Parasitol Res (1993) 79:42–48

Parasitology Research

Further evidence for the occurrence of a distinct strain of *Echinococcus granulosus* in European pigs

J. Eckert[1], R.C.A. Thompson[2], A.J. Lymbery[3], Z.S. Pawlowski[4], B. Gottstein[1], U.M. Morgan[2]

[1] Institut of Parasitology and WHO Collaborating Centre for Parasitic Zoonoses, University of Zurich, CH-8057, Zurich, Switzerland
[2] Institute for Molecular Genetics and Animal Disease and WHO Collaborating Centre for Echinococcosis, School of Veterinary Studies, Murdoch University, Western Australia, 6150
[3] Institute for Molecular Genetics and Animal Disease and Division of Animal Production, Western Australian Department of Agriculture, South Perth, Western Australia, 6151
[4] Clinic of Parasitic and Tropical Diseases, Academy of Medicine, Przybyszewskiego 49, 60–355 Poznan, Poland

Accepted: 15 September 1992

Abstract. The morphology, adult development and genetic characteristics of *Echinococcus granulosus* isolated from pigs in Poland were examined and compared with those of other recognised strains of *E. granulosus*. The isolates were characterised by their distinct morphology, rapid maturation and unique DNA hybridisation profiles. The form of *E. granulosus* that occurs in European pigs may therefore be a distinct strain that can be separated morphologically and genetically from other strains and that exhibits features of epidermiological significance, including a rapid rate of development in dogs and an apparent low infectivity to humans and domestic ungulates.

Echinococcus granulosus exists as a number of well-defined, genetically distinct strains that are known to differ morphologically as well as in characteristics of epidemiological significance (Eckert and Thompson 1988; Thompson and Allsopp 1988; Thompson and Lymbery 1988; Eckert et al. 1989). Strains adapted to sheep, horses, cervids, cattle and camels have been well characterised, whereas forms in pigs, goats and buffaloes require further study (Thompson and Lymbery 1990a).

Although pigs are known to be susceptible to infection with the common sheep strain of *E. granulosus* (Kumaratilake and Thompson 1984a, b), the cysts that develop are rarely fertile and the pig is not thought to play a major role in the perpetuation of this form of *E. granulosus*. Extensive studies in eastern Europe (Czechoslovakia, Bulgaria, Hungary, Yugoslavia, Poland and the former Soviet Union; e.g. see Shablovskaya et al. 1989) have suggested that the form of *E. granulosus* in pigs is different from that occurring in sheep on the basis of a variety of criteria, including morphology, antigenic characteristics, infectivity trials and development (reviewed in Eckert and Thompson 1988; Thompson and Lymbery 1988). This view is supported by morphological observations and experimental host-specificity experiments on *E. granulosus* of pig origin from the southeastern United States (Hutchison and Bryan 1960; Hutchison 1970), where infection was introduced in imported swine in the late nineteenth century (Schwabe 1986), presumably from Europe.

It appears that where a dog/pig cycle operates in eastern Europe, it may be the most important cycle for perpetuating the parasite (Eckert and Thompson 1988). However, there is circumstantial evidence suggesting that *E. granulosus* of pig origin may have a low infectivity to humans, as in parts of Poland where pigs and dogs are commonly infected, cases of human hydatid disease are rare (Pawlowski 1985). A similar situation has been reported from the Ukraine (Shablovskaya et al. 1989).

The aim of the present study was to substantiate previous observations on *E. granulosus* of pig origin by directly examining the morphology, adult development and genetic characteristics of porcine isolates of the parasite from Poland.

Materials and methods

Morphological studies

Each of two 4-month-old helminth-free Appenzeller dogs was infected with approximately 1 ml of sedimented protoscoleces from fertile hydatid cysts of *Echinococcus granulosus* isolated from the liver of pigs slaughtered in Poznan, Poland. Each dog was infected with parasite material isolated from two different groups of pigs. Over 95% of the protoscoleces used for infection were motile and had flame-cell activity. About 50% of the protoscoleces were given in a gelatine capsule perorally, and the rest were mixed with some canned meat and offered to the dogs.

