Serological Detection of *Capillaria hepatica* by Indirect Immunofluorescence Assay

MARTINA JUNCKER-VOSS,^{1*} HEINRICH PROSL,¹ HELGA LUSSY,² ULRIKE ENZENBERG,³
HERBERT AUER,⁴ AND NORBERT NOWOTNY²

Institute of Parasitology and Zoology¹ and Institute of Virology,² University of Veterinary Medicine, A-1210 Vienna, Schönbrunner Tiergarten Ges.m.b.H., A-1130 Vienna,³ and Department of Medical Parasitology, Clinical Institute of Hygiene, University of Vienna, A-1095 Vienna,⁴ Austria

Received 25 January 1999/Returned for modification 16 March 1999/Accepted 19 October 1999

In this paper, a serological assay for the detection of antibodies to *Capillaria hepatica*, a zoonotic parasite, is described. In the past, the only way of detecting *Capillaria hepatica* was to perform a liver biopsy. The indirect immunofluorescence (IIF) assay, based on liver sections of naturally infected mice and human serum samples, is suitable for detecting early stages of human infections and for screening purposes. No cross-reactivity with other parasitic infections was detected. We have applied the IIF assay to serum samples of 60 employees of the Zoological Garden of Vienna, Schönbrunn, Austria, and found one positive and one questionable sample.

Capillaria hepatica (Bancroft, 1893) (2) is a zoonotic liver nematode of mammals of worldwide distribution. The parasite frequently causes hepatic infections in rodents and lagomorphs. In humans, infections are rarely found. To our knowledge, only 37 reports of human infections have been published so far; however, human infections may lead to severe disease and death; approximately half of the cases have been diagnosed by liver biopsy, and half of them have been diagnosed at autopsy (Table 1). The life cycle of this parasite, which reveals a marked affinity for the liver (32), is a direct one. Following ingestion of embryonated eggs, larvae hatch in the cecum and reach the liver via the portal vein system. They mature in the parenchyma of the liver and mate, leading to the production of eggs an average of 28 days after mating. Sexually mature males are found in the livers of mice, 15 (18) to 18 (34) days postinfection (p.i.), and sexually mature females are found 15 (18) to 20 (34) days p.i. The life spans of adult organisms are short. References in the literature range from 18 (23) to 60 (34) days p.i. in mice. Eggs deposited in the liver parenchyma remain there and develop only to the eight-cell stage. Further cleavage does not take place until they are released from the liver tissue. Eggs reach the environment only through the decay of an infected carcass or when they are excreted in the feces either of carnivores or of scavengers feeding on infected mammals. Eggs embryonate and become infective in the soil, under optimal conditions, such as exposure to air with a high relative humidity and temperatures of 25 to 30°C, within 5 to 8 weeks. Embryonated eggs remain viable for 25 months at room temperature. The life cycle is completed when a new host ingests the parasite (34).

It has to be noted that the ingestion of nonembryonated eggs does not lead to hepatic capillariasis. In such cases, ova just pass through the gastrointestinal tract of the host following ingestion of uncooked or poorly cooked livers of infected animals and are excreted in the feces. In such spurious infections there are no definite clinical signs (12, 19, 21). Reports of spurious infections must be kept well apart from reports of genuine infections. In the former, eggs of *C. hepatica* are found

in stool, in the latter, they are not. Genuine infections cause severe hepatic disease in humans and animals. Persistent fever, hepatomegaly, and leucocytosis with eosinophilia are reported to be the symptom triad of hepatic capillariasis. Anemia, pulmonary symptoms, splenomegaly, enlargement of the kidneys, and wasting may also occur. Unsanitary practices, poor hygienic conditions, and the presence of dense rodent populations are predisposing factors for genuine human infections. Children or adults with the habit of eating soil (geophagia, pica) (9, 11, 12, 27), as well as children with soil-to-hand-to-mouth contact, are especially at risk.

For differential diagnosis, infections with *Toxocara canis* or *Toxocara cati* (the dog and cat roundworms, respectively; symptom, larva migrans visceralis), *Fasciola hepatica*, and *Echinococcus granulosus* or *Echinococcus multilocularis* must also be considered.

