Animal models of intestinal nematode infections of humans

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SUMMARY

In this paper we discuss several established and potential animal models for human parasitic infection, with a focus on rodent, pig and primate models and the nematodes *Ascaris*, *Trichuris* and *Toxocara* spp. Firstly, we discuss the relevance of choosing a suitable animal host to fit the particular study hypothesis, and the interaction between mathematical modelling and animal models. Secondly, we review the use of animal models for the study of nutrition-parasite interaction, evaluation of treatment and control strategies, and bacteria-parasite interactions. We show that rodent, pig and primate models are all very useful in parasitological research, and that each model has its limitations. However, based on recent experience with the pig-*Ascaris* and pig-*Trichuris* models, a more extensive use of the pig-parasite model is advocated, especially for the study of the interaction between human malnutrition and helminth infection, and congenital helminth infection.

Key words: Animal models, rodents, pigs, primates, helminths, Ascaris, Trichuris, Toxocara, malnutrition, control strategies, congenital infection.

INTRODUCTION

Knowledge concerning parasites infecting humans in endemic areas can be gained both through study of the parasite in the human population (field studies, case reports) and by studying the parasite (or its close relatives) in an animal model. Data generated by both approaches can subsequently be applied in mathematical modelling. In many well-established animal models, field observations and mathematical theory have driven laboratory experimentation, and in turn laboratory observations have stimulated field studies (Scott & Tanguay, 1994). Studies in animal models have obvious advantages over human field studies: animal caretaking is relatively easy and less expensive per case for equivalent data (except in primate studies), and experimentation on animals is easier to control and less problematic ethically, especially when conducted in animals bred to be consumed by humans.

Wessler (1976) suggested that the definition of an animal model for the study of human diseases is: 'A living organism with an inherited, naturally acquired or induced pathological process that in one or more respects resembles the same phenomenon in man'. This describes adequately animal models of human parasitic infection. The choice of animal model will depend on the type of human infection to be studied, and on the hypotheses to be tested in the model. In parasitic infections, the choice also depends on whether appropriate animal host–parasite combinations already exist or can be established experimentally; an appropriate model should mimic (a) the human host, (b) the parasite, and (c) the human host–parasite system or the way in which the host and parasite interact.

Choice of the appropriate host to model a particular parasitic infection of humans is best made by examining the immunological, physiological, anatomical and metabolic similarities that each have, since all of these may influence experimental results and are unlikely to be favoured equally in a specific animal model. Finding parasites of animals equivalent to the four major intestinal nematodes of humans is not difficult: helminth genera like *Trichuris* (Beer, 1976), *Ascaris* (Anderson, 1995), *Strongyloides* (Georgi, 1982) and *Ancylostoma* (Schad, 1979) occur as closely related species in animals and man. Other genera like *Toxocara* and *Trichinella* are zoonotic parasites, although this does not exclude the possibility of marked differences in infection biology in the human and animal hosts. But perhaps the most important issue is that animal models should be chosen from naturally occurring host–parasite systems whenever possible. *Trichuris muris* in the mouse, *Ascaris suum* in the pig and *Ancylostoma caninum* in the dog are well described infections that include a naturally occurring parasite species closely related to human parasite species, and they therefore serve as suitable models for

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the corresponding human infection. Other natural models like *T. suis* and *S. ransomi* in young pigs have been suggested previously (Stephenson, 1987*b*; Holland, 1987).

Only when the life cycle and dynamics of parasite infection in the animal host are well described and well understood can valid conclusions be drawn from the model. In addition, the model may actually provide new knowledge about the natural animal host–parasite system used to model human infection. An interesting example is the recent discovery of a new predilection site of *A. suum* larvae in the pig. While most textbooks until then had stated that after hatching the larvae penetrate the wall of the small intestine *en route* to the liver, Murrell *et al.* (1997) discovered that *A. suum* instead penetrates the wall of the caecum and colon. Based on these results, the authors proposed the possibility of similar lifecycles and liver pathology for *A. suum* and *A. lumbricoides*, at the same time pointing out a dearth of knowledge about the migration of *A. lumbricoides* in the human host.

The choice of animal model depends in part on the questions it should try to answer, and a distinction between simple and more complex models can be made. Basic parasitological research often will focus on certain mechanisms or factors determining parasite infection or host response (e.g. infectivity, antibody response, cytokines, genetic differences) and will attempt to identify/quantify the effect of a single factor on these. More complex models try to mimic the natural situation of parasite infection more closely and in more detail (e.g. distribution, transmission dynamics) and will inevitably need to concentrate on the interaction between different infection parameters. In the case of human parasite infection, a simple model could in many cases use rodents or other hosts, while the complex model will require the use of more closely related animals like pigs or primates, which allow study of all aspects of the population dynamics of a particular infection (see below).

We describe and discuss here several examples of successful animal models for human parasite infections. We limit ourselves to animal models of the most important nematodes infecting humans and, drawing upon many years of research experience at the Danish Centre for Experimental Parasitology, we will focus primarily on *Ascaris*, *Trichuris* and *Toxocara*. Using these and other nematode species as examples, we first discuss two more general issues: (1) the choice of appropriate animal hosts for models of human parasite infection, and (2) the interaction between animal models and mathematical modelling. Subsequently, we discuss 3 specific areas of interest in modelling parasitic diseases of humans: (a) the impact of host nutrition and diet, (b) the development of treatment and control strategies, and (c) interaction of parasites with other pathogens. Finally, we outline some perspectives for the use of animal models in medical parasitology.

HOST ANIMAL

The choice of animal species as a model for human host–parasite relationships depends on the purpose of the study and the parasite to be modelled. Whether similarity in host physiology or the existence of a natural animal host–parasite relationship should be favoured in order to test a particular hypothesis and conduct feasible experiments is a problem that can be solved in many ways, but which experimental host is chosen will importantly influence results obtained.