Dedicated to Prof. K.T. Friedhoff on the occasion of his 60th birthday

Correspondence to: R.C.A. Thompson

Table 1. Development of *Echinococcus granulosus* of pig origin in artificially infected dogs at 34 days post-infection

Dog	Mean length (mm)	Percentage of worms at different stages of segmentation				Percentage of worms at different stages of maturation			
		S+1	S+2	S+3	S+4	T+FG	U+C	U+TE	U+SE
1	1.9±0.3 (1.1–2.9)	–	14.2	85.3	0.5	9	41	45	5
2	1.9±0.3 (1.1–2.7)	–	10.8	89.2	–	6	30	64	–

Numbers in parentheses represent ranges. S+1, Scolex with one segment; S+2, scolex with two segments; S+3, scolex with three segments; T+FG, testes containing spermatozoa, ovary, uterine streak and other female genitalia; U+C, developing eggs in the uterus; U+TE, "thin-shelled" (partly developed embryophore) eggs with a fully formed oncosphere in the uterus; U+SE, thick-shelled eggs in the uterus

The dogs were maintained in large stables according to the Swiss animal-protection regulations and received canned meat, dry dog food and water. Both dogs were euthanasically killed on day 34 post-infection (p.i.) by the i.v. injection of an overdose of sodium pentobarbital (Vetanarcol, Veterinaria AG, Zurich). Subsequent autopsy and worm recovery procedures were carried out as previously described (Eckert et al. 1989).

Echinococcus worms were fixed and stained as detailed by Eckert et al. (1989). In addition, some worms were used unfixed for immediate staining in lactic acid carmine. Hook and strobilar measurements were undertaken as previously described (Thompson et al. 1984; Kumaratilake et al. 1986). From these measurements and equivalent data reported by Thompson et al. (1984), Kumaratilake et al. (1986) and Eckert et al. (1989), Euclidean distances (Burr 1968) were calculated as a measure of overall phenotypic dissimilarity between isolates of *E. granulosus* from pigs, horses, sheep, cattle and camels. The matrix of Euclidean distances was clustered using the UPGMA strategy (Sneath and Sokal 1973) to produce a phenogram of relationships between isolates.

DNA studies

DNA was extracted as previously described from the following isolates of *E. granulosus*: horse, cattle and camel origin according to Vogel et al. (1990) and sheep and pig origin according to Yap et al. (1988). DNA samples were double-digested with *Eco*R1 (Amersham) and *Pst*1 (Promega). Restriction fragments were separated by agarose gel electrophoresis [0.65% agarose in 0.04 M TRIS acetate, 0.001 M ethylenediaminetetraacetic acid (EDTA), pH 8.0]. After electrophoresis, the DNA within the gels was depurinated by immersion in 0.25 M HCl for 15 min, rinsed briefly in distilled H_2O and then soaked for 30 min in denaturing solution (0.5 NaOH, 1.5 M NaCl). Finally, the gels were rinsed and equilibrated for a further 30 min in neutralising solution (0.5 M TRIS, 1.5 M NaCl, 0.001 M EDTA, pH 7.5) at room temperature. Denatured DNA fragments were transferred from agarose to nylon membranes (Hybond N, Amersham) by Southern transfer in 20×SSC (3 M NaCl, 0.4 M trisodium citrate, pH 8.0). After transfer membranes were rinsed in 0.4 M NaOH for 10 min and blotted dry.

The DNA probes used in this study were pAL1, a fragment of genomic *E. multilocularis* DNA cloned in Bluescript (Vogel et al. 1990), and pREG395, a fragment of *E. granulosus* ribosomal DNA cloned in a PBR322 plasmid (Yap 1988). Both probes were labeled in a nick-translation procedure using [α-^{32}P]-deoxycytidine 5′-triphosphate (dCTP; 3000 Ci/mmol, Amersham) to a specific activity of approximately 10^7 cpm/μg. Hybridisation of probes to parasite DNA fragments on the nylon membranes was carried out in rapid hybridisation solution (Amersham) for 16 h at 50° C using the Hybrid-ease hybridisation chamber (Edwards Instrument Company). Following hybridisation, membranes were washed twice in 2×SSC, 0.27×sodium dodecyl sulphate (SDS) for 5 min each and twice in 0.2×SSC, 0.1%m SDS for 5 min, all at room temperature. Membranes were then washed twice in 0.1×SSC, 0.1% SDS at 50° C for 5 min each. Finally, air-dried membranes were exposed overnight at −70° C to Amersham Hyperfilm in a cassette with an intensifying screen.

The number of base substitutions/nucleotide (or the proportion of nucleotides substituted) between isolates was estimated from restriction-fragment data by the method of Nei and Li (1979). All fragments were weighted equally, regardless of the intensity of hybridisation, and fragment polymorphisms were assumed to arise only from base substitutions. The matrix of base-substitution differences was clustered using the UPGMA strategy to produce a phenogram of relationships between isolates.

