Chickens and pigs as transport hosts for *Ascaris*, *Trichuris* and *Oesophagostomum* eggs

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SUMMARY

Ten chickens and 2 pigs were fed non-embryonated eggs of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum*. Each chicken was fed approximately 15000 eggs of each parasite species while approximately 300000 eggs were given to each of the pigs. After passage in chickens 8·3% of *O. dentatum* eggs were recovered in faeces compared to 61·1% and 41·6% of *A. suum* and *T. suis* eggs, respectively. After passage in pigs the percentages were 38·4%, 49·1% and 30·3%, respectively. After embryonation in the laboratory, 1000 eggs of each parasite species having passed through chickens or pigs or having been kept in the laboratory as controls were fed to groups of 6 pigs to check the infectivity. The number of *A. suum* recovered from pigs was similar in the 3 groups with 34·0, 52·8 and 41·8%, respectively. The recovery of *T. suis* in the pig passage group was 54·0% which was significantly lower than the recovery in the chicken passage group (81·8%) and the laboratory group (88·0%). The number of *O. dentatum* recovered was not significantly different among the 3 experimental groups, the percentage recovery being 30·5, 9·2 and 28·5%, respectively. One explanation for the lower infectivity of *T. suis* in the pig passage group may be that the eggs have been sublethally damaged through their passage. The results demonstrate that chickens and pigs can act as transport hosts for *A. suum*, *T. suis* and *O. dentatum*, and it is highly probable that these domestic animals are able to act also as transport hosts for the human parasite equivalents. This will have important consequences for the environmental and behavioural strategies in human helminth control programmes.

Key words: chickens, pigs, transport hosts, *Ascaris suum*, *Trichuris suis*, *Oesophagostomum dentatum*.

INTRODUCTION

Infections with *Ascaris lumbricoides* and *Trichuris trichiura* are the most prevalent human intestinal helminth infections in the world, with high prevalences and intensities among children below the age of 10–12 years (Crompton, 1989; Bundy & Cooper, 1989). Transmission occurs by ingestion of infective eggs in soil, food, water or other objects contaminated with excreta. Especially for pre-school children most infections are believed to take place in, or close to, the house compound in which children live and play.

Hookworms are believed to infect approximately 1 billion people worldwide (Stoltzfus & Dreyfuss, 1998). Most hookworm infections are acquired by L_3 larvae penetrating the host skin on contact with faeces contaminated soil or vegetation. All *Necator americanus* infections occur in this way while infections with *Ancylostoma duodenale* may also be acquired by ingestion of the infective larvae in soil, water or on raw vegetables. Although *Oesophagostomum* spp. normally infects ruminants, pigs and monkeys, *Oesophagostomum bifurcum* is a frequent human parasite in some parts of West Africa (Polderman & Blotkamp, 1995). Eggs of *O. bifurcum* are indistinguishable from those of hookworms, the L_1 larvae develop into an infective L_3 larvae in the soil and the adult parasite lives in the intestinal lumen of the host. Although not fully elucidated in humans, the infection is believed to occur by ingestion. Thus, *Oesophagostomum* spp. have a very similar life-cycle to that of *A. duodenale*.

In theory, infections with *A. lumbricoides*, *T. trichiura* and hookworm can be controlled if a hygienic latrine is constructed and the necessary health education is provided for all members of the family to ensure that the latrine is used. However, control efforts will be jeopardized if parasite eggs are spread to the surroundings by domestic animals such as chickens and pigs.

Chickens are prevalent in Tanzania where most families have 10 chickens or more (Minga *et al.* 1989). They move freely around in almost every compound constantly searching for food in the yard, refuse dump and among human faeces. Thus, it is unavoidable that chickens accidentally take up parasite eggs, which have been deposited in human

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faeces. Embryonated eggs of *A. suum* are known to hatch inside chickens, migrate in the form of larvae migrans and accumulate in livers and lungs (Permin *et al*. 2000). Non-embryonated *Ascaris* and *Trichuris* eggs (probably of human origin) have been found in chicken droppings and experimentally it has been demonstrated that although a majority of *A. lumbricoides* eggs were destroyed in the chicken gut those which passed through were able to develop to the embryonated stage (Otto, Cort & Keller, 1931). Non-embryonated hookworm eggs (possibly *N. americanus*) passed through young and adult chicken guts and were able to hatch and develop to the L_3 stage (Ackert, 1922).