All together, only 37 cases of hepatic capillariasis in humans have been reported between 1924 and 1996 worldwide, with 15 of them having been in Europe. However, human infections with *C. hepatica* probably occur more frequently, showing a milder or even subclinical course (as was demonstrated by different authors who incidentally detected the infection) (Table 1). Until now, the only way of diagnosing *C. hepatica* infections was taking and examining liver biopsy samples.

We report in this paper a serological assay which requires only a serum sample of the patient. The assay is easy to perform and can be used for detecting early stages of human infections as well as for screening purposes.

Six-micrometer cryocut sections were prepared from livers of naturally infected mice, which showed a massive infestation with nonembryonated *C. hepatica* eggs. The mice originated from the natural population of house mice (*Mus musculus*) in the Zoological Garden of Vienna, Schönbrunn, Austria. During 1996 and 1997, mice were trapped and examined for the presence of *C. hepatica* in their livers, both macroscopically by looking for the characteristic yellowish-white lesions and by the tissue press technique. The presence of the parasite was confirmed by recovery of the typical bipolar plugged eggs in the liver preparations. Hepatic lesions consisted of minimal to severe replacement of liver tissue by *C. hepatica* eggs. Nonembryonated eggs from infected livers were recovered by peptic digestion, and their morphological characteristics were examined microscopically. Eggs showed the characteristic features

* Corresponding author. Mailing address: Institute of Parasitology and Zoology, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria. Phone: 43 1 25077 2220. Fax: 43 1 25077 2290. E-mail: Martina.Juncker@vu-wien.ac.at.

TABLE 1. Cases of human *C. hepatica* infestation reported in the literature

Reference	Age	Sex ^a (no. of patients)	Region	Therapy	Outcome
19	20 years	M	India	None	Died
20	17 months	F	Louisiana	None	Died
21	7 years	F	Maryland	Chloroquine	Died after 2 years
30	60 years	M	Turkey	None	Incidental detection
11	15 months	F	Hawaii	Diethylcarbamazine	Died after 4 months
9	15 months	F	South Africa	Antibiotics, cortisone, antimony	Survived (15 years)
33	2 years	F	Louisiana	None	Died, but incidental detection
5	20 months	M	Illinois	Dithiazanine iodide	Survived (10 months)
15	5 years	F	South Africa	None	Incidental detection
26	22 months	F	Mexico	Unknown	Unknown
25	25 years	F	Brazil	None	Incidental detection
6	11 months	M	Ivory Coast	Antibiotics, cortisone	Died, but incidental detection
8	3 years	F	Italy	Some anthelmintic drugs	Unknown
10	Unknown	F	Canada	Unknown	Died
28	17 months	F	South Africa	Diethylcarbamazine, antimony	Survived (1 year)
29 ^b	19–83 years	F (5), M (4)	Czechoslovakia	None	Incidental detection
31	2 years	M	Mexico	Thiabendazole, cortisone	Survived (1 month)
24	39 years	M	Brazil	Pyrantel	Survived (8 years)
1	27 years	F	Nigeria	None	Unknown
3	1 year	F	Switzerland	Thiabendazole	Survived
17	2 years	F	Yugoslavia	Levamisole	Survived
22	18 months	F	Germany	Ivermectine, thiabendazole, cortisone	Survived
27	26 years	M	Japan	None	Survived
7	14 months	F	Korea	Thiabendazole	Survived
16	32 years	F	Japan	Surgery	Survived
4	19 months	M	Mexico	Unknown	Unknown
13	54 years	F	Mexico	None	Incidental detection
14	6 years	M	India	Antimonials	Survived
35	60 years	F	Greece	Some anthelmintic drugs	Survived

^a M, male; F, female.^b Nine cases.

described by Calle (5). They were oval and showed a double-layer shell with radial striations in the outer shell. The average size of the nonembryonated eggs ($n = 100$) was 55.8 by $30 \mu\text{m}$ (ranges, 49.2 to $61.5 \mu\text{m}$ [length] and 24.6 to $41.0 \mu\text{m}$ [width]). Several (42.7%) of the 166 examined livers proved positive. In the infected livers, no larval stages or adult parasites were found except in one case. In this case, a live nematode was slightly connected to the surface of the liver of a mouse. The nematode was identified as *C. hepatica* on the basis of its morphological characteristics (34).

