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ORIGINAL ARTICLE

Dogs and pigs are transport hosts of *Necator americanus*: Molecular evidence for a zoonotic mechanism of human hookworm transmission in Ghana

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Abstract

Hookworm infection (*Necator americanus* and *Ancylostoma* spp) causes significant morbidity in resource-limited countries. Dog and pig ownership is associated with human infection, although the mechanism through which animals increase risk remains unknown. We first confirmed this association in Kintampo North, Ghana, using a retrospective analysis and serology, followed by a prospective molecular study of animal faeces. As a proxy of exposure to dog faeces, we analysed immunoreactivity of human serum to the zoonotic nematode *Toxocara canis*. Anti-*Toxocara* antibodies were present in 62% of samples (n = 89), and reactivity was associated with dog ownership. A subsequent prospective study revealed that 43% of dog and 56% of pig faecal samples contained hookworm eggs by microscopy. PCR analysis confirmed the presence of *N. americanus* DNA in 47% of samples from dogs and 56% pig samples. Nematode larvae were successfully cultured from samples collected from 36 dogs and seven pigs. These results demonstrate that dogs and pigs have a likely role in the transmission of *N. americanus* in endemic communities.

KEYWORDS

dog, Ghana, hookworm, *Necator americanus*, pig, zoonoses

1 | INTRODUCTION

Human hookworm infection represents a significant health burden in low- and middle-income countries in Africa and Asia, causing up to 65% of the morbidity attributable to soil-transmitted helminth (STH) infections (Bartsch et al., 2016; Jourdan, Lamberton, Fenwick, & Addiss, 2018; Pullan, Smith, Jasrasaria, & Brooker, 2014). Infection in children leads to anaemia and cognitive impairment, reinforcing a cycle of poverty and underdevelopment, especially in Sub-Saharan Africa (Fenwick, 2012; Gyorkos et al., 2018; Pullan, Gitonga, Mwandawiro, Snow, & Brooker, 2013). The majority of human hookworm infections are caused by *Necator americanus*, which is acquired through skin contact with third-stage larvae that develop from eggs excreted in the faeces of individuals harbouring adult worms (Loukas et al., 2016). Humans are considered the definitive host for *N. americanus*, which has only rarely been detected in other mammalian species in non-experimental settings (Hasegawa et al., 2014, 2017; Orihel, 1970). It is therefore widely assumed that *N. americanus* transmission depends exclusively on human factors and behaviour that can be altered by treatment of infected individuals and improved sanitation. Periodic deworming and implementation of community-based sanitation programmes have been proposed as a means of reducing global morbidity and transmission of hookworm and other STH infections (Campbell et al., 2017; Drabo et al., 2016; Marocco, Bangert, Joseph, Fitzpatrick, & Montresor, 2017; Nikolay et al., 2015; Strunz et al., 2014; Worrell et al., 2016). However, prevalence often persists and transmission continues, even in communities where one or both of these interventions have been implemented (Allen & Parker, 2016; Campbell, Nery, McCarthy, et al., 2016; Kepha et al., 2017; Pullan et al., 2014). The modest progress towards global

control of hookworm and other STH infections suggests that our current knowledge and understanding of hookworm epidemiology and pathogenesis may not be complete (Campbell, Nery, Doi, et al., 2016; Coffeng et al., 2017; Humphries, Nguyen, Boakye, Wilson, & Cappello, 2012; Toor et al., 2018).

Despite the absence of a known zoonotic reservoir, epidemiologic studies have found that dog and pig ownership are each associated with a higher risk of hookworm infection (Corrales, Izurieta, & Moe, 2006; Moll, McElroy, Sabogal, Corrales, & Gelting, 2007; Ngui, Lim, Traub, Mahmud, & Mistam, 2012). In Malaysia, rural dog owners are more than four times as likely to be infected with hookworm as non-dog owners, and in Central American sites, Corrales et al. observed that children living in compounds with pigs had 150% higher prevalence of hookworm than children living in compounds without pigs (Corrales et al., 2006; Moll et al., 2007). Likewise, in previous studies in the Kintampo North Municipality of central Ghana, children in pig-owning households were found to have a 77% greater probability of harbouring *N. americanus* hookworm infection (Humphries et al., 2013).

In the current study, we defined a potential immunoepidemiologic link between exposure to dog faeces and hookworm infection status in a retrospective study. We then prospectively examined whether dogs and pigs excrete viable hookworm eggs in a non-experimental setting, in order to assess the potential of domesticated animals to serve as transmission vectors of *N. americanus*. These data suggest that improved animal husbandry has the potential to augment current hookworm control efforts, which rely primarily on targeted chemotherapy to school-age children. Our study also sheds light on the biology and ecology of *N. americanus*, adding a new dimension to the existing paradigm of human hookworm transmission and disease.

