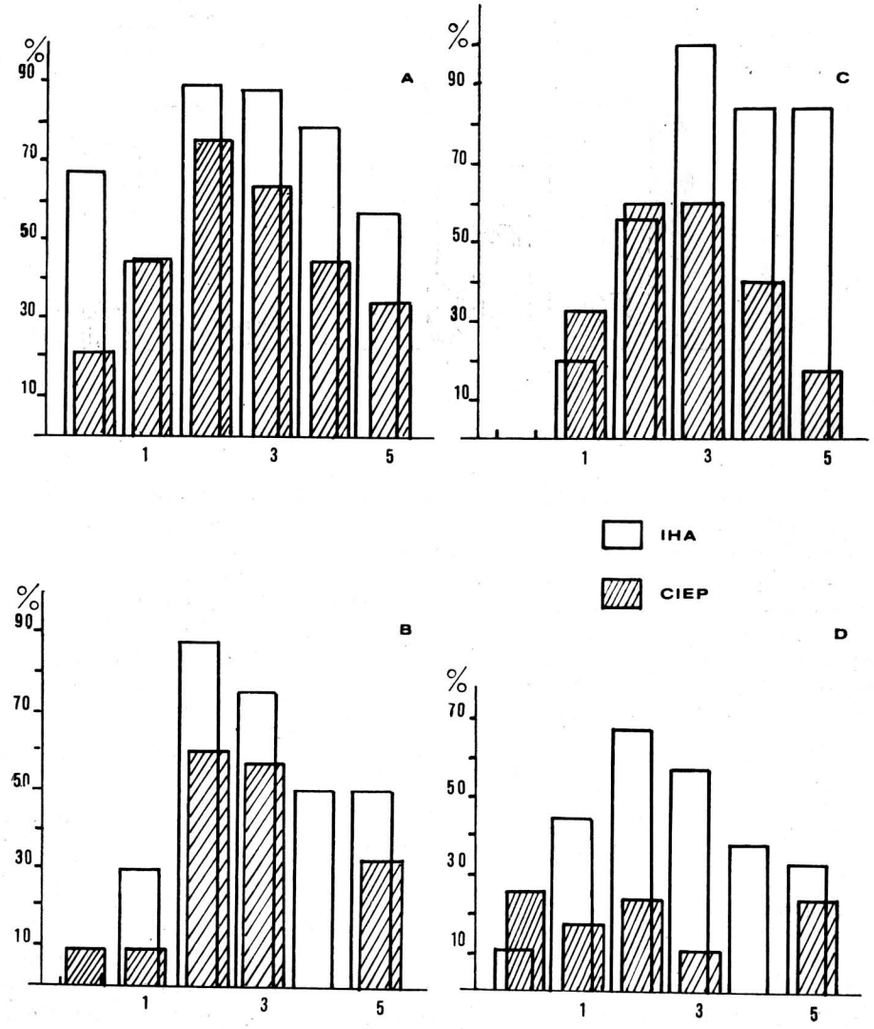

Fig. 1. Detection of positive reactions in the first five weeks after infection. A — *Ascaris suum* (5 000 eggs), B — *A. suum* (10 000 eggs), C — *A. suum* (20 000 eggs), D — *Toxocara canis* (5 000 eggs).

RESULTS

The results obtained are summarized in Tables 1 and 2. Figs. 1—2 show the dynamics of positive reactions with rabbit sera in indirect haemagglutination test and counterimmunoelectrophoresis in the first 5 weeks after infection. It is evident that IHA is most effective from the 2nd to the 4th week p. i. After this period the positivity of sera decreases, even if the increased antibody titre mostly persists till the end of the experiment.

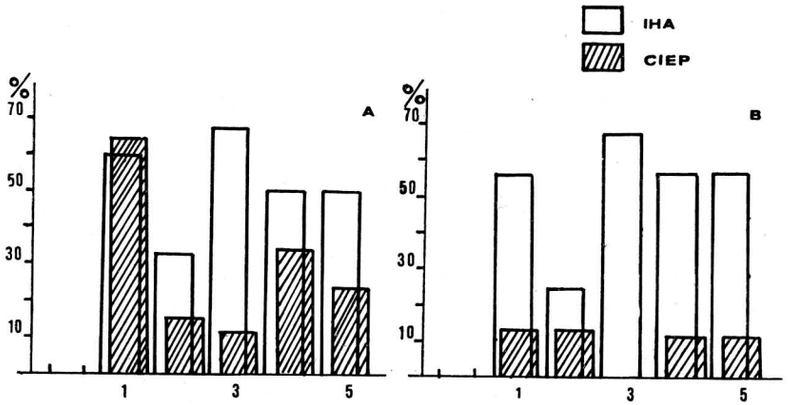

Fig. 2. Detection of positive reactions in the first five weeks after infection. A — *Toxascaris leonina* (5 000 eggs), B — *T. leonina* (10 000 eggs).

There is also a marked difference in the reactivity of experimental animals in the groups infected with *T. leonina*. In spite of the fact that a heterologous antigen was used, the number of positive reactions is markedly higher in IHA (compare Tables 1 and 2). In all groups infected with *A. suum* the peak of positive sera appears in the second and third week after infection when CIEP is used. Table 2 shows that there is no relationship between the intensity of infection and number of positive results in the groups. The number od positive sera is increased only by repeated infections (44—71 % in case of *A. suum* infection). This fact is at variance with the results of IHA, where the number of positive sera markedly increases in the group with stronger infection (68—94 %).

The control sera and sera from experimental groups reacted in CIEP in 7.04 % (5/75) and in IHA in 35 %. In 14 cases the titres were higher than 1 : 4, in 11 cases 1 : 4 and in 15 cases 1 : 2. The control sera were not tested with other antigens than those used in the experiment.