Because of the high rate of infestation of house mice with *C. hepatica* in the Zoological Garden of Vienna, Schönbrunn, Austria, and the resulting high probability of a severe contamination of the environment, there was a potential risk for the employees of the zoo. Therefore, we initiated a serological survey, using the assay described below. Sixty employees agreed to participate in the study.

Cryocut sections of mice livers heavily infested with nonembryonated *C. hepatica* eggs were fixed for 10 min on glass slides with precooled acetone. Subsequently, the human serum samples to be examined were added to the sections in dilutions of 1 in 40, 1 in 80, and 1 in 160 (in phosphate-buffered saline [PBS]) and incubated for 45 min at 37°C . Following a triplicate washing procedure in PBS, a fluorescein isothiocyanate-conjugated antihuman immunoglobulin G antiserum (Sigma, St. Louis, Mo.) was added to the sections at a dilution of 1 in 80. After another 45-min incubation period at 37°C , the sections were again washed three times with PBS, covered with buffered glycerol (glycerol-PBS, 9:1), and examined in a microscope under UV light. A serum sample taken from a convalescent 1-year-old child from Switzerland who had suffered from severe hepatic capillariasis (3) served as positive control serum,

and serum samples from three healthy colleagues at the University of Veterinary Medicine, Vienna, Austria, were used as negative controls. Because hepatic capillariasis is a rare infection in humans, it was not possible to obtain sera from other patients. However, to further evaluate the sensitivity of the assay, we tested serum samples of four mice with verified *C. hepatica* infection and three negative control mice.

One employee of the zoo showed antibodies to *C. hepatica*, with a titer of 1 in 40 (Fig. 1a), and another one was questionably positive at the 1 in 40 dilution; the other samples proved negative (one of the negative serum samples is shown in Fig.

FIG. 1. IIF assay of sections of *C. hepatica* eggs to detect antibodies to *C. hepatica* in human serum samples. (a) Serum with antibodies to *C. hepatica*. Arrows indicate the highly specific fluorescence of inner structures. (b) Negative serum. Microphotograph magnification, $\times 100$.

1b). In addition to the bright fluorescence, which is seen all over the sections of the parasite eggs when antibodies to *C. hepatica* are present in the serum, we especially draw the reader's attention to the fluorescing inner structures of the eggs, which seem to be highly specific. During the evaluation of the assay, about 10% of the serum samples showed a low-level nonspecific fluorescence (which was seen only in serum dilutions of <1 in 20). When we used the recommended serum dilutions of 1 in 40 and higher, however, we could clearly discriminate between sera with antibodies to *C. hepatica* and negative sera. The serum of the convalescent Swiss child with diagnosed hepatic capillariasis repeatedly showed a titer of 1 in 320 in the indirect immunofluorescence (IIF) assay. All four examined serum samples of mice with *C. hepatica* infection proved positive, with titers of 1 in 80 to 1 in 640; although mouse sera were tested on mouse tissue, there was surprisingly little background staining. The three clinically negative mice were also negative by the IIF assay.

The blood parameters of the two individuals seropositive and questionably positive for *C. hepatica* were normal, and they showed no increase in the activities of the liver-dependent enzymes. Two months later, blood was again taken from them and analyzed by the above-described assay. For the man who initially showed an antibody titer of 1 in 40, the same titer was determined 2 months later, while the other individual with the borderline titer of 1 in 40 tested negative. The two employees will also be monitored in the future, but a transient or subclinical infection with *C. hepatica* seems to be possible in these cases.

To further assess the specificity of the assay, serum samples of patients with documented infections of *E. granulosus*, *E. multilocularis*, *F. hepatica*, *Schistosoma mansoni*, *T. canis*, *Trichuris trichiura*, and *Trichinella spiralis* were tested for cross-reactivity with *C. hepatica*. All sera proved negative for antibodies to *C. hepatica* at dilutions of 1 in 20 and 1 in 40. Furthermore, to check the other possibility of cross-reaction, the sera of the two employees who were positive and questionably positive for *C. hepatica* were tested for the presence of antibodies to *E. granulosus* (enzyme-linked immunosorbent assay [ELISA] and Western blotting), *E. multilocularis* (ELISA and Western blotting), *F. hepatica* (ELISA), *S. mansoni* (ELISA), *T. canis* (ELISA and Western blotting), *Trichuris trichiura* (ELISA), and *Trichinella spiralis* (ELISA). No antibodies to any of the above-named parasites were found in the sera.