Impacts

- Ownership of domesticated animals has long been associated with human hookworm infection, although the mechanism of transmission remains unknown.
- This paper confirms the epidemiologic link between dog and pig ownership in hookworm endemic communities in Kintampo North, Ghana.
- For the first time, both dogs and pigs have been shown to excrete viable human hookworm eggs (*Necator americanus*) in a non-experimental setting, suggesting that animal behaviour plays a direct role in parasite transmission.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

Ethical approval for all human subject research was obtained from the Human Investigation Committee at Yale University (HIC#1306011926) and the Institutional Review Board at the University of Ghana's Noguchi Memorial Institute for Medical Research in Accra.

2.2 | Retrospective modelling of hookworm infection status and domestic animal ownership

The epidemiology of *N. americanus* hookworm infection in communities along a 90-km stretch of highway in the Kintampo North Municipality, located in the Brong Ahafo Region of central Ghana, has been characterized previously in a number of field studies (Orr et al., 2019; Humphries et al., 2011, 2013, 2017). Epidemiological data collected during a 2010 study (Humphries et al., 2013) were analysed using regression modelling to determine whether individual animal ownership and community rates of animal ownership were independent risk factors contributing to the prevalence and intensity of hookworm infection. Logistic and ordinal regression modelling that assessed the impacts on prevalence and intensity of infection was also applied and produced comparable results, with the same terms reaching significance. To find a best fit model, AIC scores were calculated for each possible model combination, including individual ownership of dogs, individual ownership of pigs, the proportion of households in one's community owning dogs and the proportion of households in one's community owning pigs, as well as the two-factor interaction terms between those variables. No interaction terms achieved statistical significance.

2.3 | Serum anti-*Toxocara* antibody testing

To verify exposure to other zoonotic nematodes transmitted by contact with animal faeces, a sub-sample of available human sera collected during the 2010 field study (45 dog owners and 48 non-dog owners) was analysed for the presence of antibodies to *Toxocara canis* (Humphries et al., 2013). Samples were kept frozen (−80°C) until analysis using the reagents and buffers contained within the *T. canis* IgG Human ELISA kit (Abcam). Briefly, serum was defrosted on ice prior to dilution (1:100) in assay buffer and addition to duplicate wells of a microtitre plate. Plates were incubated at 37°C, wells were washed, and horseradish peroxidase-conjugated protein A solution was added. Wells were washed again after incubation at 37°C and bound protein detected with TMB substrate. Following the subsequent addition of stop solution to all wells, absorbance (OD_{450}) was measured using a Spectramax 190 microtitre plate reader (Molecular Devices) and the mean of duplicate readings was used to determine the level of response. Samples were considered positive if the absorbance value measured more than 10% above the value for the cut-off control sample provided with the kit and included in each run. Four samples (one from a dog owner and three non-dog owners)

lacked valid negative control values and were therefore not included in the bivariate analyses.

2.4 | Field site and subjects

In 2013, a prospective field study was conducted in eight rural villages along the main Kintampo road. Following completion of a brief questionnaire, permission was obtained from residents to collect faecal samples from dogs (n = 64) and pigs (n = 20) owned by individuals living within the study villages. Care was taken to collect fresh faecal samples, which were processed on the day of collection in order to preserve the integrity of nematode eggs.

2.5 | Hookworm egg isolation and larval culture

Animal faeces were first examined for the presence of parasite ova using the Kato–Katz method of slide preparation and light microscopy (Katz, Chaves, & Pellegrino, 1972). Eggs were then purified from hookworm-positive samples using serial buffer suspension and centrifugation, followed by filtration (Humphries et al., 2013; Reiss, Harrison, Bungiro, & Cappello, 2007). Purified eggs were kept frozen (−20°C) until further processing by genomic DNA (gDNA) extraction and analysis. The remaining faeces were cultured in charcoal, and viable larvae were isolated using a Baermann apparatus (Viglierchio & Schmitt, 1983). Viability was assessed by light microscopy, and all recovered larvae were frozen at −80°C prior to transportation from Ghana to New Haven.