Moreover, pigs scavenge around in the compound and non-embryonated eggs of *A. suum* and *T. suis* are regularly found to pass through the intestine of pigs (Boes, Nansen & Stephenson, 1997; Boes *et al*. 1998). Likewise, non-embryonated hookworm eggs were able to pass through young pigs and subsequently to hatch and reach the infective stage (Ackert & Payne, 1922; Chandler, 1924).

Although eggs of *Ascaris*, *Trichuris* and hookworm seem to develop normally after passage, we do not know whether they retain their infectivity. If chickens and/or pigs are able to act as transport hosts for *A. suum*, *T. suis* and *O. dentatum* it is also very likely that they are able to act as transport hosts for *A. lumbricoides*, *T. trichiura*, *O. bifurcum* and the human hookworms. This will have important consequences for the environmental and behavioural control strategies of these parasites in humans.

The objective of this study was to investigate, whether non-embryonated eggs of *A. suum*, *T. suis* and *O. dentatum* are capable of passing through the intestine of chickens and pigs, respectively, and if so, whether they then develop normally and retain infectivity for pigs.

MATERIALS AND METHODS

Infection material

Pigs were infected orally with embryonated *A. suum* (CEP-strain, Roepstorff & Murrell, 1997), *T. suis* (CEP-strain, Roepstorff & Murrell, 1997) or *O. dentatum* (EH-strain, Roepstorff, Bjørn & Nansen, 1987) eggs and at patency faeces were collected and the non-embryonated eggs recovered by washing the faeces through a series of sieves, collecting the eggs on a 20 μm sieve (Roepstorff & Nansen, 1998). The eggs from the faeces were then divided into 3 parts. One part was kept in the laboratory (laboratory control), one was fed to chickens (chicken passage) and one to pigs (pig passage).

Passage of eggs in chickens and pigs

Ten 20-week-old chickens were fed approximately 15000 non-embryonated eggs of *A. suum*, *T. suis* and *O. dentatum* by means of a pipette placed behind the tongue. The chickens were kept in individual cages and faeces were collected every 12 h for 48 h. Faeces from each 12-h period were mixed and weighed and parasite eggs present were estimated using the McMaster concentration technique described by Permin & Hansen (1998). Similarly, two 7-week-old pigs were fed approximately 300000 non-embryonated eggs of *A. suum*, *T. suis* and *O. dentatum* by stomach tube. Faeces were collected every 12 h during the first 48 h and treated as above. The egg doses for the passage were selected in order to be sure that enough eggs could be recovered for the infectivity study. Because of the bigger size and the higher stool volume of the pigs, these were fed more eggs than the chickens.

Embryonation

The *A. suum* and *T. suis* eggs which had passed through the chickens and pigs were collected, concentrated by sieving (see above) and embryonated in vermiculite (Bie and Berntsen A/S, Denmark) at 25 °C for 12–16 weeks. Vermiculite was added to optimize conditions for embryonation of the eggs (Burden & Hammet, 1976). The *O. dentatum* eggs were cultured in a mixture of vermiculite and helminth-free pig faeces for 3 weeks (Roepstorff & Nansen, 1998). The pig faeces were added in order to avoid starvation of the *O. dentatum* larvae. The larvae were recovered using Baermann's method, transferred to water and kept at 10 °C until the infectivity test in pigs.