Results

Biological characteristics of metacestodes

Metacestodes of *Echinococcus granulosus* are mostly found in the liver of pigs and are less frequently encountered in the lung and other internal organs. The 13 cysts used in our trials had been isolated from pig livers, their

Table 2. Rostellar hook characteristics of protoscoleces and 34-day-old adult *Echinococcus granulosus* of Polish pig origin

Characteristics	Protoscoleces	Adult worms
Large hooks:		
Total length (μm)	29.1±1.3 (26.2–31.1)	35.4±1.5 (31.5–38.9)
Blade length (μm)	13.1±0.7 (11.8–14.5)	11.8±1.2 (5.3–16.6)
BL/TL (%)	44.0±2.5 (40.0–49.0)	33.4±3.9 (27.0–43.0)
Small hooks:		
Total length (μm)	24.4±1.3 (21.4–26.7)	28.4±1.7 (23.7–32.1)
Blade length (μm)	9.2±0.6 (8.4–10.3)	8.5±0.9 (5.9–11.1)
BL/TL (%)	37.8±4.0 (33.0–42.0)	30.9±3.8 (21.0–40.0)
Total number of hooks	33±3.5 (30–38)	33±2.1 (26–39)
Shape of hooks	Smooth outline	Smooth outline
Arrangement of hooks	Large and small hooks alternate	

Data represent mean values±SD; ranges are given in parentheses. *BL*, Blade length; *TL*, total length

Table 3. Morphological characteristics of adult *Echinococcus granulosus* of Polish pig origin in comparison with data published by Vogel (1957) for isolates of German pig origin

	E. granulosus of Polish pig origin	*E. granulosus* of German pig origin (from Vogel 1957)	Affinity[c]
Total number of hooks	33 ± 2.1 (26–39)	Not given	Camel, cattle, sheep
Hook dimensions (μm)	35.4 ± 1.5 (large hooks) (31.5–38.9)	37.2 (large hooks) (34.0–39.8)	Camel
Outline of hooks	Smooth	Smooth	Camel, cattle, horse
Total worm length (mm)	1.9 ± 0.3[a] (1.1–2.9)	3.27[a] (2.1–5.0)	Camel, sheep, horse
Length of terminal segment (mm)	1.0 ± 0.2[a] (0.5–1.5)	1.9[a] (1.0–3.2)	Camel, sheep
Terminal segment as % total length	52%	55–61%	Camel
Maximum number of segments	3 (95%)	3	Cattle, horse
Position of sexually mature segment	Usually penultimate	Penultimate[b]	Cattle, horse
Position of genital pore:			
Penultimate segment	60% slightly anterior to middle of segment	Slightly anterior to middle	Camel
Terminal segment	80% posterior to middle of segment	Posterior to middle	Cattle, sheep, horse
Number of testes	54 ± 3.0 (50–60)	44 (38–52)	Sheep
Distribution of testes	Throughout segment (1 or 2 rows behind vitelline gland)	Throughout segment[b]	Camel, sheep, horse
Shape of cirrus sac	Spherical to piriform	Piriform to spherical	Camel, cattle
Size of cirrus sac (μm)	68.0 ± 6.0 × 60.0 ± 4.0 60.0 – 80.0 × 65.0 – 70.0	Not given	Camel
Shape of ovary	Compact without lobules	Slightly lobed in outline	Sheep
Female reproductive system	No loops in female ducts; Mehlis' gland readily visible	Not given	Cattle, sheep, horse
Maturation	Rapid	Not given	Cattle > pig > camel > horse > sheep

[a] Note the differences in the ages of worms after infection: Polish isolate, 34 days; German isolate, 51–64 days
[b] Derived from a photograph
[c] Compared with data from Thompson et al. (1984), Kumaratilake et al. (1986) and Eckert et al. (1989)

diameters ranged from about 3 × 4 to 6 × 7 cm, and 12 of them (92%) contained viable protoscoleces.

Biological characteristics of adult worms

The two dogs were heavily infected with *Echinococcus*, harbouring 10050 and 17850 worms, respectively. The developmental characteristics of *E. granulosus* of Polish pig origin recovered from dogs are presented in Table 1. The mean total length of worms from both dogs at 34 days p.i. was 1.9 mm. Over 80% of the worms from both dogs had three segments and the majority had embryonated thin-shelled eggs in fully dilated uteri. A few worms in dog 1 were fully mature, containing thick-shelled eggs (Fig. 1a). These isolates of *E. granulosus* of Polish pig origin therefore exhibited a very rapid rate of maturation similar to that of cattle isolates (see Table 3).