Here we consider 3 types of animal hosts as models of parasitic diseases of humans: rodents, pigs and primates. While rodents are commonly used to study specific host–parasite interactions such as immune response, parasite fecundity and survival, and genetic effects (Wakelin & Blackwell, 1988; Scott & Tanguay, 1994; Grencis, 1996), pigs and primates are often though not always more appropriate and complete models because of their many similarities with humans (Miller & Ullrey, 1987; Swindle, 1992; Willingham & Hurst, 1996; Urban *et al.* 2000). For the same reason, ruminants and carnivores can be considered less suitable models for human disease, although dogs have been used as models for infection with the hookworm *Ancylostoma* spp. (Schad, 1979; Carroll & Grove, 1984). However, whether rodents, pigs, primates or other animals are the animal host of choice depends on many factors.

Rodent models are by far the most popular and the most frequently used animal models for many aspects of human disease. Mice, rats, guinea pigs and rabbits are relatively easy to keep and handle, they are less expensive, and reproduce rapidly and in large numbers. Perhaps the best known rodent-parasite models are those of *T. muris* (Wakelin & Blackwell, 1988), *Heligmosomoides polygyrus* (Scott & Tanguay, 1994) and *Trichinella spiralis* (Wakelin & Lloyd, 1976) in mice while the *T. canis*-mouse model represents both a true paratenic host in the life-cycle of the mouse as well as a model system (Dubinsky *et al.* 1995). However, rodent models may have their limitations in parasitological research due to host physiology, parasite size constraints and a relatively short host life span. Size limitations are most evident for *Ascaris* and *Toxocara* parasites, which are the largest intestinal nematodes, and normally do not complete their life cycle in rodents. Larval parasite stages, however, are able to migrate and establish in rodents, e.g. *A. suum* larvae in guinea pigs (Roberts, 1934) and mice (Eriksen, 1981; Slotved *et al.* 1998) and *T. canis* in mice (Abo-Shehada, Al-Zubaidy & Herbert, 1985; Abo-Shehada & Herbert, 1985).

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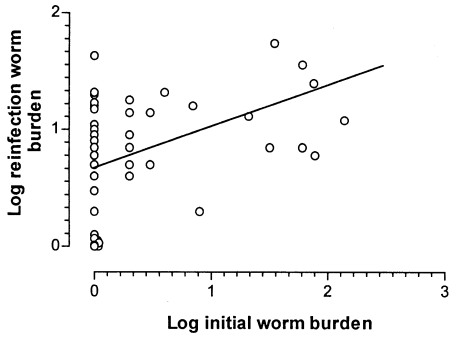

Fig. 1. Evidence for predisposition to *Ascaris suum* infection in naturally infected pigs. The data show that initial worm burdens for individual pigs are highly significantly correlated with worm burdens acquired following anthelmintic treatment and a period of reinfection (Spearman rank correlation $r_s = 0{\cdot}39$, $P < 0{\cdot}01$, $n = 49$). Data reproduced from Boes *et al.* (1998).

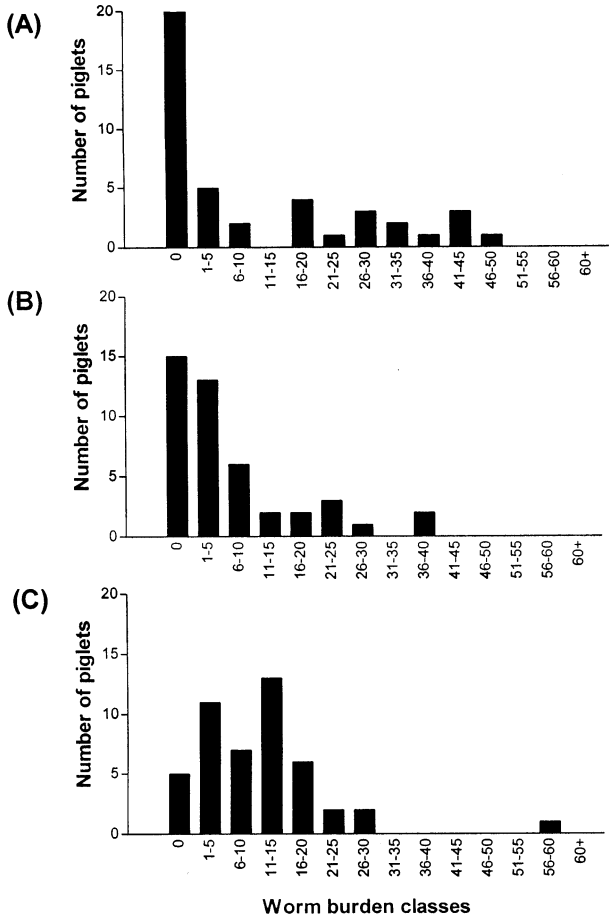

Fig. 2. Worm burden distributions in piglets experimentally infected with *A. suum*. (A) The combined data from piglets from helminth-naive sows serving as controls for short-term or long-term exposed sows ($n = 53$); (B) Piglets from sows that were exposed during gestation only with increasing doses of *A. suum* eggs twice weekly (short-term exposure, $n = 44$). (C) Piglets from sows that were exposed before and during gestation with 10000 *A. suum* eggs twice weekly (long-term exposure, $n = 47$). Data reproduced from Boes *et al.* (1999).

Thus, larval migration in rodents may be used in infectivity studies not requiring patency. Alternatively, migration of larvae may be used as a model for in which to study intestinal immunity in the early phase of *A. suum* infection (Slotved *et al.* 1998).