Infectivity test in pigs

Sixty-three parasite-naïve pigs (7 weeks old) were infected as shown in Fig. 1. For each parasite, 6 pigs were inoculated with 1000 eggs/larvae which have passed through chickens, 6 pigs with 1000 eggs/larvae which have passed through pigs and 6 pigs with 1000 eggs/larvae from the laboratory control. For each parasite, 3 pigs were kept together with the experimental pigs to control for possible pen contamination. Before installation of the pigs, the pens were thoroughly cleaned and the surface heated with a burner to obtain parasite-free pens. The pigs infected with *A. suum* were slaughtered on day 12 post-inoculation (p.i.) when the large majority of larvae have finished migration and accumulated in the small intestine and before the initiation of expulsion (Roepstorff *et al*. 1997). The *A. suum* larvae were isolated from the small intestinal contents using the agar technique (Slotved *et al*. 1997). Pigs inoculated with *O. dentatum* were slaughtered on day 28 p.i. and the worms were isolated from 10 % of the large intestinal contents by the agar technique (Slotved *et al*. 1996). *T. suis*-inoculated pigs were killed on day 42 p.i. and *T. suis* were recovered

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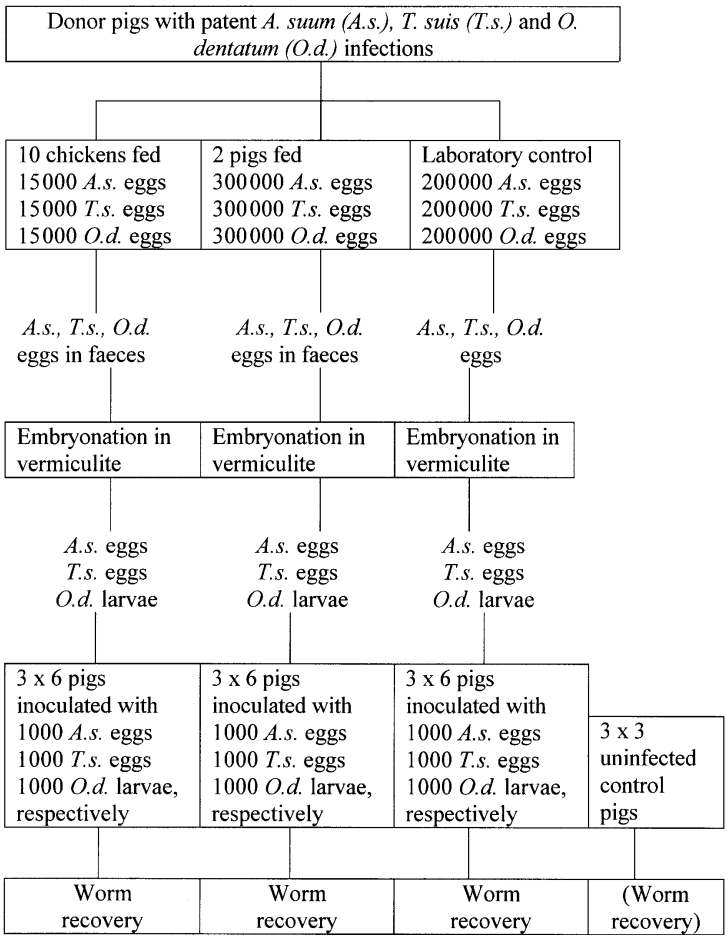

Fig. 1. Experimental design.

by microscopical examination of 20 % subsamples of the large intestinal contents after washing on a 250 μm sieve (Roepstorff & Nansen, 1998).

Statistics

Statistical analysis was carried out using SPSS™ software. Differences in eggs recovered and in worm burdens among experimental groups were assessed using the non-parametric Kruskal-Wallis test as none of these variables were normally distributed.

RESULTS

Recovery of eggs after passage in chickens and pigs

The percentage recovery of non-embryonated eggs of *O. dentatum* after passage through chickens was significantly lower (8·3 %) than the percentage recovery of *A. suum* and *T. suis* eggs (61·1 % and 41·6 %, respectively) (Table 1). All chickens excreted *A. suum* eggs during the first 12 h while only 2 continued to do so during the following 12 h (Table 2). Nine out of 10 chickens passed *T. suis* eggs during the first 12 h (1 did not pass *T. suis* eggs at all) while only 1 chicken discharged eggs during the 12–24 h period. Only 3 out of 10 chickens excreted *O. dentatum* eggs and they only did so during the first 12 h. The percentage recovery after passage in 2 pigs was rather similar for the 3 species of eggs, namely 49·1, 30·3 and 38·4 %, respectively (Table 1). All 3 types of eggs passed through both pigs, however, while *A. suum* and *T. suis* eggs were excreted both after 36 and 48 h, the excretion of *O. dentatum* eggs stopped completely after 36 h (Table 2).