2.6 | Hookworm DNA analysis

Genomic DNA was extracted from egg samples using the QIAgen DNA Stool Extraction Kit (QIAGEN) following manufacturer's instructions. Purified gDNA was kept frozen (−20°C) until molecular analysis. To determine whether faecal samples from dogs and pigs contained human hookworms, two species-specific PCR methods were used to amplify *N. americanus* sequences from egg gDNA (Monti, Chilton, Qian, & Gasser, 1998; Zhan, Li, Xiao, Zheng, & Hawdon, 2001). The first PCR amplified a region of cytochrome *c* oxidase subunit 1 (*cox-1*) (Li et al., 2004; Zhan et al., 2001) using oligonucleotide primers specific for *N. americanus* (NaF: 5′-TTCGTTTGGAGTTGGCT-3′ and NaR: 5′-TAGCTCCAGCCAAAACT-3′). The second PCR amplified regions of the internal transcribed spacer-2 (*ITS-2*) of rDNA using the *N. Americanus*-specific primer jmNA (5′-CGTTAA CATTGTATACCTGTACATAC-3′) in combination with the NC2 primer (5′-TTAGTTTCTTTTCCTCCGCT-3′) (Monti et al., 1998). Positive PCR controls were prepared using gDNA prepared from *N. Americanus* collected from human subjects in Kintampo (Humphries et al., 2011, 2013). Negative controls lacking DNA template were included in each assay. All PCR products of the appropriate size were purified from agarose gel slices using a QIAGEN PCR Purification kit. Sanger sequencing of purified PCR products was carried out by the DNA Analysis Facility at Yale University.

2.7 | Phylogenetic analysis

Genomic DNA sequences were used to construct a phylogenetic tree using maximum likelihood as implemented in the Mega7 software package (Kumar, Stecher, & Tamura, 2016). In addition to the sequences obtained from dog and pig samples, alignments included sequences from *N. americanus* that were collected from children residing within the endemic communities of Kintampo (Humphries et al., 2011, 2013). Sequences captured by PCR using ITS-2primers included 12 that were amplified from human faeces, three sequences that were amplified from dog samples and one that was amplified from a sample collected from a pig. Additional sequences from the NCBI database were also included for comparison, including ITS-2 from hookworm species *Ancylostoma caninum* accession #JQ812694; *Ancylostoma ceylanicum* accession #AF263490, *Ancylostoma duodenale* accession #EU344797 and *N. americanus* accession #s AJ001599, #Y11734 and AJ001600. Accounting for gaps and missing sequence data, a total of 200 positions were present in the final sequence alignment. For the cytochrome oxidase I analysis, 10 sequences corresponding to hookworm samples collected from humans were aligned with four dog samples and one pig sample. Additional sequences from the NCBI database for cytochrome oxidase I were also included from *N. americanus* (accession # AF303142.1) and the *Ancylostoma* species (*A. caninum* accession #U57030.1, *A. ceylanicum* accession # LC036568.1, and *A. duodenale* accession # EU007447.1). A total of 257 positions were present in the final sequence alignment.

2.8 | Statistical analysis

Statistics were computed in either R (https://www.r-project.org/about.html; R Foundation for Statistical Computing) or Stata (version 14.0, 2013.; Stata Corp). All bivariate analyses (including Pearson's chi-square and Kruskal–Wallis rank-sum tests for non-parametric data) were two-tailed.

3 | RESULTS

3.1 | Ownership of dogs and pigs is associated with an increased risk of hookworm infection

Retrospective analysis of data collected during a 2010 study of hookworm epidemiology (Humphries et al., 2013) demonstrated that children from pig-owning households were significantly more likely to be infected with hookworm than those living in households without pigs (68% of pig owners infected vs. 35% of non-owners; $\chi^2 = 13.3$; $p < .001$). Individual dog ownership was also a significant

predictor of hookworm infection status in this population (48% vs. 34%; $\chi^2 = 4.46$; $p = .03$), and the proportion of dog-owning households in a community was also associated with hookworm infection risk (Figure 1; logistic regression, $p = .01$). Over the range of dog ownership rates (8%–62%) found in our study communities, the expected hookworm prevalence more than doubled from 25% to 54%.

When considering both individual pig/dog ownership and community ownership rates in a single model, individual pig ownership and the proportion of households within a community that owned dogs were also significantly associated with infection intensity, as measured by eggs per gram of faeces (ordinal regression, $p < .03$ for both). The best fit ordinal regression model included individual household pig ownership and the proportion of households in the community that own dogs, but not individual-household dog ownership. Taken together, these results suggested a causal role for dogs and pigs in hookworm transmission in Kintampo.

3.2 | Anti-*Toxocara* antibodies are associated with hookworm infection status

Using samples collected during the 2010 Kintampo study, we conducted a retrospective seroepidemiological analysis of anti-*Toxocara* antibodies as a putative marker of human exposure to dog faeces. Of the 89 samples (45 dog owners and 48 non-dog owners) tested, 55 (62%) were seropositive for *T. canis*. A higher proportion of dog owners tested positive (56% vs. 38%, $p = .1$), and dog owners as a group also exhibited significantly higher levels of anti-*Toxocara* antibodies compared with non-dog owners (Figure 2a; *t* test $p = .02$). A chi-square analysis revealed that subjects seropositive for *T. canis* were also more likely to be infected with hookworm than those who were seronegative (43.6% vs. 20.6%; $p = .03$).

