MORPHOLOGICAL CHARACTERIZATION OF ADULT 
ECHINOCoccus GRANULOSUS AS A MEANS OF 
DETERMINING TRANSMISSION PATTERNS

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ABSTRACT: Host induced changes in morphological characters of the rostellar hooks of Echinococcus granulosus were used to determine the origin of infection in definitive hosts in rural areas of southeastern Australia where wild and domestic cycles of transmission may interact. The morphological characters studied vary depending on the species of intermediate host (macropod marsupials, sheep) in which protoscoleces develop, and these characters are retained in adult worms. It was therefore possible to determine whether definitive hosts (dingoes and foxes) acquired infection by consuming protoscoleces of E. granulosus from macropods or from sheep. The results correlated well with the known distribution of intermediate hosts and illustrate the practical value of such morphological markers in epidemiological studies.

Echinococcus granulosus is maintained in 2 cycles of transmission on the Australian mainland (Thompson and Kumaratilake, 1982). One cycle principally involves domestic sheep as the major intermediate host, whereas the other involves numerous species of macropod marsupials (kangaroos and wallabies) as intermediate hosts. The potential for interaction between these cycles via a variety of carnivorous definitive hosts, including domestic dogs, feral dogs, dingoes (Canis familiaris dingo), and red foxes (Vulpes vulpes), represents a significant limitation to the institution of effective control strategies (Thompson, 1992), yet the frequency of any such interaction is not known.

Thompson and Kumaratilake (1982, 1985) suggested, on the basis of differences in hook characters (Kumaratilake and Thompson, 1984a), strobilar characteristics, and protein profiles (Kumaratilake and Thompson, 1984b) between parasites derived from sheep and macropods, that the 2 cycles of transmission represented 2 genetically different strains. Subsequent studies (Lymbery et al., 1990; Hope et al., 1991), however, have found no evidence of genetic differences between parasites from different intermediate hosts in Australia, and more detailed morphological analyses indicate that differences in hook characters are host induced (Hobbs et al., 1990).

Hobbs et al. (1990) found that protoscoleces from sheep and macropods (and other intermediate hosts) differed markedly in both the number and size of hooks and that these differences were due to the influence of the host. In addition, the larval hook outline was found to remain visible within the adult hook, and larval hook characters remain largely unchanged by passage through different definitive hosts (Hobbs et al., 1990). Taken together, these findings suggest that the hook characters of adult worms from naturally infected definitive hosts could be used to determine the intermediate host from which infection was acquired.

In this study, hook measurements of protoscoleces from sheep and macropods were used to establish criteria to type adult worms of E. granulosus from feral dogs, dingoes, and foxes, in order to identify the most likely source of infection of these naturally infected definitive hosts.

MATERIALS AND METHODS

Dogs, dingoes, and/or foxes were trapped in 7 sites in the state of New South Wales (NSW) in southeastern Australia (Fig. 1). The elevation of the collection sites ranges from 500 to 1,000 m above sea level with peaks up to 1,400 m in some areas. Much of each site consists of native hardwood forest interspersed with plantations of pine trees. The forested areas are crossed by numerous bush tracks. All the sites are covered by snow for part of the winter and are popular for recreational pig hunting with packs of trained dogs. Sheep raising country is either within or adjacent to each collection site except for the area within Kosciusko National Park (Fig. 1). The areas of greatest sheep raising activity are Braidwood and Bombala.


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Adult worms were removed from the intestinal contents of dingoes and foxes caught by professional trappers of the Rural Lands Protection Board between 1988 and 1991. As soon as possible after the animals were shot each small intestine was removed, slit longitudinally, and the contents were stripped out into clean water. The contents were rinsed through a 350-µm sieve with running water to separate the worms from the intestinal mucus. The helminths were backwashed off the sieve and preserved in 10% formalin.