Morphological characteristics of protoscoleces and adult worms

The rostellar hook characteristics of protoscoleces and adult intestinal stages of Polish pig origin are shown

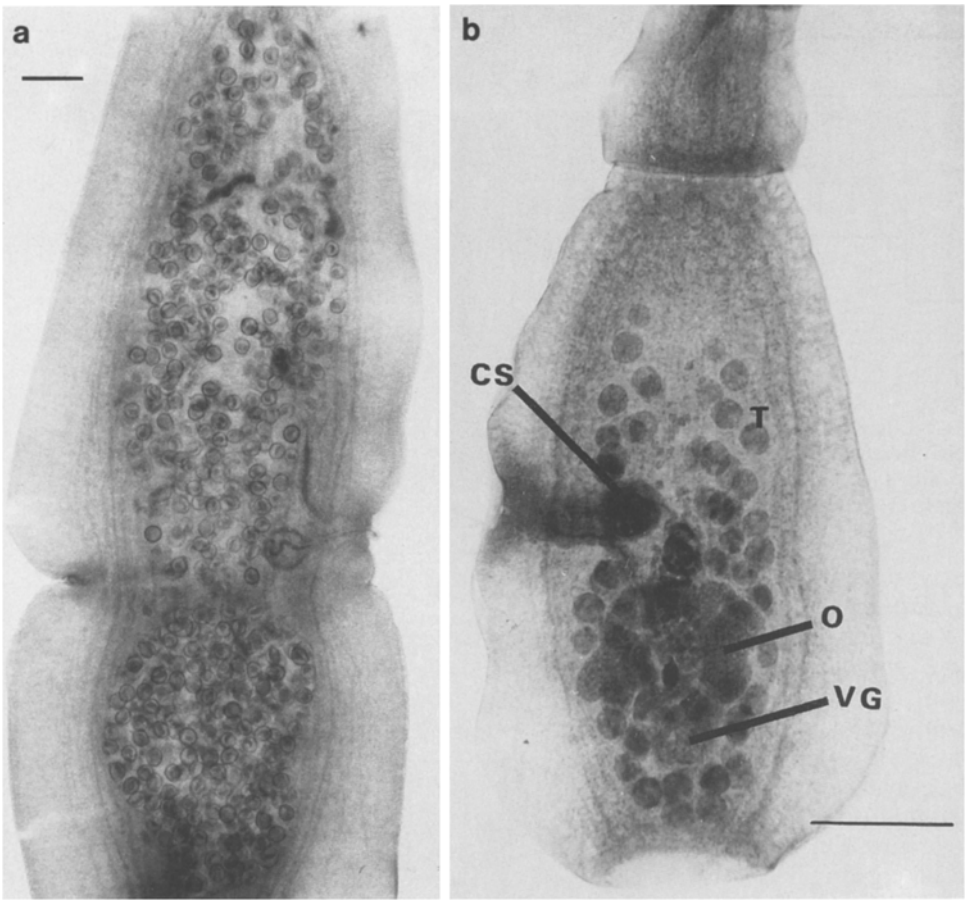

Fig. 1. a Terminal segment of *Echinococcus granulosus* of pig origin, recovered from an experimentally infected dog at 34 days p.i. Thick-shelled eggs are present in the fully dilated uterus. Bar = 0.1 mm. **b** Mature segment of *E. granulosus* of pig origin, recovered from a dog at 34 days p.i. *O*, Ovary; *VG*, vitelline gland; *T*, testes; *CS*, cirrus sac. Bar = 0.1 mm