To confirm assay specificity and eliminate the possibility of cross-reaction between antibodies to *T. canis* and hookworm, we compared the OD values obtained using the commercially available *Toxocara* ELISA to values measured against hookworm antigens using a previously described laboratory-based ELISA assay (Humphries et al., 2011, 2013, 2017). Among the identical serum samples, there was no correlation in a linear regression between anti-*Toxocara* and anti-hookworm antibody responses (Figure 2b; adjusted $R^2 = -.004$, $p = .45$), confirming that the *T. canis* results were not influenced by cross-reactivity with hookworm antigens in the serum samples tested. Of note, none of the study subjects from whom these serum samples were drawn were infected with *Ascaris lumbricoides* or *Trichuris trichiura* (Humphries et al., 2013).

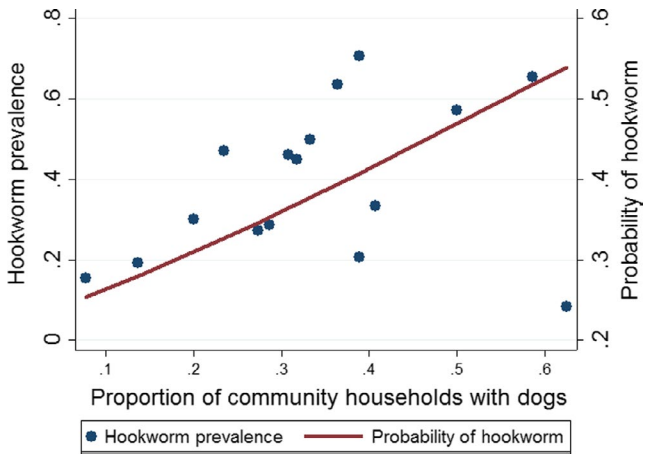

FIGURE 1 Living in a community with a higher proportion of dog ownership is associated with a higher probability of hookworm infection. The scatterplot represents the prevalence of hookworm infection in communities with the given proportion of households with dogs. The predicted line was derived based on logistic regression of probability of hookworm infection as a function of community prevalence of dog ownership ($p = .01$). The line indicates that the probability of hookworm infection correlates with the prevalence of dog ownership within the communities studied

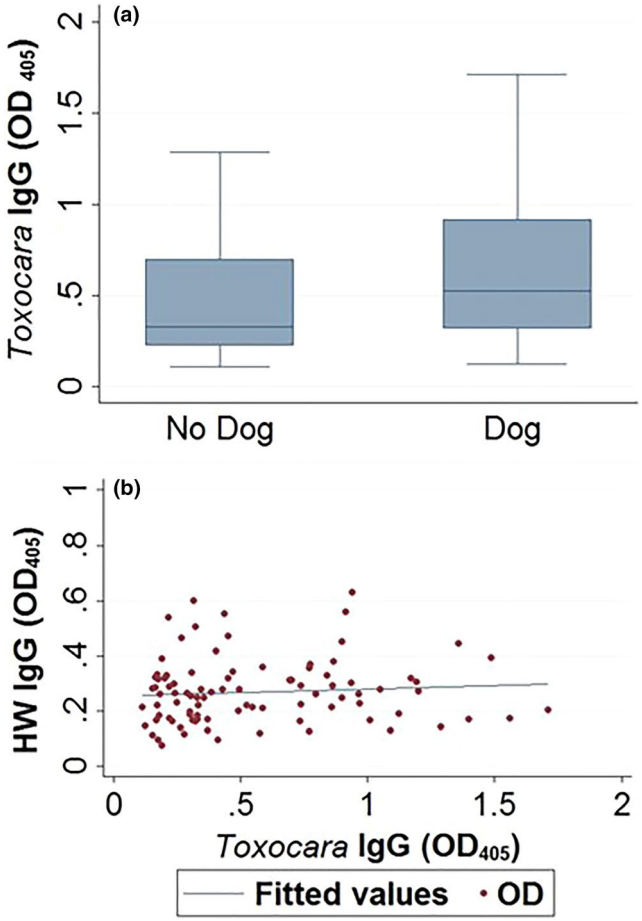

FIGURE 2 Children in the Kintampo North Municipality show evidence of exposure to the dog nematode *Toxocaracanis*. (a) Analysis of seroreactivity (Kruskal–Wallis rank-sum test) shows higher antibody responses among dog owners than for non-dog owners ($p = .02$). (b) Linear regression of *Toxocara* and hookworm (HW) antibody levels (OD values) for study samples shows no relationship (adjusted $R^2 = -.004$; $p = .45$), indicating a lack of cross-reactivity between the two ELISA assays

3.3 | Dogs and pigs excrete the human hookworm *N. americanus*

In 2013, a prospective field study was conducted across eight villages in Kintampo North. Faecal samples collected from 64 dogs and 20 pigs were examined by light microscopy for hookworm eggs using the Kato–Katz method. As shown in Figure 3, hookworm eggs were identified in 43/64 dog (67%) and 9/20 pig (45%) samples. To assess the viability of observed hookworm eggs, positive faecal samples from dogs and pigs were cultured using the Baermann method. Viable nematode larvae were successfully cultured from 80% of dog (n = 36) and 78% of pig (n = 7) faecal cultures studied (Figure 3).

