The prevalence, organ distribution and fertility of cystic echinococcosis in feral pigs in tropical North Queensland, Australia

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ABSTRACT

An investigation was carried out to study the prevalence of Echinococcus granulosus hydatidosis in feral pigs (Sus domesticus) in the Charters Towers region of tropical North Queensland. Data were collected from a total of 238 carcasses, which were hunted and shot in the Burdekin River catchment area. Organs of the abdominal, thoracic, and pelvic cavities were examined for the presence of hydatid cysts. In the laboratory, cysts and hydatid cyst fluids were examined under a stereoscopic binocular microscope and a compound microscope.

An overall prevalence of E. granulosus hydatid cysts in feral pigs was found to be 31.1 %. There was no significant difference in either sex or age between infected and non-infected feral pigs. The predilection sites of cysts were livers (23 %) and lungs (62 %), with more cysts in lungs (252) than livers (48). The ratio of livers to lungs infected with fertile cysts was 1:4 compared to 1:8 sterile cysts. The overall fertility of cysts was 70.1 %. The percentage of fertile cysts in liver and lung was 79.2 % and 68.7 %, respectively. The diameter of fertile cysts ranged from 15 to over 60 mm. There was no significant difference in size between fertile and non-fertile cysts in lungs. The high prevalence rate and fertility of cysts in feral pigs confirm that feral pigs can take part in the sylvatic cycle of the parasite in the region. The public health significance of this observation is potentially very important.

Keywords: Australia, Echinococcus granulosus, feral pigs, North Queensland, organ distribution, prevalence, sylvatic hydatidosis

INTRODUCTION
Echinococcosis/hydatidosis is a zoonotic disease caused by tapeworms of the genus Echinococcus that occurs throughout the world and causes considerable economic losses and public health problems in many countries. Taxonomically only four species are accepted and regarded as valid. These are Echinococcus granulosus, Echinococcus multilocularis, Echinococcus oligarthus and Echinococcus vogeli (Thompson 1986). The disease which is caused by E. granulosus has a broad geographical distribution, with highly endemic areas in countries of Africa, Latin America, Australia and Asia (Soulsby 1986). It is considered endemic in the entire Mediterranean zone including Egypt and all countries in the Middle East (Haridy, Ibrahim & Morsy 2000; Anderson, Ouhelli & Kashani 1997 cited by Dalimi, Motamedi, Hoseini, Mohammadian, Malaki, Ghamari & Ghaffari Far 2002). The domestic dog as a definitive host of the adult E. granulosus plays the most important role in the spread of infection via contamination of

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environment creating the domestic life cycle. The maintenance of *E. granulosus* in wild or feral animals without the involvement of humans or domestic ungulates demonstrates the existence of sylvatic cycles.

There is evidence of the sylvatic cycle in Australia, North America, and in East and South Africa (Rausch 1986). Based on morphological, developmental and biochemical criteria Thompson & Kumaratilake (1982), Thompson (1987), Lymbery & Thompson (1989) and Thompson & Lymbery (1990) classified *E. granulosus* in Australia into three intraspecific variants, namely the Tasmanian sheep strain, the Australian mainland strain and the Australian sylvatic strain. The sylvatic cycle exists in North Queensland between dingoes and macropods (Banks 1985). Kumaratilake & Thompson (1982) found two infected feral pigs in Western Australia, but none of the five cysts were fertile. Subsequently, Thompson (1987) reported that 12 out of 25 feral pigs trapped were infected with the parasites. Sakamoto, Tani, Hutchison, Copeman, Thompson & Sakamoto (1989) and Hutchinson & Copeman (1988) conducted epidemiological and histopathological studies in North Queensland and suggested that feral pigs could play an important role in the sylvatic cycle of the parasite. The sample size taken during these studies was small.

The objective of this study was to further investigate the prevalence, organ predilection and cyst fertility levels of hydatid disease in feral pigs in tropical North Queensland.

### MATERIALS AND METHODS

#### Study area

This study was conducted in the Charters Towers region of Dalrymple Shire in sub coastal North Queensland, approximately 120 km inland from Townsville (19.35° S, 146.78° E). The total area was estimated to be up to 30,000 km² in extent and is in the seasonally dry tropics with one annual wet season, which is generally between December and April. The start of the wet season may vary depending on the monsoon. The mean annual rainfall in Charters Towers is 570 mm. The annual average daily maximum and minimum temperatures are 27.4 °C and 12.7 °C, respectively.

#### Study design and sampling procedure

Studies to establish the prevalence of hydatid disease in animals depend mainly on collection of data obtained from animals slaughtered in abattoirs. In wildlife reliable and accurate records to study this disease depend on the number of animals which can be trapped or killed for survey purposes. Limitations of surveillance data for cystic hydatidosis have been reviewed recently (Schantz 1997). Therefore, this study was designed on the use of a chiller depot which has been assigned permanently in the study area to store the carcasses of feral pigs shot in the field.