Macropods and a feral pig were shot in the same areas in which the dingoes and foxes were caught. The liver and lungs of these intermediate hosts were examined as soon as possible for hydatid cysts that then were transported to Murdoch University. Hydatid cysts from the livers and lungs of sheep were supplied from abattoirs in southeastern New South Wales. We do not know if any of the sheep originated from the same areas where wildlife species were collected, but the hook characters of E. granulosus from sheep in eastern Australia have not been found to vary significantly (Hobbs et al., 1990). Each cyst was treated as a separate sample (Lymbery and Thompson, 1990).

Adult worm scoleces and protoscoleces were squashed under coverslips in polyvinyl lactophenol on microscope slides. Six worms or protoscoleces were examined from each host (where possible). The number of hooks was determined, and the length of 3 large hooks was measured as described by Hobbs et al. (1990). For adult worms, the embedded total length of hooks, equivalent to the length of the larval hook (Hobbs et al., 1990), was measured.

**RESULTS**

There were significant differences in both the number and length of hooks ($t = 6.97$, $P < 0.001$ for number of hooks; $t = 5.95$, $P < 0.001$ for length of hooks) between protoscoleces from sheep (number of hooks $35.3 \pm 0.66$, length $25.8 \pm 0.21 \mu m$, $n = 16$) and macropod marsupials (number of hooks $51.3 \pm 2.87$, length $22.7 \pm 0.57 \mu m$, $n = 9$). In general, protoscoleces from macropods had greater numbers of smaller hooks than those from sheep (Fig. 2). This was also

**FIGURE 1.** Map of study sites showing proximity to national parks.

**FIGURE 2.** Scatterplot of the length of the large hook vs. number of hooks for Echinococcus granulosus protoscoleces from 22 sheep (87 protoscoleces), 8 macropod (48), and 2 pig cysts (12). The straight lines mark the 90th percentiles along the axis between the mean sheep and mean macropod, i.e., 90% of sheep values are above line A; 90% of macropod values are below line B.
found by Hobbs et al. (1990), although they reported more variation among isolates from macropods, with some being morphologically indistinguishable from sheep isolates.

To establish a typing criterion, a line was drawn between the mean values of the sheep and macropod hooks. Two lines were drawn perpendicular to the axis of this line, line A such that 90% of sheep protoscoleces were above the line, and line B such that 90% of the macropod protoscoleces were below the line (Fig. 2).

Protoscoleces of pig origin (from only 2 hydatid cysts) had more hooks than those from sheep and larger hooks than those from macropods, but they were not as distinct as the other 2 groupings.

For the next phase of the present study in which adult worms from dingoes, feral dogs, and foxes were examined, we used the lines shown in Figure 2 as limits to determine the origin of the intermediate host from hook number and size. Worms with larval hook characters to the left of line A were considered to be of sheep origin; those with hook characters to the right of line B were considered to be of macropod origin. Isolates with hook characters between lines A and B were considered to be indeterminate.

In all, 271 worms were examined from 69 definitive hosts (60 feral dogs or dingoes and 9 foxes). Fourteen percent of all worms were typed morphologically as of sheep origin, 61.3% were of macropod origin, and 24.8% were indeterminate. The number of worms of each type is shown by collection site in Table I. The sites with fewer worms of sheep origin were Kosciusko National Park and the adjacent Brindabella, whereas in Taralga and Braidwood more than 50% of the worms were of sheep origin (Table I).

Table II shows the type of worms found in each species of definitive host by study site. A large proportion of dingoes/dogs carried worms of both sheep and macropod origin (27%), and this is probably an underestimate as only 1–6 worms could be typed for each host. Although only 9 foxes were examined, most had worms solely of macropod origin, with 1 fox at Braidwood having worms solely of sheep origin and 1 from Kosciusko with a mixed infection.

**DISCUSSION**

The measurement of larval hook characters in adult worms offers a useful tool for determining both the route of transmission of *E. granulosus* and the role of feral carnivores in stock losses. Simply by collecting worms from any carnivore it may be possible to deduce which intermediate hosts it has eaten, not just at its last meal but over a period of many months.

