2
3 A hyperendemic focus of porcine cystic echinococcosis in the Banke
4 District of Nepal
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20 Short title: Porcine cystic echinococcosis in Banke District, Nepal
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22 ABSTRACT

23 Detailed post mortem analyses of 68 free-ranging, slaughter-age pigs from two sites in the
24 Banke District of Nepal identified 36% as being infected with *Echinococcus granulosus*. The
25 cysts ranged from infertile, immature cysts a few millimetres in diameter to fertile cysts >10
26 centimetres in diameter. PCR RFLP and DNA sequencing identified the cysts as being *E.*
27 *granulosus sensu stricto*. The Banke district has recently been identified as having a high
28 prevalence of porcine cysticercosis. These data suggest that cestode zoonoses in this, and
29 possibly other parts of Nepal may be a serious concern for human health. An assessment of the
30 level of human cystic echinococcosis and neurocysticercosis, in the region is warranted and the
31 introduction of control measures are required to limit the parasites' transmission.

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33 *Keywords: Echinococcus granulosus, pig; Nepal*

Cystic echinococcosis is a zoonotic disease with a worldwide distribution. The disease is most prevalent in pastoral communities, often transmitted by sheep as intermediate hosts (Deplazes et al., 2017). However, the disease may be transmitted by a wide variety of domestic livestock and wild animals (Thompson, 2017). The aetiological agent of 88% of human cystic echinococcosis is *Echinococcus granulosus sensu stricto* (Alvarez Rojas et al., 2014). Almost all the remaining proportion of human cystic echinococcosis is caused by a closely related species within the *E. granulosus sensu lato* group, *Echinococcus canadensis* (genotypes G6 and G7). Pigs are one of the principal animal intermediate hosts for *E. canadensis* (genotype G7), although they may also be infected with *E. granulosus sensu stricto* (Cardona & Camino, 2013).

Several reports have identified Nepal as being endemic for cystic echinococcosis (CE). Joshi et al. (1997) reviewed studies undertaken as early as 1973 on echinococcosis in Nepal, particularly in abattoirs and hospitals in Kathmandu city, Nepal. Hydatid cysts were found in slaughtered animals, including in 5% of water buffalos, 3% goats, 8% sheep, and 7% pigs. During 1994/5, 120 operations for CE were carried out in Kathmandu hospitals and high levels of serological positivity in tests for hydatid disease were found in Nepalese people (Joshi et al., 1997).

Detailed necropsy investigations were undertaken by Poudel et al. (2019) on 68 free-roaming, slaughter-age pigs from two areas of the Banke District of Nepal, 33 from Udaypur Village Development Committee (VDC) and 35 from Hirminiya & Betahani VDC enumerating infections with *Taenia solium*. Here we report the findings of these necropsies in relation to *E. granulosus* infections. The necropsies included palpation and slicing at approximately 5 mm of both the liver and the lungs. Twenty-five animals (36%) were identified as having CE cysts by macroscopic appearance, thirteen from Udaypur VDC, and twelve from Hirminiya & Betahani VDC. The average age of the infected animals was 18

59 months (range 10-34 months). Similar infection rates were seen in both males and females.

60 Twenty animals were found to have cysts exclusively in the lungs, two had cysts exclusively
61 in the liver and three had cysts in both lungs and liver. Cysts ranged in size from a few
62 millimetres to more than 8 centimetres in diameter (Fig. 1). Not all specimens were

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Fig. 1. Representative images of cystic echinococcus cysts (A-F) identified in the liver or lungs of free-ranging, slaughter-age pigs from the Banke District of Nepal. Fertile cysts are shown in panels D and E; protoscolices shown in panel F and insert.

investigated to identify the presence of protoscolices, however protoscolices and brood capsules were noted in some of the larger cysts (Fig. 1F). DNA samples from 16 animals were analysed by PCR RFLP as previously described by Gauci et al. (2019) and were confirmed to have been *E. granulosus sensu stricto* (genotype G1).

There are few reports of *E. granulosus sensu lato* occurring in Nepal. Zhang et al. (2000) investigated the genotype of cystic echinococcosis in specimens from Nepal and found both the G1 genotype (*E. granulosus sensu stricto*) and the G5 genotype (*Echinococcus orthleppi*) in buffalo, sheep and goats. Two human cysts were studied, both of which were the G6 genotype (*E. canadensis*). Zhang and colleagues did not investigate parasite samples from pigs. Joshi and Yamasaki (2010) reported the presence of two pancreatic CE cysts in Nepalese pigs which were identified as being *E. granulosus sensu stricto* (genotype G1).

Nepal is a developing country with a GDP ranking of 162 out of 187 nations globally ranked by the International Monetary Fund (<https://www.imf.org/en/Countries/NPL>). The Nepalese population suffers from a substantial burden of parasitic zoonoses, including CE (Devleeschauwer et al., 2014). Joshi and Yamasaki (2010) reported a nation-wide prevalence of CE in Nepal of 55 per 100,000 population based on hospital in-patient records. Little is known about regional differences in the prevalence of CE in Nepal, however the high prevalence of *E. granulosus sensu stricto* in pigs in the Banke District warrants further investigation of human CE cases in the region and, possibly, implementation of control measures. Two zoonotic diseases that are highly prevalent in free-ranging pigs in the Banke District of Nepal are caused by cestode parasites, *E. granulosus* and *T. solium*. There are

effective and commercially-produced vaccines against both infections (Craig et al., 2017; Poudel et al., 2019). Potentially, a program of pig vaccination may reduce the incidence of both CE and neurocysticercosis in communities in this region.

Author's contributions

MD, IP, KS, DKS and MWL undertook the necropsies. CGG and AJ undertook the genotyping. All authors contributed to drafting the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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