Feral pigs are hunted for sport and for export as game meat to Europe especially to Germany. Field-shot animals were identified with ear tags with code numbers and deposited the same day in the chiller depot after their intestinal tracts were removed. The weight of each carcass was measured and it and the hunter’s registration number were recorded on tags which were fastened to each carcass. Data, such as mass, sex, age group and physical condition of the carcasses were collected on a monthly basis. For subsequent meat inspection prior to export certification hearts, the lungs, livers, kidneys and spleens were not removed from carcasses.

Age estimations were based on dentition. The animals were assigned to one of two broad age groups, i.e. 9–15 months as young, and above 16 months as adult. Data on suckling or weaner pigs were not available. Organs of the abdominal, thoracic and pelvic cavities were examined for the presence of hydatid cysts by observation and palpation, with special emphasis being placed on the livers and lungs. Hydatid lesions were differentiated from other cystic parasitic diseases based on FAO/UNEP/WHO guidelines (1981), and were removed intact from the infected organs, stored on ice and transported to a laboratory in Townsville within 4 h.

In the laboratory, the size of the cysts was measured. Small lesions < 5 mm diameter and those that could not be confidently differentiated as hydatids, were excluded. The presence and number of cysts were recorded according to organ distribution. The contents of each cyst were then aspirated with a hypodermic syringe, transferred into vials or petri dishes and examined under a stereoscopic binocular microscope for the presence of brood capsules. Drops of hydatid cyst fluid (HCF) were also mounted on slides with cover slips and observed under a compound microscope for the presence (fertile cysts) or absence (sterile cysts) of protoscolices. A fertile cyst was recorded as viable if it contained protoscolices showing flame-cell activity. Cysts which contained no protoscolices or were calcified, were considered as infertile. The cysts were then incised with
a scalpel to determine the stages of degeneration. All were examined for the presence of an identifiable laminated membrane indicating that the cyst was of *Echinococcus* origin. This structure could be detected in hydatid cysts which were either live or partially necrotic, and it appeared to persist for some time after death of the parasite (Banks 1985).

Age and sex, predilection sites, fertility and sterility of cysts were factors which were considered for statistical analysis. The chi-square method of analysis was employed to determine statistical differences in the fertility of cysts in both sexes and age groups of the pigs. Numbers and sizes of the cysts were calculated and differences tested for statistical significance using a one-way analysis of variance using Statistix version 3.5 (Analytical Software, St. Paul, MN, USA).

**RESULTS**

All feral pigs were of the same black type (Fig. 1). A total of 238 feral pigs was examined, 137 being male and 101 female. Of these 103 were classified as young and 135 as adult. The overall prevalence of *E. granulosus* was 31.1% (74/238) with the age group prevalence of the disease being 31.1% in both young and adult feral pigs alike. The sex-specific prevalence was 32.1% and 29.7% for male and female feral pigs, respectively (Tables 1 and 2).

In all 74 of the infected feral pigs 56 lungs, 17 livers and one kidney were found harbouring one or more cysts. The prevalence of *E. granulosus* metacestodes in the principal sites of predilection was calculated to be 56/74 (75.7%) in the lung and 17/74 (22.93%) in the liver. Mean numbers of cysts per organ were 2.8 in livers and 5.5 in lungs (Table 3). A single sterile cyst was found in a kidney. No cysts were found in the hearts or other organs. The frequency distribution of the number of cysts found in infected pigs shows the level of infection.

A total of 301 confirmed hydatid cysts was found in the livers and lungs of all the infected feral pigs (Table 3). The percentage of fertile cysts in the livers and lungs was 79.2% and 68.7%, respectively (Table 3). The overall fertility rate of cysts in livers and lungs was 70.1%. Live protoscolices were found in 19/38 (50%) of all fertile liver cysts and 109/173 (63.2%) of all fertile cysts from lungs. The ratio of livers to lungs infected with fertile cysts was about 1:4 compared with 1:8 for sterile cysts. The diameter of fertile cysts ranged from 15–60 mm (Table 4). Of all cysts in either organ only a very few cysts showed necrosis and calcification. The mean size of sterile cysts from the livers (33.7 mm) was significantly larger than that of the fertile cysts (21.0 mm) (Table 4) but in lungs there was no difference between the two types (mean 31 mm). There was no significant difference in the fertility of cysts in pigs of either sex (*P* = 0.803).

