

RESEARCH NOTE

Attempted infection of the rhesus monkey (*Macaca mulatta*) with the British horse strain of *Echinococcus granulosus*

R. C. A. THOMPSON

School of Veterinary Studies, Murdoch University, Murdoch, Western Australia, 6153

and J. D. SMYTH

Department of Zoology and Applied Entomology, Imperial College, London, SW7 2BB

Attention has been drawn to the fact that the occurrence of the cystic stage of *Echinococcus granulosus* in > 60% of slaughtered horses represents a potential public health hazard (Thompson and Smyth, 1974). Williams and Sweatman (1963) regard this organism as a separate sub-species, *E. granulosus equinus* distinct from *E. granulosus granulosus*, but we prefer at this stage to refer to it as the horse "strain" which exhibits some small morphological differences from organisms of sheep origin.

In spite of the alarming increase in equine hydatidosis, public health figures have not revealed any increase in the number of cases of human hydatidosis concurrent with the increase in prevalence of the disease in horses. This does not necessarily indicate the non-infectivity of the horse strain to man, for other factors may account for its non-appearance in the human population to date (Thompson and Smyth, 1975). In an attempt to throw some light on this problem, a preliminary study of the infectivity of the horse strain of *E. granulosus* to nonhuman primates has been carried out.

The primate chosen for this study was the rhesus monkey (*Macaca mulatta*). Natural infections of hydatid disease have been widely reported in this species (Lambert, 1918; Heller, 1927; Torrance, 1937; Allen, 1957; Healy and Hayes, 1963; Ilievski and Esber, 1969; Houser and Paik, 1971; Tate and Rubin, 1973) and fertile primary cysts of a human strain of *E. granulosus* have been reared in it experimentally (Hutchison, 1966).

Four mature female rhesus monkeys were maintained at the Huntingdon Research Centre and monitored throughout this study. Serum samples were taken from each animal both before, and at regular intervals during, the six-month infection period. Serum from this source was incorporated into the daily routine testing of patients' sera for hydatidosis by the Pan American Zoonosis Center, using the indirect haemagglutination (IHA), latex agglutination (LA) and immunoelectrophoresis (IEP) tests. Serum was also assayed for the presence of the liver enzymes, glutamate pyruvate transaminase (GPT) and leucine amino peptidase (LAP), as Bundesen and Janssens (1971) have shown that the migration of taeniid oncospheres through the liver causes a leakage of liver enzymes into the blood.

Prior to infection, X-rays of the thorax and abdomen of all four monkeys did not reveal the presence of hydatid cysts. The IHA, LA and IEP tests also proved negative for antibodies to *E. granulosus*.

Two monkeys each received approximately 10 gravid proglottids orally. The worms were of experimental horse/dog origin, which had been grown from a mature to gravid state *in vitro* according to the technique of Smyth and Howkins (1966). The gravid prog-

lottids contained normal shelled oncospheres whose viability and activity were tested by placing in an artificial hatching and activation medium (Heath and Smyth, 1970).

The two remaining monkeys each received 0.5 ml packed protoscoleces, removed from fresh horse hydatid cysts, by intraperitoneal injection. The protoscoleces were viable, as judged by their infectivity to secondary rodent hosts.

The IHA, LA and IEP tests for hydatidosis in the sera from the four monkeys during the course of infection proved negative, except for a low titre (1 : 32) observed two weeks post infection in one animal that had received a secondary infection.

Results concerning the presence of the liver enzymes GPT and LAP (Table 1) showed no observable increase above the normal range throughout the infection period.

TABLE 1

Primary and Secondary Infection of Rhesus Monkeys with *E. granulosus* of Horse Origin
The Results of Assaying Serum Samples for the Liver Enzymes Serum Glutamate
Transaminase (GPT) and Leucine Amino Peptidase (LAP)

Days post infection	GPT (mU/ml) ¹ CODE*				LAP (GR units) ² CODE*			
	A1 (1°)	A2 (1°)	B1 (2°)	B2 (2°)	A1 (1°)	A2 (1°)	B1 (2°)	B2 (2°)
0	24	32	50	15	173	224	211	137
4	20	32	21	15	140	174	182	95
8	36	32	26	22	112	158	181	89
12	19	39	24	18	182	230	232	139
15	26	33	20	17	172	215	207	138
19	20	19	25	22	139	178	170	112
22	23	25	22	19	230	196	194	155
25	26	35	19	15	181	224	196	149
29	24	26	17	15	230	266	269	177
57	20	28	21	17	126	202	165	100
85	20	25	17	13	208	236	255	168
113	22	24	24	21	175	171	230	146
141	22	22	22	19	171	232	250	139
169	36	36	32	40	258	282	300	230

1. Normal range for rhesus monkey = < 50 mU/ml.

2. Normal range for rhesus monkey = 30-350 GR units.

*A1 (1°) and A2 (1°) received *E. granulosus* eggs orally. B1 (2°) and B2 (2°) received protoscoleces by IP injection.

At autopsy, 187 days after infection, no developing hydatid cysts were found in any of the monkeys. Unfamiliar lesions of an unknown etiology, and all structures suspected of being of parasitic origin, were found on histological examination to be unrelated to *E. granulosus*.