Two assumptions are made in using this technique. First, that the intermediate host confers consistent and distinguishable changes in larval hook characters and, secondly, that these changes are visible in the adult worms. The second assumption appears justified. Hobbs et al. (1990) found that larval hooks are embedded within adult hooks and remain largely unchanged after experimental passage through dogs, dingoes, and foxes. This study extends these results from experimental infections to field data.

The first assumption is only partly valid because we found some overlap of measurements of hook number and size of protoscoleces from macropods and sheep, although 90% of each group was typed correctly using the criteria. Hobbs et al. (1990) collected protoscoleces from throughout Australia and noted significant dif-
ferences due to macropod species in different geographic areas. This study has used only "local" samples and thus shows less overall variation in hook characters than the previous study.

Electrophoretic analysis (Lymbery et al., 1990) showed no significant genetic difference between protoscoleces from different hosts, indicating that the morphological differences were host induced. However, when larval hook characters were measured in adult worms, 25% of worms were neither clearly of sheep origin nor of macropod origin in their characters. These indeterminate worms may originate from other intermediate hosts (for example in Fig. 2 many of the pig protoscoleces fall in the indeterminate region) or may represent overlapping extremes in the distributions of hook characteristics of macropods and sheep.

There is evidence of variation among species of macropod (Hobbs et al., 1990). Further studies with larger samples and including other species of macropods are needed to clarify the extent of host-induced variation. Studies in endemic areas in other parts of the world also would be of value in determining whether other species of intermediate host induce useful phenotypic changes in Echinococcus. This would be of particular value in areas where cycles of transmission may overlap and/or where wild and domestic cycles operate. For E. granulosus, this would apply to endemic areas in Africa and China, whereas with Echinococcus multilocularis a morphological marker to identify a particular prey species would be of great value in central Europe where the epidemiology of infection is complicated by the involvement of several rodent intermediate hosts (Eckert and Thompson, 1988).

As well as providing a measure of the source of infection for a particular definitive host, morphological typing as described here can give a general indication of the mode of transmission of the parasite. For example, in all except 2 areas (Taralga and Braidwood; Table I) more worms of macropod origin than of sheep origin were found in definitive hosts. This pattern is consistent with the known distribution of intermediate hosts in these areas. In particular, Kosciusko and Brindabella, with very low proportions of worms of sheep origin, are within a National Park and are the most isolated from sheep-raising areas. In contrast, collection sites in Taralga and Braidwood are situated in predominately sheep-raising areas.

The dual infection rate of 27% (Table II) infers considerable overlap of domestic and sylvatic cycles, so attempts to reduce parasite numbers must be aimed at both cycles of transmission. It should be recognized, however, that where dual infections occur, the proportion of prey species cannot be estimated with any precision, as the relationship between the number of hosts consumed and the number of worms present from those hosts at a given time is likely to be affected by numerous other variables, such as differential rates of infection among intermediate host species, differential viability (for example most pig cysts are infertile), and differences in the numbers of protoscoleces consumed from each host.

The measurement of morphological traits that are largely affected by environment usually has been considered to be of little value. Indeed, much effort has been put into identifying and avoiding such traits, because they give a misleading picture of genetic relatedness (Rausch, 1953; Thompson, 1982; Thompson and Lymbery, 1988, 1991). For example, it was suggested (Thompson, 1982) that some of the confusion surrounding the taxonomy of Echinococcus in Argentina may have been the result of morphological differences that were shown to be host induced (Schantz et al., 1976). However, the present study has shown that such traits can reveal useful information about the previous environment of individual organisms. In this case the hook characters of adult E. granulosus can suggest which intermediate hosts carnivores have consumed. The use of such morphological markers are thus of considerable practical value as it is not possible to develop molecular markers for such environmentally induced traits.

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LITERATURE CITED


Schantz, P. M., C. Colli, A. Cruz-Reyes, and U. Prezioso. 1976. Sylvatic echinococcosis in Argentina. II. Susceptibility of wild carnivores to Echinococcus granulosus (Batsch, 1786) and host-induced morphological variation. Trophcmedizin und Parasitologie 27: 70–78.