Species identification of hookworm eggs excreted by dogs and pigs in Kintampo was evaluated using PCR of gDNA extracted from hookworm positive samples (Humphries et al., 2013). As shown in Figure 3, 20/43 (47%) of purified egg samples from dogs and 5/9 (56%) of samples from pigs contained *N. americanus*. Sanger sequencing of PCR products successfully validated the PCR results in seven of the 20 dog samples (35%) and 2/7 (29%) pig samples. Failed sequencing reactions likely resulted from low amounts of purified PCR product serving as template DNA (<10 ng) for Sanger sequencing.

Molecular Evolutionary Genetic Analysis (MEGA) version7 computer software (Kumar et al., 2016) was used to construct separate dendrograms for *ITS2* and *COI* sequences amplified from human- and animal-derived hookworm samples. As expected from prior investigations (Humphries et al., 2011, 2013), sequences in PCR-amplified products from human faecal samples collected in Kintampo are most consistent with *N. americanus*, the predominant human species endemic in these communities (Figures 4 and 5). In addition, individual sequence alignments of the *ITS2* and *COI* PCR amplicons from pig and dog hookworm samples confirm that these isolates are most

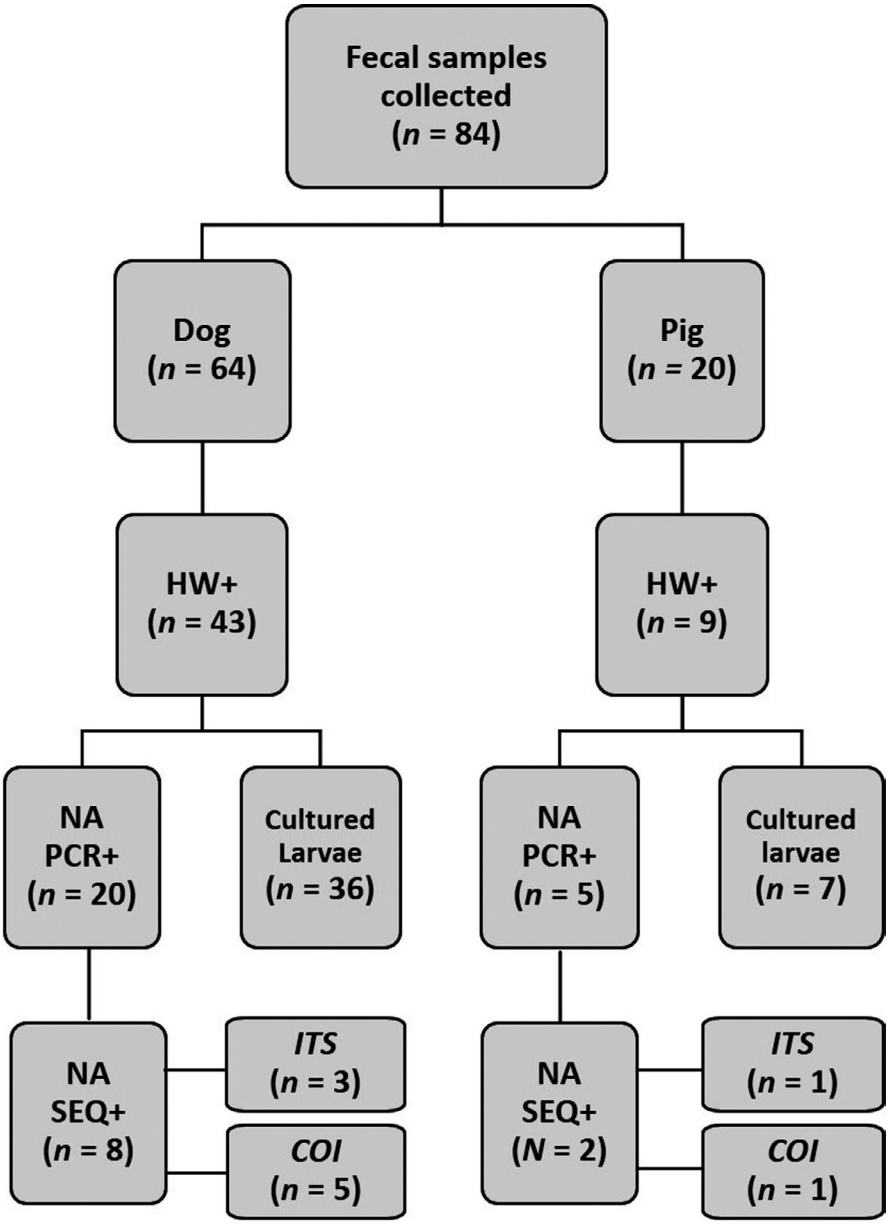

FIGURE 3 Flow chart describing collection and analysis of faecal samples from dogs and pigs