Infectivity of eggs after passage in chickens and pigs

Table 3 summarizes the number of *A. suum*, *T. suis* and *O. dentatum* recovered from the recipient pigs. The number of *A. suum* recovered on day 12 p.i. was similar in the 3 experimental groups (chicken passage, pig passage and laboratory control groups, $P = 0{\cdot}16$), the percentage recovery being 34·0, 52·8 and 41·8 %, respectively. The percentage recovery of *T. suis* in the pig passage group was 54·0 %, which was significantly lower ($P = 0{\cdot}03$) compared to the recovery in the chicken passage group and laboratory group, respectively (81·8 and 88·0 %). The number of *O. dentatum* recovered on day 28 p.i. was lowest in the pig passage group, the percentage recovery being 30·5, 9·2 and 28·5 %, respectively. However, this difference was not statistically significant ($P = 0{\cdot}42$). The pen control pigs had no infection of either *T. suis* or *O. dentatum*, while the 3 pen control pigs had a few *A. suum* worms each (1, 1, 11).

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Table 1. Percentage recovery of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* eggs after passage through the intestine of chickens and pigs, respectively

(Chickens were given approximately 15000 eggs of each parasite species and approximately 300000 eggs were fed to pigs.)

Exp. group	No. of animals	*A. suum* recovered in % mean (S.D.)	*T. suis* recovered in % mean (S.D.)	*O. dentatum* recovered in % mean (S.D.)	*H* values*	*P* values*
I Chicken passage (~ 15000 eggs)	10	61·1 (37·8)	41·6 (29·9)	8·3 (14·6)	12·79	0·002
II Pig passage (~ 300000 eggs)	2	49·1 (16·1)	30·3 (7·0)	38·4 (12·4)	Not done	Not done

* Kruskal-Wallis test of difference in percentage recovery among the different parasite groups.

Table 2. Relative percentage recovery at 12, 24, 36 and 48 h of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* eggs after passage through the intestine of chickens and pigs, respectively

Exp. group	No. of animals	Recovery time (h)	Eggs recovered in % of total number of eggs found after passage (no. of animals excreting eggs)		
			A. suum	*T. suis*	*O. dentatum*
I Chicken passage	10	12	91·9 (10)	91·0 (9)	100 (3)
		24	8·1 (2)	9·0 (1)	0
		36	0	0	0
		48	0	0	0
II Pig passage	2	12	20·8 (2)	52·4 (2)	50·4 (2)
		24	34·7 (2)	25·8 (2)	25·9 (2)
		36	42·1 (2)	17·7 (1)	23·7 (2)
		48	2·4 (1)	4·1 (2)	0

Table 3. Percentage recovery of *Ascaris suum*, *Trichuris suis* and *Oesophagostomum dentatum* in recipient pigs inoculated with 1000 infective eggs/larvae of each parasite species which have passed through chickens, pigs or been kept in the laboratory before embryonation

Exp. group	No. of pigs	Mean ± S.D.		
		Percentage recovery of *A. suum* at day 12 p.i.	Percentage recovery of *T. suis* at day 42 p.i.	Percentage recovery of *O. dentatum* at day 28 p.i.
I Chicken passage (1000 eggs/larvae)	6	34·0 ± 20·2	81·8 ± 27·3	30·5 ± 36·3
II Pig passage (1000 eggs/larvae)	6	52·8 ± 14·5	54·0 ± 10·1	9·2 ± 10·3
III Laboratory control (1000 eggs/larvae)	6	41·8 ± 18·6	88·0 ± 20·8	28·5 ± 30·6
H values*		3·67	7·09	1·74
P values*		0·16	0·03	0·42

* Kruskal-Wallis test of difference in percentage recovery among the different passage group.