An important exception is the whipworm *Trichuris* where the human parasite *T. trichiura* can be modelled by naturally occurring *T. muris* infections in mice. In this model it has been possible to study one of the main characteristics of *T. trichiura* infection in humans, namely predisposition to infection (Bundy, 1988). Some individual mice are predisposed to trichuriasis, being unable to express protective immunity (Else, Wakelin & Roach, 1989). Predisposition has also been shown in infections with the mouse hookworm *H. polygyrus* (Scott, 1988), a laboratory model for direct life-cycle nematodes infecting man. Tanguay & Scott (1992) further developed this model to study the importance of host heterogeneity in generating parasite aggregation. Heterogeneity in acquired resistance and, less consistently, host behaviour were suggested to contribute significantly to variability in parasite burden (Tanguay & Scott, 1992). Both mouse models are useful for detailed immunogenetic studies, but for direct comparison with human parasite infection the *T. muris*-mouse model might be preferred to study, for example, intestinal pathology.

Despite the clear advantages of rodent models in terms of costs, ease of handling and reproductive capacity, the results will have to be validated in the model target, i.e. man (which is rarely possible) or in a more closely related animal model. The primate model would of course be ideal as for obvious reasons it is considered closest to man, and has successfully been applied in e.g. human immunodeficiency virus (HIV) research (e.g. Le Grand, Vaslin & Dormont, 1998). However, due to cost, complex logistics, and ethical considerations, primate models have rarely been used in parasitological research.

Experimental *Schistosoma mansoni* and *S. japonicum* and malaria have been studied in small numbers of chimpanzees (Sadun *et al.* 1966; von Lichtenberg *et al.* 1971), and the immune response to *S. mansoni* and *S. haematobium* infections has been studied systematically in baboons (Sturrock *et al.* 1985; James *et al.* 1986), but research on these justifiably high-profile tropical diseases is able to attract funding much more easily than are the intestinal nematodes.

In spite of that fact, Urban *et al.* (2000) were able to inoculate *Erythrocebus patas* monkeys with *A. suum* eggs to observe migration and development of larvae, clinical effects on the host and immunity to infection in a primate model of human zoonotic *Ascaris* infection. Their results showed that *A. suum* was able to migrate via the hepatic-pulmonary-

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Table 1. Number of *Toxocara canis* larvae per g tissue in selected organs/muscles of pigs inoculated orally with 60000 *T. canis* eggs

Organ/muscle	Weight (g)	Day 7 p.i.[d] Larvae/g tissue Mean (range)	Weight (g)	Day 14 p.i. Larvae/g tissue Mean (range)	Weight (g)	Day 28 p.i. Larvae/g tissue Mean (range)
SI[a] (in total)	–[e]	8·33 (0–17)	–	0	–	0·33 (0–2)
LN[b] (SI)	–	86·62 (39·6–126·4)	–	4·57 (0·9–9·6)	–	0·11 (0·0–0·4)
LN (LI[c])	–	10·13 (1·4–20·2)	–	0·83 (0·0–2·1)	–	0·30 (0·0–1·4)
Liver	554	0·80 (0·2–1·8)	656	0·01 (0·0–0·03)	653	0·01 (0·0–0·01)
Lungs	297	3·33 (1·3–5·1)	425	6·58 (0·8–15·9)	296	1·33 (0·6–2·5)
Brain	46	0	75	0·26 (0·0–0·7)	55	0·07 (0·0–0·4)
Kidney	117	0·04 (0·0–0·1)	137	0·09 (0·0–0·4)	138	0·01 (0·0–0·02)
Diaphragm	84	0·15 (0·1–0·3)	79	0·02 (0·0–0·04)	106	0·04 (0·0–0·1)
Masseter	36	0·03 (0·0–0·1)	58	0·03 (0·0–0·08)	60	0·01 (0·0–0·06)
Tongue	81	0·03 (0·0–0·1)	95	0·01 (0·0–0·02)	84	0·01 (0·0–0·03)
Heart	110	0	76	0·01 (0·0–0·3)	111	0
Eyes	10	0	11	0	11	0

[a] small intestine; [b] lymph nodes; [c] large intestine; [d] post infection; [e] not weighed. Numbers from contents of the small intestine are in total. Adapted from Helwigh *et al.* (1999).

intestinal route in *E. patas*, but larvae were delayed in their development and kinetics in monkeys compared to *A. suum*-infected pigs, and only a few developed into adult worms. In addition, *E. patas* showed strong innate and acquired immunity against migrating *A. suum* larvae (Urban *et al.* 2000). Interestingly, the primate model as described by these authors offers both a comparison with human *Ascaris* infection and the pig-*Ascaris* model, and, as suggested by Murrell *et al.* (1997), may be used fruitfully to study the migration of *A. lumbricoides*.

The primate model is often considered unattractive because it is very expensive. However, as Stephenson (1987*b*) points out, the scientific value of one study in an appropriate animal model may be many times greater than dozens of studies in parasite-animal systems that are very unlike the human-parasite system. However, economic and practical considerations (e.g. housing restrictions for primates due to zoonotic risk factors) as well as immunological, physiological, anatomical and metabolic similarities have led Stephenson (1987*b*), Willingham & Hurst (1996) and others to propose the pig as a model for human parasite infections. Pigs are very appropriate models for many human diseases and are used widely in biomedical research (e.g. Book & Bustad, 1974; Swindle, 1992), and in recent years they have been increasingly employed in parasitology. One of the best-studied parasites infecting humans is *A. lumbricoides* which is very closely related to the pig parasite *A. suum* (Anderson, 1995). In a population biology study, Boes *et al.* (1998) demonstrated that the degree of overdispersion of *A. suum* worm burden distributions in continuously exposed pigs is very similar to that of *A. lumbricoides* in humans. Furthermore, initial worm burdens and those on reinfection were significantly correlated (Fig. 1) suggesting that individual pigs are predisposed to heavy or light infection (Boes *et al.* 1998), a feature that is common in human *Ascaris* infections in endemic areas (Keymer & Pagel, 1990; Crompton, 1994).