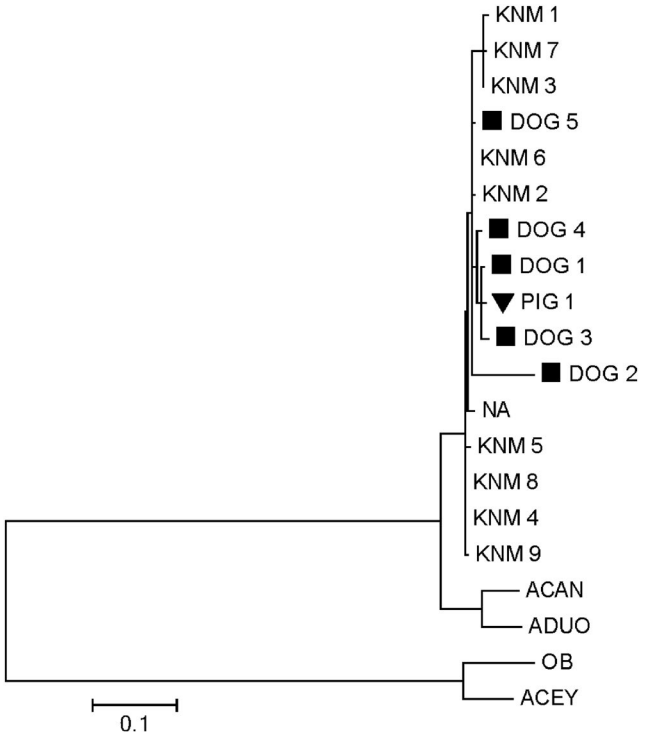

FIGURE 4 Sequences obtained for cytochrome *c* oxidase I amplicons were used to evaluate the maximum-likelihood phylogenetic tree. The tree with the highest log-likelihood (−1754.3057) is shown. Branch lengths in the tree are scaled to reflect the number of substitutions per site. The analysis involved 20 nucleotide sequences including samples from five dogs (■) and one pig (▾). All positions with gaps and missing data were eliminated, resulting in 257 positions in the final sequence alignment. Evolutionary analyses were conducted using the MEGA7 software. ACAN, *A. caninum* sequence from NCBI database; ACE, *Ancylostoma ceylanicum* sequence from the NCBI database; ADUO, *Ancylostoma duodenale* sequence from NCBI database; DOG, faecal sample collected from a domesticated dog; KNM, Kintampo North Municipality human samples; NA, *Necator americanus* sequence from NCBI database; OB, *Oesophagostomumbifurcum* sequence from NCBI database; PIG, faecal sample from a domesticated pig