DISCUSSION

The present study showed that non-embryonated eggs of *A. suum*, *T. suis* and *O. dentatum* are able to embryonate, to develop normally and to become infective to pigs after they have passed through the digestive tract of chickens as well as of pigs. This means that chickens and pigs can act as transport hosts for the pig intestinal helminths and, therefore, probably also for the human equivalents.

Approximately half of the *A. suum* and *T. suis* eggs were lost in the chicken gut while more than 90 % of the *O. dentatum* eggs were lost. Otto and co-workers (1931) found recovery rates of 16·5, 33·5 and 40·5 %

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of *A. lumbricoides* eggs in 3 experimental chickens and the high loss was explained by the grinding action of the gizzard. Similarly, less than 10 % of the hookworm eggs ingested by chickens produced infective larvae in a study in Trinidad (Ackert, 1922). The author explains this failure by mechanical damage of eggs in the gizzard, injury from the high level of ammonium in chicken faeces and malnutrition of the larvae in the faeces. Our results correspondingly showed that the thin-shelled *O. dentatum* eggs are more vulnerable to the passage through the chicken and/or to the stay in the chicken faeces. Starvation of the *O. dentatum* larvae was avoided by adding pig faeces from helminth-negative donors to the vermiculite during embryonation.

Slightly fewer than 50 % of eggs of all 3 helminths were recovered after passage through the digestive tract of pigs in the present study. This is in contrast to earlier studies which found that all *Ascaris* and *Trichuris* eggs (Ramsey, 1924 cited by Otto *et al.* 1931), > 99 % of *N. americanus* eggs (Jones, 1976) and 95 % of the *O. bifurcum* and *N. americanus* eggs were destroyed through passage in pigs (Steenhard *et al.* 2000). At the other extreme, a very high proportion of hookworm eggs swallowed by pigs were recovered in pig faeces (Ackert & Payne, 1922; Ramsey, 1924 cited by Otto *et al.* 1931). Also, more recent studies have shown that significant numbers of *A. suum* (Jones, 1976) and *A. suum* and *T. suis* eggs (Boes *et al.* 1997; Boes *et al.* 1998) might pass through the intestine without being destroyed.

Although passage through chickens reduced the number of parasite eggs, the eggs which have passed successfully, developed to normal infectivity. Thus, those eggs, which have not been destroyed in the gizzard or during their stay in the faeces, are unharmed by the rather short passage through the digestive tract. In contrast, the passage through the pig had a deleterious effect on *T. suis* infectivity, but not on *A. suum* and *O. dentatum* infectivity. One explanation could be that some of the *T. suis* eggs were sublethally damaged during passage through pigs, e.g. by the low pH in the stomach or by digestive enzymes perhaps in combination with the prolonged stay (1–2 days) at body temperature. Passage through chicken and pigs in our experiment took place during early embryonation, and in a laboratory study Burden & Hammet (1976) showed that even though *T. suis* eggs were able to embryonate under different physical/chemical conditions, these conditions severely influenced the later infectivity to pigs. However, it is difficult to explain why the same phenomenon does not exist for eggs of *A. suum* and *O. dentatum*. Even if the hypothesis of sublethal damage is true, it is not known whether passage through pigs will influence the infectivity of *T. trichiura* to the human host.

We conclude that chickens and pigs can act as transport hosts for *A. suum*, *T. suis* and *O. dentatum* and it is highly probable that these domestic animals are able to act also as transport hosts for the human parasite equivalents. Although the number of eggs is reduced through the passage, it is possible that domestic animals may transport eggs from places away from human contact to places where children and adults, unaware of the possible risks, have contact with contaminated soil. This is particularly true with respect to chickens because these domestic animals are allowed to move around inside the houses. The results of the present study have therefore important consequences for the environmental and behavioural strategies in human helminth control programmes.

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