Another significant result from the pig-*Ascaris* model was that maternal exposure to *A. suum* infection may have no direct influence on worm burdens but instead increased the prevalence of infection, at the same time decreasing the degree of parasite aggregation in infected piglets (Fig. 2; Boes *et al.* 1999). This finding is important for the understanding of *A. lumbricoides* population biology, since most children in endemic environments will be born to exposed mothers. It was concluded that *A. suum* infection in continuously exposed pigs is a good model for *A. lumbricoides* population dynamics in humans (Boes *et al.* 1998).

A model that has not been explored extensively but certainly deserves more attention is that of *T. suis* infection in the pig as a model for *T. trichiura* in humans (Holland, 1987). Both *Trichuris* species are very closely related (Beer, 1976) and occupy similar anatomical sites in the body of their host, namely the mucosa of the large intestine. For example, pigs infected with *T. suis* and *Schistosoma japonicum* and fed low or high protein diets (Johansen *et al.* 1997), and pigs made deficient in iron and vitamin A then infected with *T. suis* (S. K. Pedersen, unpublished results), have been used as models for infection in malnourished human hosts. There is much and varied research to be done using this model (see below), which at the same time may provide important information for veterinary parasitology.

Toxocara canis, the dog ascarid, possesses the capacity to migrate to various parts of the human body, causing visceral or ocular larva migrans syndrome (Glickman, Schantz & Cypess, 1979; Molk, 1983). The seroprevalence of *T. canis* can be

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as high as 18% in the human population (Barringa, 1988) but the number of cases of larva migrans is much lower, suggesting that hitherto unknown aspects of parasite behaviour and host response determine the severity of infection (Helwigh, 1998). This observation merits further study of the parasite in an animal model. For example, Cox & Holland (1998) reported that the number of larvae recovered from the brain of *T. canis*-infected mice was associated with behavioural changes which might favour parasite survival and hence transmission to the final host. Recently attempts have been made to study the migratory capacity and patterns of *T. canis* in the pig model. Helwigh, Lind & Nansen (1999) infected pigs with high doses of eggs and demonstrated that *T. canis* larvae could be recovered from a variety of body parts, including eyeballs and brain tissue (Table 1). In contrast to mice (Cox & Holland, 1998), the number of larvae recovered from brain tissue was very low, suggesting that in pigs, accumulation in the brain does not occur and that this may more realistically reflect the human situation, given the differences in brain size between mice and pigs/humans. In addition, *A. suum* infection superimposed on a primary *T. canis* infection in pigs resulted in more severe liver pathology and serological cross-reaction than occurred with either infection alone (Helwigh, 1998). These results suggest that *T. canis* infections in pigs may serve as a suitable model for the study of human toxocariasis, and of concurrent helminth infection.

STATISTICS AND MATHEMATICAL MODELLING

Parasitological data collected in field studies can be used for mathematical modelling. The objective of mathematical modelling is to build a simplified representation of a complex system that exists in the real world in order to examine possible outcomes of various interventions, for example chemotherapy regimens for parasite control programmes, which are too costly or impractical for various reasons or possibly unethical to test, or to test often, in the real world system (Morris & Marsh, 1994).

May & Anderson (1979) and Anderson & May (1985, 1991) developed mathematical models to investigate the population dynamics of human helminth infection. Using these models they were able to evaluate relationships between host immunity, prevalence and intensity of infection, and the impact of control strategies in the community. The results of these and other models have since been validated by researchers in the field, allowing further modification and improvement. In this instance, mathematical models may indicate how best to allocate available resources and identify achievable objectives in case of helminth control programmes (Crompton, 1994; Chan *et al*. 1994). In addition, mathematical modelling helps identify both the data to be collected and the target population, and thereby stimulates and guides further experimental work.

It is not always feasible or ethically justifiable to test model assumptions in the field, and animal models may offer a good alternative. Once the appropriate animal model has been established it is possible to manipulate parasite, host and possibly their interaction, which will provide the necessary feedback for the mathematical model. In human parasitic infections, the relative contributions of exposure and host susceptibility are difficult to assess (Bundy & Medley, 1992), and the advantage of the animal model is a greater degree of control over the different parameters under study. Susceptibility and predisposition in human helminth infection can be studied quite effectively using the available immunological techniques (e.g. Palmer *et al*. 1995; McSharry *et al*. 1999), whereas the relative contribution of parasite exposure can only be studied experimentally in animal models. In addition, possible genetic factors influencing protection from helminth infection have been identified in humans (Bundy 1988; Holland *et al*. 1992), and since this factor is Human Leucocyte Antigen (HLA) associated, an investigation of a similar role of SLA in porcine helminth infection may be worthwhile.

For example, continuous exposure of pigs to *A. suum* is a good model for *A. lumbricoides* infection in humans living in endemic areas (Boes *et al*. 1998). In this model it will be possible to investigate the impact of different levels of exposure, different treatment regimes, and different host nutritional deficiencies – and their interaction – on *Ascaris* infection and its negative sequelae, as feeding nutrient-deficient diets, withholding chemotherapy, and frequent sampling (blood, faeces and biopsies, as well as post mortem pathology) are conditions to which humans cannot be subjected for obvious reasons. In addition, while mathematical modelling is relatively inexpensive, data collection in the community for validation of the model is often expensive and laborious. These considerations clearly advocate their use in the validation of mathematical models.