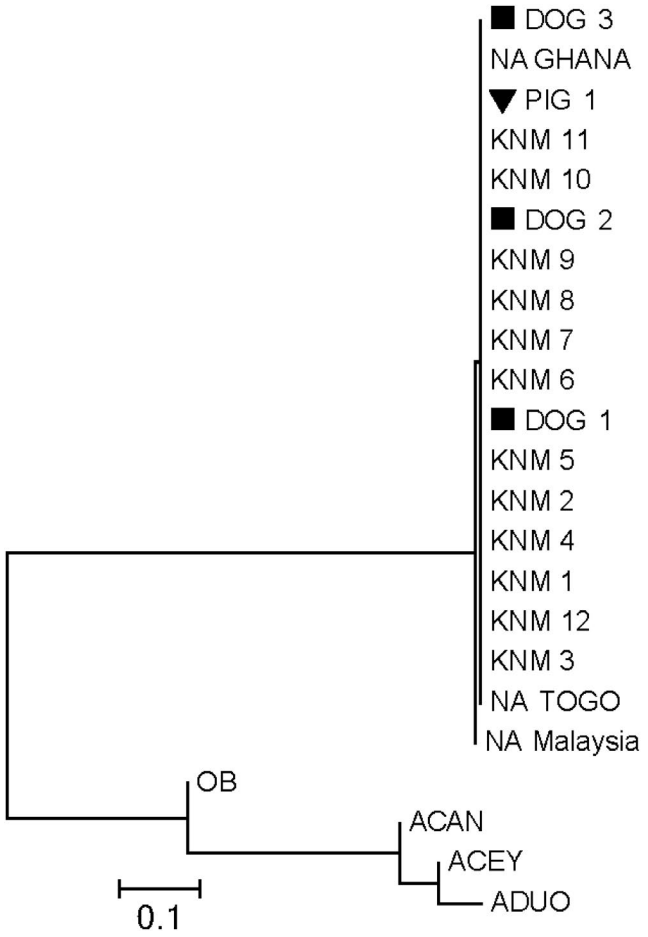

FIGURE 5 Sequences obtained for *ITS2* amplicons were used to evaluate the maximum-likelihood phylogenetic tree, based on the Tamura-Nei model. The tree with the highest log likelihood (−788.3545) is shown. Branch lengths in the tree are scaled to reflect the number of substitutions per site. The analysis involved 23 nucleotide sequences including samples from three dogs (■) and one pig (▾). All positions with gaps and missing data were eliminated, resulting in 210 positions in the final sequence alignment. Evolutionary analyses were conducted using MEGA7 software. Abbreviations as in Figure 4 legend, in addition to NA Ghana, *Necator americanus* sequence from NCBI database; NA Malaysia, *N. americanus* sequence from NCBI database; NA Togo, *N. americanus* sequence from NCBI database

closely related to *N. americanus* with greater than 97% sequence identity seen in all but 1 sample, which showed 90% sequence identity to *N. americanus*. By contrast, DNA sequences from nematode species known to infect dogs and pigs were not as closely related. These combined results confirm that domesticated dogs and pigs living in Kintampo are most likely excreting the human hookworm *N. americanus* in a natural, non-experimental setting.

4 | DISCUSSION

We provide here molecular evidence that supports a biological mechanism through which domesticated dogs and pigs contribute to the transmission of human hookworm in Ghana. First, we retrospectively analysed 2010 study date for a potential epidemiological link between dog/pig ownership and hookworm infection status. Regression modelling showed that pig and dog ownership was positively associated with hookworm infection status among individuals surveyed in the Kintampo field study. Moreover, pig and dog ownership was also associated with a greater intensity of human hookworm infection, suggesting that interaction with domesticated animals leads to higher cumulative worm burdens at the individual level and community level. This result has particular public health significance in light of the association between intensity of infection and morbidity (anaemia, growth delay) associated with hookworm (Pullan et al., 2013).

Using samples collected during the 2010 study, retrospective analysis revealed a high seroprevalence (62%; 55/89) of anti-*Toxocara* antibodies in Kintampo North, thus establishing high rates of exposure to the zoonotic nematode that causes visceral and ocular

larva migrans in people. Humans acquire this zoonotic infection through direct contact with eggs excreted by infected dogs, and therefore, seroreactivity to *T. canis* could be considered a surrogate marker of individual exposure to canine faeces. The observation that antibodies to *T. canis* correlate with *N. americanus* infection status is novel, and experimental data presented here support that this is not due to lack of assay specificity or antigen cross-reactivity (Figure 1).

We next undertook a prospective field study to assess whether dogs and pigs were harbouring human hookworms in a natural, non-experimental setting. The molecular data reported here confirm the association of hookworm infection and exposure to domesticated animals by providing evidence that dogs and pigs commonly excrete the human hookworm *N. Americanus* into the environment. The PCR data also provide a potential mechanism to explain our observation that pig owners and children living in communities with high dog ownership rates have nearly double the risk of hookworm infection.

Previous associations between pig ownership and hookworm infection status have been reported in Latin America, Africa and Asia, supported by experimental data from studies in which *Necator* or *Ancylostoma* eggs were fed to pigs (Ackert & Payne, 1922a; Buckley, 1935; Jones, 1976; Steenhard et al., 2000). In 1922, Ackert reported that pigs in Trinidad excreted intact eggs within days of being fed faeces from a human infected with *N. americanus*. In Papua New Guinea, Jones showed that *Ancylostoma braziliense* hookworm eggs fed to pigs survived transit through the gut, although the yield was significantly less than *Ascaris* eggs. More recently, Steenhard et al. confirmed that pigs in Northern Ghana were capable of excreting viable hookworm eggs after being fed human faeces, with the yield of infectious larvae representing approximately 5% of the estimated inoculum. Interestingly, to our knowledge this is the first report of *N. americanus* identified in dog faeces, thus implicating a second commonly domesticated animal in the transmission of this hookworm species. As with the pig, however, it has previously been demonstrated under experimental conditions that human hookworm eggs fed to dogs are excreted intact and develop into third-stage larvae (Chandler, 1924).