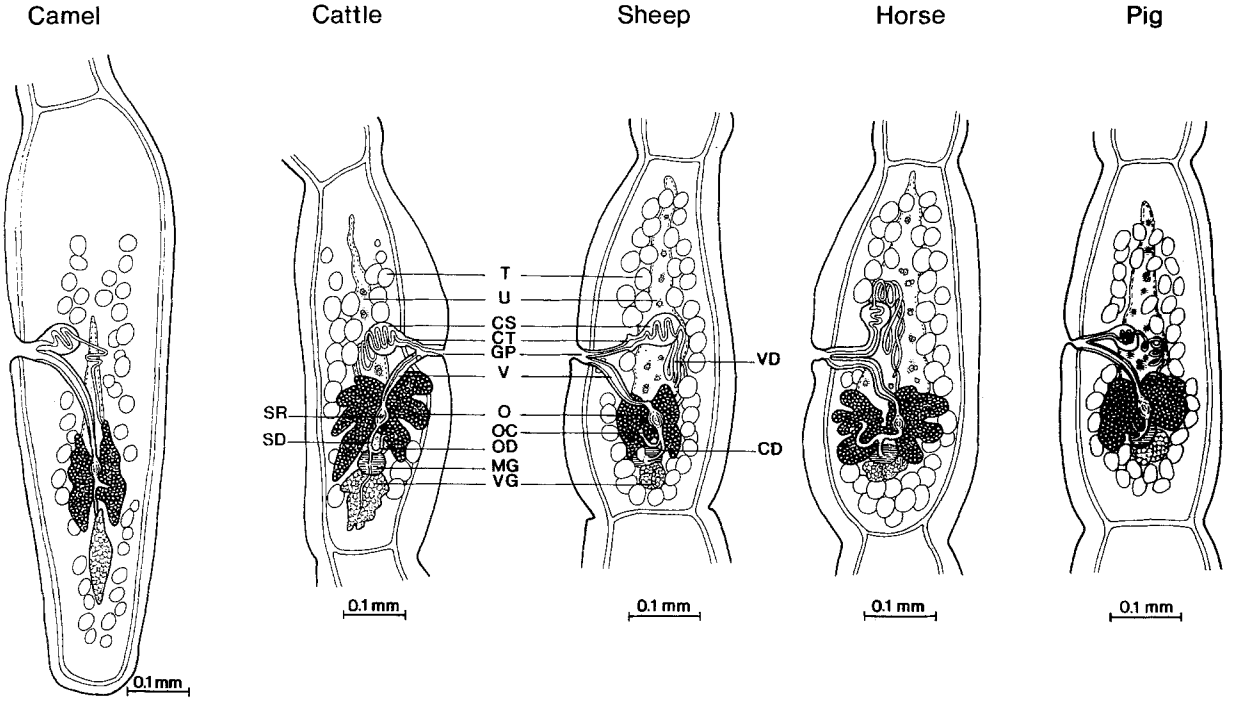

Fig. 2. Comparative major morphological characteristics of mature proglottids of various "domestic" strains of *Echinococcus granulosus*. *CD*, Common duct ; *CS*, cirrus sac; *CT*, cirrus tube; *GP*, genital pore; *MG*, Mehlis' gland; *O*, ovary; *OC*, oocapt; *OD*, oviduct; *SD*, seminal duct; *SR*, seminal receptacle; *T*, testes; *U*, uterus; *V*, vagina; *VD*, was deferens; *VG*, vitelline gland (modified from Eckert et al. 1989)

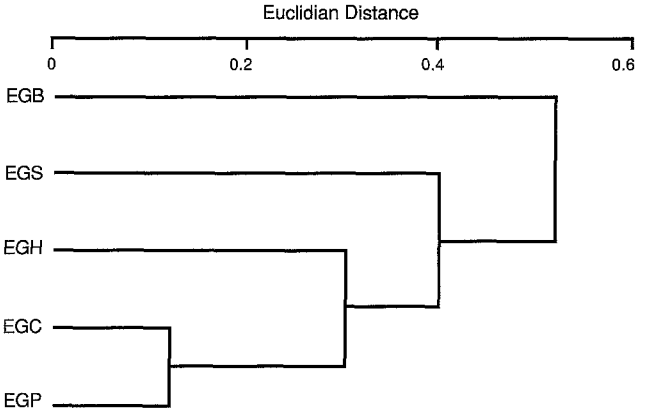

Fig. 3. Phenogram of overall morphological dissimilarity between isolates of *Echinococcus granulosus* from different hosts, clustered by UPGMA from a matrix of Euclidean distances. *EGB*, Cattle isolate; *EGS*, sheep isolate; *EGH*, horse isolate; *EGC*, camel isolate; *EGP*, pig isolate

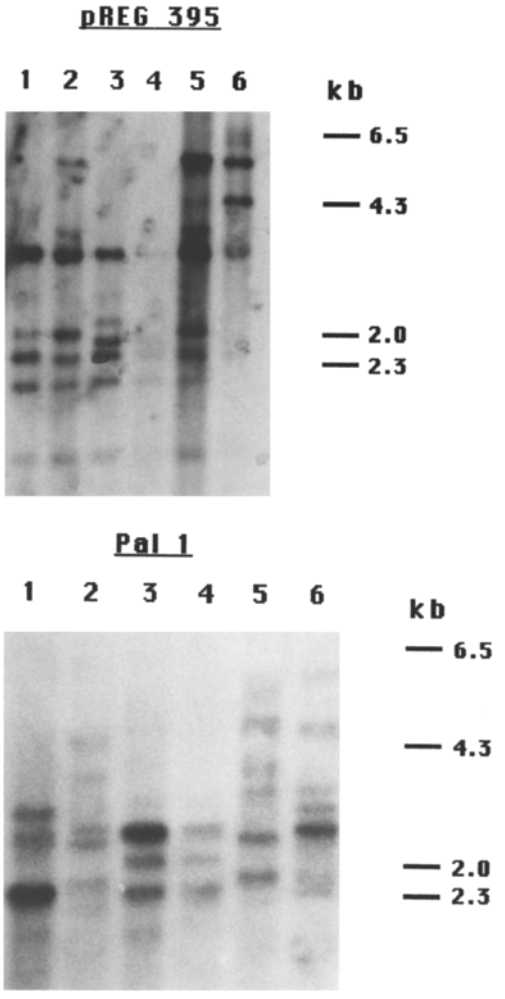

Fig. 4. Southern blot analysis of genomic double DNA digested with *Eco*R1 and *Pst1* from different *Echinococcus granulosus* isolates using two probes (pREG 395 and pAL1). *1*, horse; *2*, sheep; *3*, cattle; *4*, camel; *5*, monkey*; *6*, pig. *This isolate is not relevant to the present study