The statistical analysis of parasitological data can be difficult due to their overdispersed distributions (Wilson & Grenfell, 1997). Human populations are far from uniform and may be difficult to stratify, which makes the inclusion of large sample sizes necessary. While one requirement of the animal model is that parasite data should behave in a manner similar to that of the human infection, another criterion is that the animal model can also be more homogeneous in order to study single factors of importance. To investigate a single factor or mechanism one has to either minimise the variation between experimental hosts, or increase group sizes when hosts are more heterogeneous, in order to be able to see clearly the physiological relationships

Table 2. Comparison of the degree of overdispersion (k) and Kendall's rank correlation (τ) between infection/reinfection worm burdens in human or pig populations continuously exposed to *Ascaris* infection. Values of $k < 1$ indicate pronounced parasite aggregation. Data are reproduced with permission from Boes (1999)

	Overdispersion (k)	Correlation (τ)
A. lumbricoides		
Thein-Hlaing *et al.* (1984)	0·46	0·16–0·41
Chai *et al.* (1985)	0·36–0·54	NA
Elkins, Haswell-Elkins & Anderson (1986)	0·81	0·16–0·20
Bundy *et al.* (1987)	0·59	0·18
Bundy, Kan & Rose (1988)	0·21	NA
Holland *et al.* (1989)	0·29–0·68*	0·12–0·33
Forrester & Scott (1990)	0·71–1·44*	0·54#
Chan, Kan & Bundy (1992)	NA	0·34–0·69#
Kightlinger, Seed & Kightlinger (1995)	0·93*	0·17–0·26
A. suum		
Eriksen *et al.* (1992)	0·07	NA
Boes *et al.* (1998)		
Trickle infection	0·26	NA
Natural exposure	0·09	NA
Natural reinfection	0·69	0·31
Coates (unpublished)	0·27–0·31	0·29

* Calculated from variance to mean ratios; #Spearman Rank Correlation; NA, not available.

between the various factors under study (Tillett & Carpenter, 1991). However, care should be taken with extrapolation of results from homogeneous models to the human field, where heterogeneity may require a complex model. The question then arises whether a complex model measuring many infection parameters and their interactions should use homogeneous or heterogeneous groups of animal hosts. The use of both homogeneous and heterogeneous groups of animals will improve model comparability, but in many cases there will be economic, logistic and ethical restrictions on the number of animals included.

In the model of *A. suum* in continuously exposed pigs (Boes *et al.* 1998), the experimental animals used were cross-bred Landrace/Yorkshire/Duroc pigs. Even though measurement of overdispersion and predisposition to infection in human populations usually is based on large sample sizes, the experiment here included only 50 heterogeneous pigs. Both worm burden distribution and predisposition in continuously exposed pigs were comparable to those reported for *A. lumbricoides* in human field studies, indicating that this application of the pig-*Ascaris* model, however heterogeneous, was a useful and appropriate model (Boes, 1999). Table 2 compares field observations on parasite aggregation and predisposition to *A. lumbricoides* infection in humans with results of experimental studies on *A. suum* infection in continuously exposed pigs (Eriksen *et al.* 1992; Boes *et al.* 1998). Similar degrees of overdispersion and predisposition were recently found in a comprehensive study using even smaller groups of pigs (S. Coates, unpublished results). However, if the aim is to investigate the host factors responsible for predisposition which are likely to have a genetic component (Keymer & Pagel, 1990), a more homogeneous group of pigs will be required. The use of littermates may not necessarily reduce variation in e.g. worm burdens and prevalence (Boes *et al.* 1999), and triple cross breed pigs probably show too much genetic variability from which to draw any valid conclusions, thus future research should try to include more homogeneous pig breeds. However, inbred animals do not necessarily show less heterogeneity in parasite burden compared with outbred animals, as has been shown in mice (Cox & Holland, 1998) and pigs (L. Eriksen, unpublished observations). Finally, attention should be given to uniformity of experimental conditions, e.g. feed, housing, bedding, etc.

NUTRITION AND DIET

Perhaps the most important factor that influences the nature and magnitude of morbidity in human helminth infections in endemic areas is host nutritional status. The impairments in immunity that occur during nutritional deficiencies will often facilitate establishment and survival of parasites (Stephenson, 1987*a*; Solomons & Scott, 1994). The vast majority of malnutrition-infection interactions

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of public health importance can be classified as synergistic, i.e. nutritional deficiencies aggravate infection and vice versa (Bundy & Golden, 1987), although some interactions, especially some which are experimentally created may be antagonistic, i.e. increasing levels of malnutrition lead to reduced severity of infection (Scrimshaw, Taylor & Gordon, 1968). Low intensities of infection are not necessarily found in field studies to be correlated with nutritional deficiency in low-risk groups: for example, hookworm but not *Ascaris* or *Trichuris* infection showed a statistically significant relationship with low iron status of lightly infected adults in Kenya (Olsen *et al*. 1998). This is due in part to measurement error and our inability to measure accurately various types of morbidity in humans, particularly in large community studies.

As a first step, nutrition-parasite models should include healthy, well-fed animals in order to know and understand how the infection behaves under optimal conditions. However, if an animal model is to have relevance for human parasite infections which occur mainly in the developing parts of the world that are characterized by poverty and nutritional deficiencies (Crompton, 1994), the next step is to incorporate the different forms of malnutrition in to the model. Thus, the model provides an opportunity to compare parasitism in well-nourished and malnourished hosts, conditions that again cannot be induced experimentally in humans. It should be borne in mind, though, that animal welfare issues are becoming increasingly important, and the purpose of malnutrition studies in animals should therefore conform strictly to applicable animal ethics legislation.