**DISCUSSION**

In this study, importance was given to feral pigs as they have the ability to transmit diseases and parasites important to livestock and humans (Kanameda...
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The use of dogs for pig hunting and the way these dogs are fed during the hunting season may contribute to the maintenance of hydatid disease in feral pigs. This situation has stimulated number of studies in different parts of Australia (Banks 1985; Baldock et al. 1985; Thompson & Kumaratilake 1982; Hutchinson & Copeman 1988; Sakamoto et al. 1989).

The present study showed an overall prevalence of 31.1% of *E. granulosus* hydatid cysts in feral pigs, which is comparable to, but slightly higher than that reported in a preliminary study in the same area which was 8/29 (27%); the number of samples were, however, small (Hutchinson & Copeman 1988). There were no statistical differences due to age or sex (Tables 1 and 2). Gemmell & Brydon (1960) found no differences in sex-specific prevalence in beef and dairy cattle in New South Wales but showed an increase in prevalence with age. Similarly, Baldock, Arthur & Lawrence (1985b) found no differences in sex-specific prevalence in Queensland cattle, but the prevalence increased linearly with age from 17 months to 4 years. Several factors may affect the variation in prevalence with origin of hosts. These include climate, stocking rate and the abundance of infected definitive and other intermediate hosts. Similar factors may be responsible for the variation in prevalence in feral pigs between Queensland and Western Australia. As very young (i.e. suckling pigs) were not sampled, as such animals are not accepted by the game processors, the true age prevalence could not be determined, but would be expected *a priori* to be similar to that in cattle.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Frequency distribution of hydatid cysts in infected feral pigs by age</th>
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<tbody>
<tr>
<td>Age group</td>
<td>No. examined</td>
</tr>
<tr>
<td>Young</td>
<td>134</td>
</tr>
<tr>
<td>Adult</td>
<td>104</td>
</tr>
<tr>
<td>Total</td>
<td>238</td>
</tr>
</tbody>
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Chi-square 2.587
Degrees of freedom 1
Significance 0.108

Thus sex and infection are independent

<table>
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<tr>
<th>TABLE 3</th>
<th>Distribution and percentage of fertile hydatid cysts per organ in infected feral pigs</th>
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<tr>
<td>Organ infected</td>
<td>No. of feral pigs</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>17</td>
</tr>
<tr>
<td>Lung</td>
<td>56</td>
</tr>
<tr>
<td>Kidney</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
</tbody>
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Degrees of freedom 1
Significance $P = 0.108$

<table>
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<tr>
<th>TABLE 4</th>
<th>Differences in the mean size (± standard error) of fertile liver and lung hydatid cysts in feral pigs</th>
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<tbody>
<tr>
<td>Cyst type</td>
<td>Mean size (mm) ± S.E</td>
</tr>
<tr>
<td>Fertile liver</td>
<td>21.6 ± 2.02</td>
</tr>
<tr>
<td>Lungs</td>
<td>31.2 ± 0.58</td>
</tr>
<tr>
<td>Sterile liver</td>
<td>33.7 ± 3.66</td>
</tr>
<tr>
<td>Lungs</td>
<td>31.1 ± 2.87</td>
</tr>
</tbody>
</table>

For cysts in the liver there was a significant difference in size due to fertility/sterility
$F (1, 26) = 7.84, P = 0.0095$

For cysts in the lung there was no significant difference in size due to fertility/sterility
$F (1, 53) = 0.9, P = 0.9680$
The lungs and livers were favoured sites for hydatid cyst development in feral pigs as has been shown to be the case with other studies in pigs or other host species. In this study cysts were found in lungs five times more often than in livers. About 13.5 percent of the total feral pigs sampled had cysts in both organs, but no cysts were found in any other organ except for a single cyst in a kidney. Banks (1985), however, found three infected feral pigs of which two harboured single hepatic cysts, one of which was fertile, and the third had disseminated infection with some fertile cysts in the liver, lungs, spleen and kidneys.

In other susceptible host species such as ungulates, hydatid cysts also occur predominantly in the lungs and livers. In cervids (Cervinus spp.) cysts are rarely found in organs other than the lungs (Rausch 1975). In macropods cysts are often found in the thoracic cavity, mainly in the lungs, although occasional cysts occur in the pleural cavity attached to the lungs by a thin peduncle (Banks 1985). In pigs the liver is often involved alone or in combination with the spleen and kidneys (reviewed in Rausch 1997). Unicystic forms of infection seem to be more common than multicystic forms. Very few multicystic forms were found in this study.

At present there are no means by which to estimate the ages of hydatid cysts in feral pigs without conducting an experimental infection study to determine growth rates of the cysts. Without this information it is not possible to determine when feral pigs became infected. While the majority of cysts observed in this study were between 21 and 30 mm in diameter there was no significant differences (P > 0.05) between the mean size of cysts in livers and lungs.