in Table 2. Further morphological features of the adult stages are summarised in Table 3 and compared with data published by Vogel (1957) for *E. granulosus* of German pig origin (also see Discussion). The relationships with isolates of *E. granulosus* from other intermediate hosts are also summarised in Table 3.

The characteristics of adult *E. granulosus* of Polish pig origin are described herein in comparison with those of parasite isolates of other host origins. On the basis of hook morphology, the isolate of pig origin is very similar to isolates from camels, although certain features are also shared with isolates from cattle, sheep and horses (Table 3). The comparative morphology of mature proglottids (Figs. 1 b, 2) shows that *E. granulosus* of pig origin can be differentiated from the cattle and horse isolates in that the latter have lobed ovaries, there are no testes behind the vitelline gland in the cattle isolate, and the cirrus tube is bent in the horse isolate. Great similarities exist between the isolate of pig origin and isolates from camels, particularly in relation to the long terminal segments. However, the former is characterised by an elongated vitelline gland and some other features. Differentiation of the pig isolate from the sheep isolate is difficult, as both isolates have a large number of testes that are distributed throughout the whole segment, and the ovary is compact with indistinct lobules. Therefore, hook characteristics have to be included as additional criteria for differentiation (rough outline and smaller hooks in isolates of sheep origin). However, it

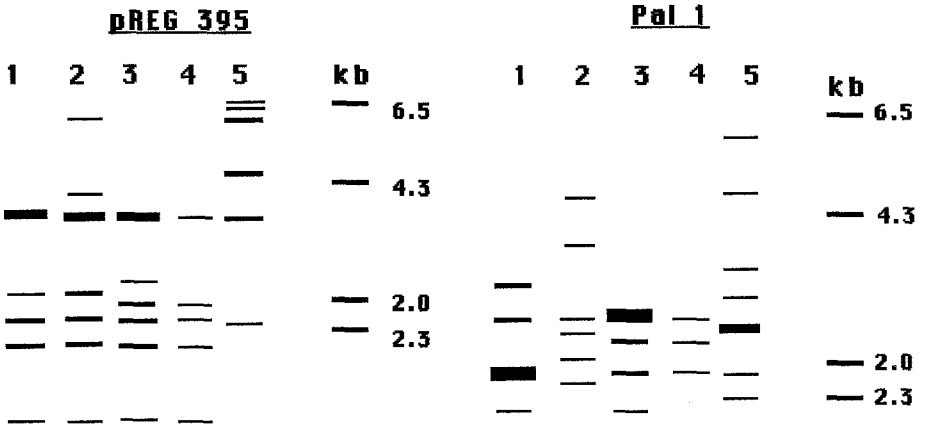

Fig. 5. Diagram showing hybridisation patterns obtained for different isolates of *Echinococcus granulosus*. *1*, Horse; *2*, Sheep; *3*, cattle; *4*, camel; *5*, pig

Table 4. The number of base substitutions per nucleotide site as estimated by the method of Nei and Li (1979) at sequences recognised by pALI (above diagonal) and pREG 395 (below diagonal) separating isolates of *Echinococcus granulosus*

EGH	0	1.000	0.016	0.032	0.104
EGS	0.01	0	0.100	0.092	0.046
EGB	0.018	0.028	0	0.009	0.104
EGC	0.013	0.023	0.005	0	0.100
EGP	0.060	0.046	0.066	0.060	0
	EGH	EGS	EGB	EGC	EGP

EGH, Horse isolate; EGS, sheep isolate; EGB, cattle isolate; EGC, camel isolate; EGP, pig isolate