Several nutritional deficiencies have been studied in the rodent model (see Solomons & Scott, 1994, for review). For example, zinc, vitamin A and protein deficiency have been shown to impair the immune response in parasitised mice (Fraker, Caruso & Kierszenbaum, 1982; Carman *et al*. 1992; Boulay *et al*. 1998), while moderate protein deficiency enhanced cell-mediated immunity in mice (Petro, 1985). Nutritional aspects of *A. suum* and *A. lumbricoides* infection and the value of the pig model for human ascariasis have been discussed by Nesheim (1985). For example, *A. suum* infections in young, malnourished pigs have been successfully used as a model for *A. lumbricoides* in growing children (Stephenson *et al*. 1980; Forsum, Nesheim & Crompton, 1981).

Relatively few studies have been undertaken using the pig-*Trichuris suis* model. Johansen *et al*. (1997) infected pigs fed low or high protein diets with *T. suis* and *S. japonicum* as a model for human protein deficiency. However, the pigs were infected with a moderate single dose of 4000 *T. suis* eggs and no significant effect on haemoglobin or serum albumin levels was detected in the *Trichuris* infected group. In contrast, preliminary results have shown that iron deficiency in young pigs permitted an increase in *T. suis* worm size and fecundity and was accompanied by hypoalbuminaemia and a decrease in haematocrit levels in the host (S. K. Pedersen, unpublished results). In addition, worm establishment and fecundity as well as serum albumin levels were lowered in vitamin A deficient piglets that were experimentally infected with *T. suis* (S. K. Pedersen, unpublished results). Both studies used low or moderate infection doses and the results indicate (a) that even subclinical *T. suis* infection may result in significant interactions with host nutritional deficiencies, and (b) a potential role for the pig model in studies of nutrition-parasite interactions.

The interaction of diet composition with parasite infection is another important area of research in which animal models are used, as it is conceivable that not only nutritional factors such as vitamins and minerals but also that dietary composition (e.g. sugars, fibre) help determine parasite establishment and survival. For example, the establishment, size and fecundity of the nodular worm *Oesophagostomum* spp. in the large intestine of pigs are promoted by an increase in dietary fibre (Petkevičius *et al*. 1995, 1997). In addition, in pigs fed low fibre diets the worms were located more distally along the large intestine compared with pigs receiving high fibre diets. These authors were not able to demonstrate an effect on *A. suum* infection using the same diets, except in one study carried out on pasture (Petkevičius *et al*. 1996). This indicates that perhaps the influence of dietary composition in monogastrics is greatest in the large intestine where fermentation products are formed, and it suggests that future studies on the influence of diet on parasite infection should include *Trichuris* because of its location in the large intestine.

TREATMENT AND CONTROL STRATEGIES

The control of parasites and parasite-induced disease by chemotherapy has been the subject of several theoretical (Anderson & Medley, 1985; Woolhouse, 1992; Medley, Guyatt & Bundy, 1993; Medley, 1994) and field studies (e.g. Elkins, Haswell-Elkins & Anderson, 1986; Bundy *et al*. 1990; Forrester & Scott, 1990; Holland *et al*. 1996; Hall *et al*. 1999). The main anthelmintic drugs used in human medicine are albendazole, mebendazole, pyrantel, levamisole and ivermectin (Van den Bossche, 1995). Most of these are also registered for use in veterinary medicine in different formulations, and animal models have a long tradition of being used for safety and efficacy trials of anthelmintic and other drugs. In addition, animal models could contribute to the development and evaluation of treatment and control strategies in two ways.

Firstly, the impact of repeated anthelmintic treatment on reinfection rates, predisposition, parasite fecundity and host performance are easily studied in an animal model (e.g. Boes *et al*. 1998). This design should be extended to malnourished hosts, since the efficacy of anti-parasitic drugs may be altered in the malnourished individual (Roe, 1985), a phenomenon that has attracted little attention from scientific or health professionals (Solomons & Scott, 1994). Furthermore, the use of anthelmintics with reduced efficacy may rapidly lead to anthelmintic-resistant parasite strains, which is by now well documented in veterinary parasitology (Prichard, 1990) and might occur in human helminths as a side effect of global mass treatment (Geerts, Coles & Gryseels, 1997; Bennett & Guyatt, 2000). Anthelmintic resistance in veterinary parasitology may in itself be a valid animal model, although some have argued that its results cannot be translated directly to community control programmes in humans, because the % coverage achieved in human communities is much lower than in groups of animals bred and maintained for human consumption and use (Savioli *et al*. 1997).

When farmers treat their livestock with an anthelminthic, they have the power to treat every one of their animals or the anthelminthic may have been added to feed consumed daily. In community treatment programmes typically at least 10% of the target group is missed, due to various reasons including absenteeism from the treatment site (e.g. primary schools), temporary or permanent outmigration, and refusal (though this is extremely rare) (Stephenson *et al*. 1993). This means that a significant proportion of the worm population remaining in the community members is not exposed to the anthelminthic drug in any one treatment cycle; these worms cannot develop resistance to an anthelminthic to which they have not been exposed, and with modern anthelminthics that produce high cure rates, the untreated worms are a large proportion of those left in the hosts to reproduce. This major difference in % coverage of the local worm population in farm animals compared with humans is essential to remember in deciding how best to use animal models of anthelminthic resistance. Furthermore, treatment is also given at intervals greater than the nematodes' generation time; this will also act against the creation of drug resistance (Savioli *et al*. 1997).

Secondly, animal models might be able to provide preliminary knowledge about the effect of combined treatment with albendazole and ivermectin on multiparasitised hosts under continuous exposure that may be useful to the Global Programme to Eliminate Lymphatic Filariasis (GPELF). The goal of the GPELF is to eliminate lymphatic filariasis as a public health problem from all endemic countries, and its primary control strategy will be antiparasitic chemotherapy (albendazole + ivermectin in Sub-Saharan Africa and diethylcarbamazine + albendazole once/year for 4–6 years) for 1 billion people over a 20 year period (WHO, 1998). Albendazole and ivermectin have different efficacies against the various nematode parasites expected to be affected by the GPELF.