DISCUSSION

Our results confirm that indirect haemagglutination test is for the time being the most sensitive serological diagnostical method for the detection of larval ascaridosis, toxocarosis and toxascaridosis. Similar results were obtained also by Bessonov (1973), Borošková (1981), de Savigny and Tizzard (1977) and others.

The higher number of positive results in control sera may be explained by a previous contact of rabbits with antigens of ascarids and antibodies against other parasites, e.g., protozoans (coccidia), the incidence of which is 100 % in rabbit breeds. According to Čatár et al. (1982) even 40 % of rabbits are infected with *Toxoplasma gondii*.

The main advantage of counterimmunoelectrophoresis, compared to other precipitation methods, is that the gel medium is disposable within a short time (60 min). The results can be read without staining and if a shortened process after Laurell (1972) is used, then even stained and dry preparations can be rapidly obtained. According to our experience, about 95 % of positive results are readable already after 1h washing in saline. It is of advantage to use more antigens (*A. suum*, *T. canis* and *T. leonina*) simultaneously in all variants of the experiment. This is possible due to the high productivity of work when this method is used. It may be supposed that the results of CIEP could be still better if a suitable anodically mobile component of antigen (or mixture of several antigens) were used. Figs. 1—2 show a good agreement between the results of IHA and CIEP. In some cases, CIEP reaches the low level of detection with IHA. The number of positive sera considerably increases in repeated infections.

СРАВНЕНИЕ ЭФФЕКТИВНОСТИ ВСТРЕЧНОГО ИММУНОЭЛЕКТРОФОРЕЗА И НЕПРЯМОЙ ГЕМАГГЛЮТИНАЦИИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЗАРАЖЕНИИ КРОЛИКОВ РАЗНЫМИ ВИДАМИ АСКАРИД

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Резюме. Сравнивали результаты встречного иммуноэлектрофореза (ВИЭФ) и реакции непрямой геммагглютинации (РНГ) на 437 сыворотках кроликов, экспериментально зараженных видами *Ascaris suum*, *Toxocara canis* и *Toxascaris leonina*. С помощью ВИЭФ овнаружено 44,0—52,3 % положительных сывороток при заражении *A. suum*, 25,5 % при заражении *T. canis* и 19,7—29,2 % при заражении *T. leonina*, тогда как с помощью РНГ — 59,8—90,2 % при заражении *A. suum*, 44,8 % при заражении *T. canis* и 59,6 % при заражении *T. leonina*. ВИЭФ оказывается удобным, скорым и несложным методом для ориентировочного обследования при заражении аскаридами.

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M. Katz, D. D. Despommier, R. Gwadz: Parasitic diseases. *Springer-Verlag, New York—Heidelberg—Berlin 1982, 264 pp., 342 Figs, 4 Color Plates, 9 Tables. Price 96 DM.*

At first sight this book differs in its arrangement from other similar manuals. It begins with helminths and this topic covers most pages of the text: out of the five parts of which the book consists, three parts (Nematodes, Cestodes, Trematodes) and 22 chapters (including 191 figures) are devoted to helminths. The arrangement of chapters is uniform and each chapter consists of a brief introduction, historical information, life cycle, pathogenesis, clinical disease, diagnosis, treatment, prevention and control, and references. The fourth part of the book comprising 14 chapters deals with Protozoa. Its arrangement is similar. Only three chapters of the fifth part are devoted to arthropods. The basic scheme of chapters is almost the same as in the previous parts. Some aspects are omitted, but some other data are added, as the role in the transmission of pathogenic agents, epidemiology of the disease and control measures. The book concludes with two appendices (Procedures suggested for use in examination of clinical specimens for parasitic infection and Table of drugs for parasitic infections) and a common subject and name index.

The figures deserve special attention. The plates showing 33 life cycles of various helminth and protozoan species are of an excellent quality. On the other hand, the figures illustrating the development of some arthropod groups are only roughly schematic. It is of interest that there are mostly photographs and only few drawings. This is to advantage but also to disadvantage. Sometimes all important details are not visible in the photograph and, moreover, some of the photographs are not sharp.

The book is intended as a manual for medical students and its format is appropriate for this purpose. A greatest part of the chapters is devoted to the questions interesting for physicians, from pathogenesis to prevention and control. The biology of the parasites is limited only to life cycles; general characteristics of even higher taxons are very brief and any data on the taxonomic classification of parasites are lacking. Some data are not correct. For example, American visceral leishmaniosis is caused by *L. chagasi* (which is now regarded as an independent species) and not *L. donovani*. Colorado tick fever is not the only tick-borne viral disease of man known in the United States. Powassan virus is another pathogenic agent. The name *Isospora hominis* was in fact used for two species of the genus *Sarcocystis*. Some species parasitic in man and closely related to those included in the book are not mentioned, as *Leishmania peruviana*, subspecies of *L. braziliensis* and *L. mexicana* from American continent, other species of *Trichinella* or *Demodex brevis*. Some commonly used names are cited either incorrectly (*Blatella*, *Dermacentor albipietis*, *Pediculus humanis*, *Phthirus*) or with an invalid generic name (*Allodermanyssus sanguineus*, *Trombicula akamushi*). According to ICZN, a comma should be written between the name of author and year.

The authors wrote the book with an intention to present basic information on parasitic diseases to medically orientated readers. This target was attained, particularly in the chapters on helminths. The arthropods were somewhat neglected. It is of advantage that each chapter is concluded with a list of references and the list of drugs in Appendix II is also very useful. The publishers paid a great attention to the publication of this valuable manual.

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