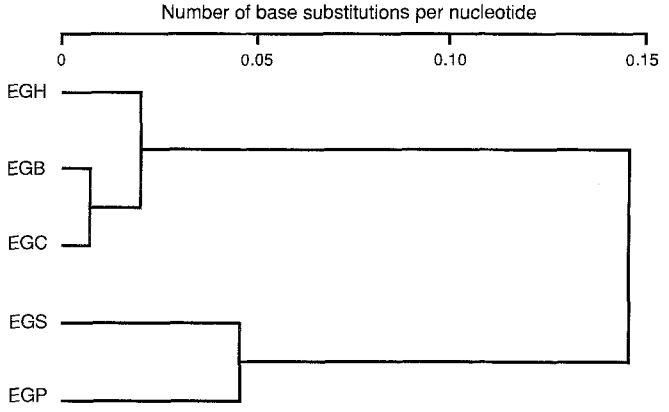

Fig. 6. Phenogram of overall genetic dissimilarity between isolates of *Echinococcus granulosus* from different hosts, clustered by UPGMA from a matrix of base substitution differences. *EGH*, Horse isolate; *EGB*, cattle isolate; *EGC*, camel isolate; *EGS*, sheep isolate; *EGP*, pig isolate

able and not always reliable (see Discussion). A phenogram of relationships between the isolates based on all morphological characters in shown in Fig. 3. This shows clearly that isolates from Polish pigs are morphologically most similar to isolates from camels.

DNA analysis

Isolates of *E. granulosus* originating from horses, sheep, cattle, camels and pigs were readily distinguished on the basis of quite distinct hybridisation patterns produced with both probes (Figs. 4, 5). Estimates of the number of base substitutions/nucleotide separating isolates ranged from 0.009 to 1.00 for pAL1 and from 0.005 to 0.06 for pREG395 (Table 4). Figure 6 shows a phenogram of relationships between the isolates based on the mean number of base substitutions/nucleotide using both probes. This indicates that isolates of *E. granulosus* from pigs are genetically most similar to isolates from sheep.

Discussion

The results of the present study support previous suggestions that there is a form of *Echinococcus granulosus* that is adapted to pigs and may represent a distinct strain. The morphology of *E. granulosus* of Polish pig origin examined in the present study conforms very closely to that generally regarded as the classic description by Vogel (1957) of adult worms isolated from dogs at 51–64 days after experimental infection with protoscoleces obtained from cysts from pig livers in northern Germany. Unfortunately, the information given by Hutchison and Bryan (1960) and Hutchison (1970) is insufficient for any comparisons.

Our morphological studies revealed that *E. granulosus* of Polish pig origin shows affinities to isolates of other hosts but that mature proglottids differ in some important features from those of cattle and horses. Most similarities exist with proglottids of *E. granulosus* of camel origin. As was to be expected, pig material developed well in dogs and exhibited a rapid rate of maturation, with thin-shelled, fully embryonated eggs being present in the majority of worms at 34 days p.i. In a few cases, worms with fully developed, thick-shelled eggs were seen. In addition, DNA analysis revealed a unique hybridisation profile for the Polish pig *Echinococcus* as compared with isolates from horses, sheep, camels and cattle, all of which possessed distinct banding patterns. Isolates from pigs were genetically most similar to isolates from sheep. The morphological studies had shown the closest similarities to the camel isolates but had also revealed affinities to the sheep isolate. Some differences between morphology and DNA analyses are not unexpected, given the frequent discordance between genetic and phenotypic rates of evolution (Avise et al. 1975; King and Wilson 1975; Nixon and Taylor 1977). In addition, we would not at this stage regard the genetic relationship shown in Table 4 and Fig. 6 as definitive. Further studies preferably using restriction-site mapping or DNA-sequencing analysis are required before we can be confident of the genetic affinities between the different strains of *E. granulosus*. The results of our DNA analyses complement those obtained by McManus and Rishi (1989) in which different DNA probes were considered to reveal distinct hybridisation profiles for isolates of *E. granulosus* from pigs in Poland and the former Yugoslavia. Our estimates of the number of base substitutions separating different isolates of *E. granulosus* were relatively large as compared with the intraspecific divergence in other organisms at either nuclear loci or the more rapidly evolving mitochondrial genome (Avise et al. 1987; Lynch and Crease 1990).

Consequently, we consider that the form of *E. granulosus* that occurs in pigs in Poland may be a separate strain. The parasite is gentically different from other strains and exhibits several characteristics of epidemiological significance. These include rapid development in dogs and a possibly low infectivity to humans and other potential domestic intermediate hosts, although these features have to be examined in further studies. On the basis of these criteria, *E. granulosus* of Polish pig origin conforms to the working definition proposed for designating strains of *Echinococcus* (Thompson and Lymbery 1988, 1990b). However, before any firm conclusions can be made, further isolates of *E. granulosus* from pigs must be examined using the DNA probes employed in the

present study, so as to determine the extent, if any, of genetic variation between different isolates.

Future work should be aimed at comparing further pig isolates of *E. granulosus* from Poland and other European countries as well as from endemic areas such as the southeastern United States, where a pig-dog cycle exists. Such studies would determine whether there is a single strain of *E. granulosus* adapted to pigs, as appears to be the case for the horse strain (Kumaratilake et al. 1986), or whether more than one strain is capable of being perpetuated in a pig-dog cycle.