INTERACTIONS BETWEEN HELMINTHS AND BETWEEN HELMINTHS AND BACTERIA

Coexistence of different helminth species or helminths and bacteria in humans as well as in animals seems to be the rule rather than the exception (Buck *et al*. 1978; Petney & Andrews, 1998). While epidemiological studies must consider possible interactions between helminths and/or bacteria, experimental studies often focus on single species infections. The introduction of a multiparasite-animal host model is therefore very relevant but requires careful consideration of experimental procedures. For example, when introducing a second parasite to an animal model it is important to include the appropriate positive and negative control groups. Otherwise it might not be possible to conclude whether an effect on the host is caused by an interaction between the different species, or by just one of the species.

Interactions between parasite species can be synergistic or antagonistic (Holmes, 1972; Christensen *et al*. 1987) and several reports on interaction between pig helminths have been published. For example, concurrent or sequential infections of pigs with the nodular worm *Oesophagostomum quadrispinulatum* significantly influenced establishment, distribution and fecundity of the sibling species *O. dentatum* (Christensen, Nansen & Barnes, 1997). Helwigh & Bøgh (1998) and Helwigh *et al*. (1999) investigated possible interactions between different helminths in the pig and observed very little interaction between *A. suum*, *O. dentatum* and *S. japonicum*, which inhabit different parts of the pig's body. Hence, development of interactions is most likely to be an indirect reaction in the host. Helwigh (1998) proposed that an area that deserves more attention is the interaction between gastrointestinal nematodes and bacteria, especially those that inhabit the same location in the host. It is conceivable that invading pathogenic bacteria may exacerbate human nematode infections, or that the presence of intestinal parasites may promote bacterial infections by lowering host resistance. These interactions, either indirect or direct, have not been studied sufficiently despite their significance for public health in endemic areas, because of technical, logistic or ethical difficulties and funding constraints (see Stephenson *et al*. Malnutrition and Parasitic Helminth Infections, this volume). However, a few examples of parasite–bacteria interaction in animal models have been described.

Mansfield & Urban (1996) studied an example of indirect interaction of helminths and bacteria, the interaction between *T. suis* and colonic bacteria in pigs and found that the bacteria increased gut pathology caused by parasite infection. They observed an increased necrotic proliferative colitis in pigs infected with *T. suis* which they attributed to worm-induced suppression of mucosal immunity to resident bacteria. Pigs infected only with *T. suis* had a significantly lower degree of intestinal pathology (Mansfield & Urban, 1996). In addition, preliminary results indicate a similar interaction causing gut pathology between *Oesophagostomum* spp. and *Salmonella typhimurium* in experimentally infected pigs (N. R. Steenhard, unpublished results). In this study, pigs harbouring *Oesophagostomum* spp. showed a higher and more prolonged excretion of *Salmonella* than pigs infected with *Salmonella* only. Furthermore, *Salmonella* bacteria were located more proximally (in the caecum and colon) in pigs with worms, compared with worm-free pigs, in which bacteria were limited to the colon only (N. R. Steenhard, unpublished results).

Direct interaction between bacteria and helminths occurs for instance when bacteria invade parasite stages present in the environment (e.g. faecal matter), and are transported into the host by the parasite. Thus, bacteria enter the host gastrointestinal tract or even the tissue, if the parasite's life-cycle includes tissue migration. For example, Adedeji, Ogunba & Dipeolu (1989) found that migrating *A. suum* larvae transported *Escherichia coli* to the lungs of pigs, and larvae of *Nematospiroides dubius* (= *H. polygyrus*) have been shown to carry *Salmonella typhimurium* into mice and aggravate the resulting Salmonella infection (Bottjer, Hirsch & Slonka, 1978).

The significant direct and indirect interactions between worms and bacteria in pigs mirror those same phenomena in parasitised humans, although the nature and extent of these interactions in humans are very poorly defined. Further, it is clear that study of helminth–bacteria interactions in pigs may be a very useful model for human infection. This is of particular importance since the study of Mansfield & Urban (1996) showed that not only pathogenic bacteria but also normally harmless resident gut flora may increase the deleterious effects of parasite infection. In addition, studies in animal models should include parasite interaction with protozoa and viruses. For example, NIH mice infected with the blood protozoan *Trypanosoma musculi* showed a high level parasitaemia following challenge infection with *T. spiralis*, whereas mice trickle-exposed to *T. musculi* without challenge did not develop parasitaemia (Chiejina & Wakelin, 1994). Interaction between *Eimeria* spp. and *T. spiralis* (Rose, Wakelin & Hesketh, 1994) and between *H. polygyrus* and *Trypanosoma congolense* (Fakae *et al*. 1994) have been described previously as laboratory mouse models for concurrent gastrointestinal nematode and protozoan infections.