The importance of animals as potential vectors of human hookworm infection has been appreciated for nearly a century (Ackert & Payne, 1923; Chandler, 1924; Goodey, 1923). Summarizing studies done by various investigators, in 1924 renowned helminthologist Asa Chandler acknowledged the significance of domesticated and wild animals in hookworm transmission as follows:

> In view of these facts, the devouring of faeces by pigs, rats, dogs or other animals, with the possible exception of chickens, cannot be looked upon as desirable on account of the destruction of so much refuse, but rather as a very dangerous process resulting in the dissemination and not the destruction of the most injurious component of the material, namely the eggs of human intestinal parasites.
>
> (Chandler, 1924)

Our findings support Chandler's proposed mechanism whereby dogs and pigs consume human faeces in places where people defecate, and then excrete viable parasite eggs in communal living areas. By transporting eggs from human faeces back to areas in proximity to where people live, domesticated animals effectively increase risk of exposure to infectious hookworm larvae among household members. This model fits with the consistently observed epidemiological link between animal ownership and hookworm prevalence and explains how a cycle of transmission could be maintained even when human defecation is primarily limited to remote locations.

Alternative theories for how *N. americanus* eggs are excreted by domesticated animals in Kintampo warrant consideration, but are unlikely. One possibility is that *N. americanus* has become adapted to dogs and pigs. This would require that dogs and pigs harbour adult *N. americanus* worms that produce viable offspring, as opposed to the animals serving as a transport host following ingestion of human faeces. This model would challenge the view that *N. americanus* is almost exclusively a parasite of humans and (rarely) non-human primates. This theory is less likely, since even in optimal experimental settings, patent infection with *N. americanus* is difficult to maintain in laboratory animals (Behnke, Paul, & Rajasekariah, 1986; Sen, 1972; Yoshida, Okamoto, Higo, & Imai, 1960).

It is also possible that the *Necator* identified in dogs and pigs from Kintampo represents a unique hookworm species, one that is distinct from the human parasite *N. americanus*. The concept of a porcine species (*Necator suillus*) was originally put forth by Ackert and Payne (1922a) and later suggested by Buckley (Buckley, 1935), based on morphologic studies in Trinidad. However, this observation has not been reported widely since and would also not explain the excretion of *N. americanus* by dogs. Rather, the DNA analyses reported here using the variable ITS2 and COX1 gene sequences (Figures 4 and 5) reveal a high degree of sequence identity with isolates collected from human study subjects in Kintampo, suggesting that the species in these animals is likely the same parasite, *N. americanus*. While the similarities in gDNA sequence do not preclude the possibility of there being distinct species of *Necator* that have adapted to dogs and pigs, the most plausible scenario remains that animals serve as transport hosts (Steenhard et al., 2000).

This study has certain limitations. First, PCR amplification with primers designed from ITS and COI sequences was not optimized to detect *A. caninum* (accession #JQ812694 and #U57030.1), and therefore, the presence of the common dog hookworm in faecal samples was not assessed. Although this prevented direct comparison of the ratio of canine to human hookworms within samples collected from dogs, it does not negatively impact the primary observation that dogs in Kintampo excrete *N. americanus*. Second, efforts to amplify species-specific hookworm DNA sequences (ITS2, COI) from cultured 3rd-stage larvae (L3) were unsuccessful, despite testing under a variety of PCR conditions. As a result, we were unable to definitively confirm that the *N. americanus* eggs detected in dog and pig faeces had the full capacity to develop into viable and presumably infectious L3. Unfortunately, the number of larvae (L3) cultured from individual animal faecal samples was typically quite

low, thereby reducing the amount of gDNA template available for extraction and amplification.

In summary, we assert that individuals who own dogs and pigs are more likely to be infected with hookworm, in which case they might contribute disproportionately to disease transmission to other community members. These findings from Kintampo, Ghana, are consistent with the rationale behind efforts to adopt a 'One Health' paradigm towards addressing the problem of parasitic diseases like hookworm, in which human and animal factors are integrated into a single, holistic model that incorporates all relevant social, behavioural, ecological and biological variables (Babo Martins, Rushton, & Stärk, 2016; Blake & Betson, 2017; Coker et al., 2011; Degeling et al., 2015; Gebreyes et al., 2014; Penakalapati et al., 2017). Such an approach would include community-based strategies that take into account transmission by both people and animals.

The augmentation of existing disease-control strategies to include enhanced deworming drug administration to adults and children from animal-owning households, or to communities with high animal ownership, could accelerate reduction in hookworm prevalence in endemic areas. Screening for animal ownership at the community level could also identify populations at higher risk for hookworm morbidity that is more likely to benefit from targeted preventive chemotherapy. Clearly, these data support the community health benefit of prompt removal of animal faeces from residential and other frequently trafficked areas; further research should investigate the health impact of interventions aimed at reducing the risk of disease exposure through education and modification of animal husbandry practices.

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CONFLICT OF INTEREST

M. Cappello provided consulting services in 2019 to Janssen Pharmaceuticals, makers of the deworming drug mebendazole. The company had no role in the funding of this study, the data analyses, or drafting of the manuscript and its conclusions. The other authors have no conflicts of interest to declare.

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