Acknowledgements. This work was supported by the Swiss National Science Foundation (project 70PP-031736) and the Australian Research Council.

References

Avise JC, Smith JJ, Ayala FJ (1975) Adaptive differentiation with little genic change between two native Californian minnows. Evolution 29:411–426

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu Rev Ecol Syst 18:489–522

Burr EJ (1968) Cluster sorting with mixed character types: I. Standardization of character values. Aust Comput J 1:97–99

Eckert J, Thompson RCA (1988) *Echinococcus* strains in Europe: a review. Trop Med Parasitol 39:1–8

Eckert J, Thompson RCA, Michael SA, Kumaratilake LM, El-Sawah HM (1989) *Echincoccus granulosus* of camel origin: development in dogs and parasite morphology. Parasitol Res 75:536–544

Hutchison WM (1960) Studies on the hydatid worm, *Echinococcus granulosus*: II. Prevalence in Mississippi. Am J Trop Med Hyg 9:612–615

Hutchison WM (1970) *Echincoccus granulosus* (Batsch, 1786) of pig-dog origin in Mississippi. J Parasitol 56:162–163

Hutchison WM, Bryan MW (1960) Studies on the hydatid worm, *Echinococcus granulosus*: I. Species identification of the parasite found in Mississippi. Am J Trop Med Hyg 9:606–611

King MC, Wilson AC (1975) Evolution at two levels in humans and chimpanzees. Science 188:107–116

Kumaratilake LM, Thompson RCA (1984a) Morphological characterisation of Australian strains of *Echinococcus granulosus*. Int J Parasitol 14:467–477

Kumaratalike LM, Thompson RCA (1984b) Biochemical characterisation of Australian strains of *Echincoccus granulosus* by isoelectric focusing of soluble proteins. Int J Parasitol 14:581–586

Kumaratilake LM, Thompson RCA, Eckert J (1986) *Echinococcus granulosus* of equine origin from different countries possess uniform morphological characteristics. Int J Parasitol 16:529–540

Lynch M, Crease TJ (1990) The analysis of population survey data on DNA sequence variation. Mol Biol Evol 7:377–394

McManus DP, Rishi AK (1989) Genetic heterogeneity within *Echinococcus granulosis* isolates from different hosts and geographical areas characterised with DNA probes. Parasitology 99:17–29

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nixon SE, Taylor RJ (1977) Large genetic distances associated with little morphological variation in *Polycelis coronata* and *Drugesin tigrina* (Planaria). Syst Zool 26:152–164

Pawlowski ZS (1985) Epidemiological basis for chemotherapy of human echinococcosis. Int J Clin Pharmacol Res 5:75–78

Schwabe CW (1986) Current status of hydatid disease: a zoonosis of increasing importance. In: Thompson RCA (ed) The biology of *Echinococcus* and hydatid disease. Allen & Unwin, London, pp 89–113

Shablovskaya EA, Bulgakov VA, Ponomareva VE, Dan'ko OP, Voloshchuk SD, Kikot' VI (1989) Hydatidosis in the Ukranian SSR. Med Parazitol Parazitarn Bol 6:49–51

Sneath PA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco

Thompson RCA, Allsopp CE (1988) Hydatidosis: veterinary perspectives and annotated bibliography. Commonwealth Agricultural Bureaux, Farnham Royal, England

Thompson RCA, Lymbery AJ (1988) The nature, extent and significance of variation within the genus *Echinococcus*. Adv Parasitol 27:209–258

Thompson RCA, Lymbery AJ (1990a) *Echinococcus*: biology and strain variation. Int J Parasitol 20:457–470

Thompson RCA, Lymbery AJ (1990b) Intraspecific variation in parasites – what is a strain? Parasitol Today 6:345–348

Thompson RCA, Kumaratilake LM, Eckert J (1984) Observations on *Echinococcus granulosus* of cattle origin in Switzerland. Int J Parasitol 14:283–291

Vogel H (1957) Über den *Echinococcus multilocularis* Süddeutschlands: I. Das Bandwurmstadium von Stämmen menschlicher und tierischer Herkunft. Z Tropenmed Parasitol 8:404–456

Vogel M, Müller N, Gottstein B, Flury K, Eckert J, Seebeck T (1990) *Echincoccus multilocularis*: characterization of a DNA probe. Acta Trop (Basel) 48:109–116

Yap KW (1988) The establishment and evaluation of molecular procedures for the characterisation of taeniid cestodes. PhD Thesis, Murdoch University

Yap KW, Thompson RCA, Pawlowski ID (1988) The development of nonradioactive total genomic probes for strain and egg differentiation in taeniid cestodes. Am J Trop Med Hyg 39:472–477