In summing up the advantages and limitations of the IIF assay described in this paper, we report that this assay offers for the first time the possibility of a large-scale screening of persons at risk for *C. hepatica* infections; it will be possible to detect infections at an early stage, thus increasing the chances for a successful treatment significantly. Furthermore, only a small amount of serum is required from the patient to carry out this assay. It is possible to perform this test in every histology lab, and the results are ready after 2 h. In our opinion, the only limitation of this assay is some nonspecific fluorescence of unknown origin at lower serum dilutions.

We thank Hans-Peter Marti, Schweizer Tropeninstitut, Basel, Switzerland, for providing the positive control serum and the two anonymous reviewers of the manuscript for their constructive criticism.

REFERENCES

- Attah, E. B., S. Nagarajan, E. N. Obineche, and S. C. Gera. 1983. Hepatic capillariasis. *Am. J. Clin. Pathol.* **79**:127-130.
- Bancroft, T. L. 1893. On the whip-worm of the rat's liver. *J. Proc. R. Soc. N. S. W.* **27**:86-90.
- Berger, T., A. Degrémont, J. O. Gebbers, and O. Tönz. 1990. Hepatic capillariasis in a 1-year-old child. *Eur. J. Pediatr.* **149**:333-336.
- Bustamente-Sarabia, J., R. Suswillo, and E. Contreras-Moreno. 1996. *Capillaria hepatica* in a 19-month old boy, third case in Mexico. *Bol. Med. Hosp. Infant. Mex.* **53**:395-400.
- Calle, S. 1961. Parasitism by *Capillaria hepatica*. *Pediatrics* **27**:648-655.
- Camain, R., X. Dor, and F. Ranavo. 1965. Infestation hépatique à *Capillaria hepatica* chez un enfant de 11 mois. *Ann. Pediatr. (Paris)* **12**:559-562.
- Choe, G., H. S. Lee, J. K. Seo, J. Y. Chai, S. H. Lee, K. S. Eom, and J. G. Chi. 1993. Hepatic capillariasis: first case report in the Republic of Korea. *Am. J. Trop. Med. Hyg.* **48**:610-625.
- Cislaghi, F., and C. Radice. 1970. Infection by *Capillaria hepatica*. First case report in Italy. *Helv. Paediatr. Acta* **25**:647-654.
- Cochrane, J. C., L. Sagorin, and M. G. Wilcocks. 1957. *Capillaria hepatica* infection in man. A syndrome of extreme eosinophilia, hepatomegaly and hyperglobulinaemia. *S. Afr. Med. J.* **31**:751-755.
- Eaton, R. D. P. 1972. Current problems in parasitology in Canadian native peoples. Proceedings of the Second International Symposium on Circumpolar Health, Oulu, Finland. *Acta Socio Med. Scand. Suppl.* **6**:249-253.
- Ewing, G. M., and I. L. Tilden. 1956. *Capillaria hepatica*. Report of fourth case of true human infestation. *J. Pediatr.* **48**:341-348.
- Foster, A. O., and C. M. Johnson. 1939. An explanation for the occurrence of *Capillaria hepatica* ova in human feces suggested by the finding of three new hosts used as food. *Trans. R. Soc. Trop. Med. Hyg.* **32**:639-644.
- Gonzalez-Barranco, D., D. Pelaez, Y. Jaramillo-Rodriguez, and H. A. Rodriguez-Martinez. 1996. Third case of human hepatic capillariasis in Mexico. *Rev. Investig. Clin.* **48**:301-305.
- Govil, H., and M. Desai. 1996. *Capillaria hepatica* parasitism. *Indian J. Pediatr.* **63**:698-700.
- Kallichurum, S., and R. Elsdon-Dew. 1961. *Capillaria* in man. A case report. *S. Afr. Med. J.* **35**:860-861.