On the basis of these results, the rhesus monkey (*M. mulatta*) does not appear to be susceptible to primary or secondary infection with the British horse strain of *E. granulosus*. No attempt by the parasite to gain even a foothold of establishment, and the virtual absence of an immune response, indicate that development in this host may not take place owing to adverse nutritional or physiological factors.

These results favour the suggestion of Nelson (1972) that the horse strain may not be infective to man, since the rhesus monkey is known to be susceptible to one strain of *E. granulosus* infective to man (Hutchison, 1966). Striking differences in the *in vitro* growth patterns of *E. granulosus* of horse and sheep origins have recently been demonstrated (Smyth and Davies, 1974) and the failure of the horse strain to become established in the rhesus monkey may be a further reflection of these differences. However, an interpretation

of these negative results, especially since only four animals were tested, is more difficult than if positive results had been obtained. Until this experiment has been repeated, and until the susceptibility of the rhesus monkey to other strains of *E. granulosus* infective to man has been assessed, the equivocality of the present results is evident.

Whatever the outcome, the existence of a significant reservoir of the parasite in Great Britain must remain a matter of concern, especially since the life cycle of *Echinococcus* could result in an altered host specificity at any time (Smyth and Smyth, 1964).

ACKNOWLEDGEMENTS

Our thanks are due to Dr. R. Heywood of the Huntingdon Research Centre, Dr. R. Rodriguez and Dr. V. M. Varela-Diaz of the Pan American Zoonosis Center, for their valuable assistance and co-operation in this project.

This study was supported by grants from the Medical Research Council and W.H.O.

REFERENCES

- ALLEN, A. M. (1957) Pulmonary hydatid disease in a rhesus monkey. *Archives of Pathology*, **64**, 148-151.
- BUNDESEN, P. G. and JANSSENS, P. A. (1971) Biochemical tracing of parasitic infections—II. *Taenia pisiformis* in rabbits—a quantitative study. *International Journal for Parasitology*, **1**, 15-20.
- HEALY, G. R. and HAYES, N. R. (1963) Hydatid disease in rhesus monkeys. *Journal of Parasitology*, **49**, 837.
- HEATH, D. D. and SMYTH, J. D. (1970) *In vitro* cultivation of *Echinococcus granulosus*, *Taenia hydatigena*, *T. ovis*, *T. pisiformis* and *T. serialis* from oncosphere to cystic larva. *Parasitology*, **61**, 329-343.
- HELLER, E. B. (1927) *Echinococcus* disease in Missouri: Incorporating report of a second case of the disease in a monkey from Kansas City Zoological Park. *Journal Missouri Medical Association*, **24**, 93-95.
- HOUSER, W. D. and PAIK, S. K. (1971) Hydatid disease in a macaque. *Journal of the American Veterinary Medical Association*, **159**, 1574-1577.
- HUTCHISON, W. F. (1966) Studies on *Echinococcus granulosus*. III. The rhesus monkey (*Macaca mulatta*) as a laboratory host for the larval stage. *Journal of Parasitology*, **52**, 416.
- ILIEVSKI, V. and ESBER, H. (1969) Hydatid disease in a rhesus monkey. *Laboratory Animal Care*, **19**, 199-204.
- LAMBERT, R. A. (1918) *Echinococcus* cysts in a monkey. *Proceedings of the New York Pathological Society*, **18**, 29-30.
- NELSON, G. S. (1972) Human behaviour in transmission of parasitic disease. In *Behavioural aspects of parasite transmission* (eds. E. U. Canning and C. A. Wright). *Zoological Journal of the Linnean Society, Supplement No. 1*, **51**, 109-122.
- SMYTH, J. D. and DAVIES, Zena. (1974) Occurrence of physiological strains of *Echinococcus granulosus* demonstrated by *in vitro* culture of protoscoleces from sheep and horse hydatid cysts. *International Journal for Parasitology*, **44**, 443-445.
- SMYTH, J. D. and HOWKINS, A. B. (1966) An *in vitro* technique for the production of eggs of *Echinococcus granulosus* by maturation of partly developed strobila. *Parasitology*, **56**, 763-766.
- SMYTH, J. D. and SMYTH, M. M. (1964) Natural and experimental hosts of *Echinococcus granulosus* and *E. multilocularis*, with comments on the genetics of speciation in the genus *Echinococcus*. *Parasitology*, **54**, 493-514.
- TATE, C. L. and RUBIN, L. F. (1973) Intraocular echinococcosis in a rhesus monkey. *Journal of the American Veterinary Medical Association*, **163**, 636-638.
- THOMPSON, R. C. A. and SMYTH, J. D. (1974) Potential danger of hydatid disease of horse/dog origin. *British Medical Journal*, **3**, 807.
- THOMPSON, R. C. A. and SMYTH, J. D. (1975) Equine hydatidosis: a review of the current status in Great Britain and the results of an epidemiological survey. *Veterinary Parasitology*, **1**, 107-127.
- TORRANCE, C. C. (1937) Hydatid disease in *Macacus rhesus*, a case report. *New York State Department Annual Report*, 1937, p. 24.
- WILLIAMS, R. J. and SWEATMAN, G. K. (1963) On the transmission, biology and morphology of *Echinococcus granulosus equinus*, a new subspecies of hydatid tapeworm in horses in Great Britain. *Parasitology*, **53**, 391-407.

Accepted 5 May, 1976.