The diameter of metacestodes of the sylvatic strain of *E. granulosus* in experimentally infected cattle increased linearly with time. Hepatic cysts increased at a rate of 0.3 mm per month, so that after 1 year the predicted mean diameter was only 3.9 mm (Banks 1985). According to Banks’ (1985) estimate, a cyst with a diameter of 5 mm could be between 1 and 2 years of age. Growth rates in sheep are believed to be similar (Sweatman & Williams 1963). Dew (1925) claimed that hydatid cysts in domestic pigs reached 40 mm in only 3 months. Unfortunately no information has been found of similar studies in feral animals. As there are host species differences and other factors, such as the immune response, environmental and climatic factors, it cannot be established if the natural growth rate of hydatid cysts in feral pigs is similar to this, or closer to that in herbivores.

The high percentage (69–80%) of fertile hydatid cysts in feral pigs was surprising in view of the limited information on the fertility rate that was obtained in previous studies, and the very low (< 1%) rate in cattle cysts in the same region. Although a high proportion of cysts were fertile, the numbers of protoscoleces observed in each cyst were relatively low, usually less than 50 and they were frequently nonviable. This precluded the possibility of having the protoscoleces typed to strain. In contrast, from a single fertile cyst of sheep origin several thousand protoscoleces can be found. In the present study, there was no statistically significant difference in the fertility of cysts in pigs of either sex (P = 0.843).

Fertile liver cysts were, with one exception, smaller than 30 mm diameter, whereas some lung cysts up to 60 mm contained protoscoleces. The reasons for the high fertility level of *E. granulosus* in feral pigs and whether feral pigs harbour the sylvatic strain of the parasite are not yet known.

The low proportion of fertile cysts in cattle (Baldock et al. 1985b; Banks 1985) suggests that cattle play little part in continuing the life cycle of the parasite. Our result supports the view that feral pigs might play an important role in maintaining the sylvatic life cycle of *E. granulosus*.

Banks (1985) noted that in those parts of northern Queensland hundreds of kilometres from the nearest sheep, no evidence was found for the existence of domestic animal cycle spill-over into wild and feral animals which share the same habitat. Since, in northern Queensland the mainland domestic strain has been identified (Baldock et al. 1985a) and propagation of the mainland domestic strain between dingoes and feral pigs has been suggested (Banks 1985) further study is clearly necessary to better understand the relationships between strains and their host assemblages.

Banks (1985) described the two most significant intermediate hosts as the black-striped wallaby (*Macropus dorsalis*) and the feral pig (*Sus domesticus*) with a prevalence of 21.8% and 9.4%, respectively. In the present study, the 31% prevalence of the disease in feral pigs was higher than that obtained previously. This demonstrates that feral pigs have a role in the epidemiology of the disease in this region. There is no direct evidence that feral pigs are a substantial diet of dingoes, in distinction to wallabies, which have been identified in stomach contents (Banks 1985). Whether feral pigs are infected from dingo faeces, and if dingoes are capable of being infected from pig-derived cysts is yet to be clarified.
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Using a chiller depot for disease surveys in wild or feral animals could be of use for the storage of animals shot in the field and it could become an economical way for collecting information. However, such surveys must be planned carefully as killed feral animals are not true random samples from the population at risk, especially where size of the population of the stock is difficult to estimate or it is impossible to apply techniques of sample size estimation. The use of tables of binomial confidence limits or capture and release techniques (Cannon & Roe 1982) would be applicable to estimate the sample size for this type of disease survey. This survey was conducted under such conditions for number of reasons. Firstly, a simple, economical and satisfactory method of surveying feral pig hydatid disease was not found. Secondly, the chiller depot serves not only as a convenient source of data and specimens, which would otherwise be logistically difficult to obtain in sufficient numbers, but it is also a source which enables the tracing of information on the habitat of the feral animals and behaviour which can be obtained by personal communication with the professional hunters.

The public health significance of this investigation is important. Hunters’ dogs which may be fed offal from killed or scavenged dead feral pigs, could become infected, and if patent infections develop, they may shed E. granulosus eggs within and around urban centres. After being voided in the faeces of a dog, eggs can contaminate extensive areas through transportation by blowflies. Thompson (1987) has suggested such a cycle already exists in Perth. No data are available on human hydatidosis in North Queensland and no pig hunting dogs were found to be infected with E. granulosus (Hutchinson & Copeman 1988), but this leaves no grounds for complacency.

Ballock et al. (1985a), in determining the origin of human infections, suggested the domestic strain of the parasite was most commonly encountered in human cases. In their study Campos-Bueno, Lopez-Abente & Andres-Cercadillo (2000) described the risk factors for hydatidosis traceable to the family to be of greater relative importance than those attributable to working directly with livestock. From the results of this study, we speculate that the sylvatic pig form may be a potential source of human cases of hydatidosis for humans.

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