- Kohatsu, H., O. Zaha, K. Shimada, T. Chibana, I. Yara, A. Shimada, H. Hasegawa, and Y. Sato. 1995. A space-occupying lesion in the liver due to *Capillaria* infection. *Am. J. Trop. Med. Hyg.* **52**:414-418.
- Kokai, G. K., S. Mistic, V. N. Perisic, and S. Grujovska. 1990. *Capillaria hepatica* infestation in a 2-year old girl. *Histopathology* **17**:275-277.
- Luttermoser, G. W. 1938. An experimental study of *Capillaria hepatica* in the rat and the mouse. *Am. J. Hyg.* **27**:321-340.
- McArthur, W. P. 1924. A case of infestation of human liver with *Hepaticola hepatica* (Bancroft, 1893) Hall, 1916; with sections from the liver. *Proc. R. Soc. Med.* **17**:83-84.
- McQuown, A. L. 1950. *Capillaria hepatica*: report of genuine and spurious cases. *Am. J. Trop. Med.* **30**:761-767.
- Otto, G. F., M. Berthrong, R. E. Appleby, J. C. Rawlins, and O. Wilbur. 1954. Eosinophilia and hepatomegaly due to *Capillaria hepatica* infection. *Bull. John Hopkins Hosp.* **94**:319-336.
- Pannenbecker, J., T. C. Miller, J. Müller, and R. Jeschke. 1990. Schwerer Leberbefall durch *Capillaria hepatica*. *Monatsschr. Kinderheilkd.* **138**:767-771.
- Pavlov, A. V. 1970. Trichocephalidae and Capillariidae of animals and man and the diseases caused by them, 404-411. In K. I. Skrjabin (ed.), *Principles of nematology*, vol. 6. Izdatel'stvo Akademii Nauk SSSR, Moscow, Russia.
- Pereira, V. G., and L. C. Mattosinho Franca. 1983. Successful treatment of *Capillaria hepatica* infection in an acutely ill adult. *Am. J. Trop. Med. Hyg.* **32**:1272-1274.
- Piazza, R., M. A. Correa, and R. N. Fleury. 1963. Sobre um caso de infestação humana por *Capillaria hepatica*. *Rev. Inst. Med. Trop. Sao Paulo* **5**:37-41.
- Romero, G., and F. Mendiola y Biagi. 1962. Eosinofilia elevada con manifestaciones viscerales. Primer caso de infección por *Capillaria hepatica* en Mexico. *Bol. Med. Hosp. Infant. Mex.* **19**:473.
- Sekikawa, H., H. Hasegawa, M. Otsuru, T. Igarashi, H. Kaneko, K. Wada, O. Sato, R. Wakakuwa, K. Tajima, and K. Homma. 1991. First human case of *Capillaria hepatica* infection in Japan. *Jpn. J. Parasitol.* **40**:528-532.
- Silvermann, N. H., J. S. Katz, and S. E. Levin. 1973. *Capillaria hepatica* infestation in a child. *S. Afr. Med. J.* **47**:219-221.
- Slais, J. 1973. The finding and identification of solitary *Capillaria hepatica* (Bancroft, 1893) in man from Europe. *Folia Parasitol. (Prague)* **20**:149-161.
- Turhan, B., E. K. Unat, M. Ynermar, and C. Sumer. 1954. İnsan karacigerinde: *Capillaria hepatica* (Bancroft, 1893) Travassos 1915. *Microbiol. Dergisi* **7**:149-159.
- Vargas-Carretero, G., H. Lopez-Martinez, R. Victoria-Victoria, and G. Hernandez-Munoz. 1979. *Capillaria hepatica*. Report of the 2nd case found in the Mexican Republic. *Bol. Med. Hosp. Infant. Mex.* **36**:909-917.
- Vogel, H. 1930. Über die Organotropie von *Hepaticola hepatica*. *Z. Parasitenkd.* **2**:502-505.
- Ward, B. L., and J. H. Dent. 1959. *Capillaria hepatica* infection in a child. *Bull. Tulane Univ. Med. Fac.* **19**:27-33.
- Wright, K. A. 1961. Observations on the life cycle of *Capillaria hepatica* (Bancroft, 1893) with a description of the adult. *Can. J. Zool.* **38**:167-182.
- Yfanti, G., E. Andreadis, C. Spiliadou, and E. J. Diamantopoulos. 1996. A woman with fever and a jejunal stricture. *Lancet* **347**:802.