IMPLICATIONS AND PERSPECTIVES

In this review, hookworm infections are conspicuous by their virtual absence, despite the fact that they cause more morbidity (anaemia and growth retardation) in humans than for example *Ascaris* or *Trichuris* (Stephenson *et al*. Malnutrition and Intestinal Helminth Infections, this volume). Apart from studies involving rodent hookworms [*Nippostrongylus brasiliensis* in rats (e.g. Kassai, 1982), *H. polygyrus* in mice (e.g. Bartlett & Ball, 1972; Scott & Tanguay, 1994), or infection with the human hookworm *Necator americanus* in mice (Wilkinson, Wells & Behnke, 1990)], the only large animal model developed for hookworm infection is that of *Ancylostoma* spp. in dogs (Schad, 1979; Carroll & Grove, 1984). This is despite the fact that human hookworms have been found in domestic pigs (Soulsby, 1982). Dogs are more omnivorous than for example cats but, in contrast to pigs, their gastrointestinal physiology is not quite comparable with that of humans. We propose that the pig model be used to study human hookworm infection, either using the human parasites *A. duodenale* and *N. americanus*, or the pig hookworm *Globocephalus urosubulatus*. Information about the prevalence of *Globocephalus* is scarce and mainly concerns wild boar where prevalences of 97% in Germany (Mennerich-Bunge, Pohlmeyer & Stoye, 1993) and 45% in the US (T. B. Stewart, personal communication) are recorded. However, hookworms are occasionally detected in domestic pigs (Jacobs & Dunn, 1969; T. B. Stewart, personal communication) indicating a potential role for a pig-hookworm model. The possibility of developing such a model deserves attention, not least because 'the global burden accruing to hookworm-infected girls and women of childbearing age, especially when pregnant, may very well be the single most important contribution intestinal nematodes make to the calculation of their global disease burden' (Stephenson *et al*. in this volume).

At our Centre, basic parasitological research is carried out in mice and pigs on parasitic nematodes of both veterinary and public health importance (*Ascaris*, *Trichuris*, *Oesophagostomum*, *Trichinella* and *Toxocara*). Furthermore, the pig model is being used successfully to study the zoonotic trematode *Schistosoma japonicum* which infects pigs and humans, and other animals, in Asia (Willingham & Hurst, 1996; Johansen *et al*. 2000). The overall goal is to increase our understanding of the parasite, its lifecycle, infection biology, pathogenicity and population dynamics. This type of research sometimes focuses on events as seen 'through the parasite's

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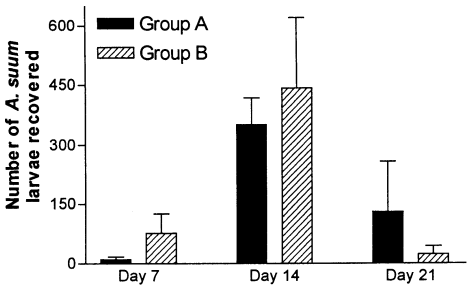

Fig. 3. Total number of *A. suum* larvae recovered from the challenge infection. Group A: Primary infection with *T. canis* and challenge infection with *A. suum*. Group B: Challenge control infection with *A. suum*. From Helwigh (1998).

eyes' and is very relevant to the study of host–parasite interactions because in field studies, this approach is often not possible (Stephenson, 1987*a*). However, research based on animal models has difficulty taking into account the human community and population level, at which infection dynamics may act differently. For example, Roepstorff *et al.* (1997) showed that although each pig became infected with *A. suum* upon experimental inoculation, the majority of pigs expelled the worms between days 14 and 21, resulting in the well-known overdispersed distribution of adult worms. It may be debatable which hosts are of most interest: those that eventually end up harbouring worms, or those that remain worm free – even after one or more rounds of chemotherapy and reinfection. But it is at the population level that the observations start, and attempts to unravel host responses controlling infection should accompany studies of the worm population.

In this context, it is important to carry out experimental studies over longer periods of time, in order to investigate long-term effects at the host population level. Many experimental studies concentrate on short-term effects, terminating studies after a few weeks post infection and often using high dose challenge infections. The results of such studies should be very carefully interpreted if conclusions are to be drawn at the host population level. For example, Helwigh (1998) investigated the effect of a primary infection with *T. canis* in pigs on a challenge infection with *A. suum*. A significantly lower recovery of *A. suum* larvae in the immunized pigs found in the beginning of the study was attributed to a temporary retardation of larval migration and not necessarily migratory inhibition, as the number of *A. suum* larvae recovered from the immunized pigs later on in the study was significantly higher compared to the control pigs (Fig. 3).

Finally, animal models may be used to study vertical transmission of parasitic nematodes. The issue is of particular importance to human parasitology, as most hosts in endemic environments/populations will be born to exposed mothers which could increase the risk of prenatal or neonatal infection. Among nematodes, *T. canis* in dogs (e.g. Lloyd, Amerasinghe & Soulsby, 1983) and *S. ransomi* in pigs (Moncol & Batte, 1966) are perhaps the best known examples of transplacental and colostral infection, respectively, but also *Ancylostoma* and *Trichinella* can be transmitted transplacentally (Lyons, 1994). In pigs, congenital infection of *S. japonicum* has recently been demonstrated (Willingham *et al.* 1999), indicating that the possibility of prenatal parasite transmission in pigs should be investigated further. So far, there is no parasitological evidence of transplacental transmission for *A. suum* (Alicata, 1961; Olson & Gaafar, 1963; Boes *et al.* 1999) or *Trichinella* spp. (Webster & Kapel, unpublished results) in pigs. However, preparturient exposure of sows to *A. suum* resulted in a change in worm burden distributions in their piglets upon neonatal challenge (Boes *et al.* 1999), suggesting that offspring born into endemic environments do not behave as parasite-naive hosts and that the worm burdens they acquire are to some extent dependent on the exposure of the previous generation. This observation may have consequences for the design of chemotherapy strategies which in human communities have been almost exclusively targeted at children.

It should be borne in mind though, that despite the many anatomical and physiological similarities, the pig placenta does not resemble the human placenta type as closely as it resembles, for example, that of guinea pigs or rabbits (Steven, 1974; Leiser & Kaufmann, 1994). This would suggest that for the study of transplacental parasite transmission, rodents or even carnivores and not pigs are the better model. Another possibility would be the primate model, but this is an expensive option with a very low yield in terms of offspring produced. Vertical transmission of *T. canis* (Tomašovičova *et al.* 1993) and *T. spiralis* (Denham, 1966; Podhajecky & Tomašovičova, 1968) in mice has been described, and recent results indicate vertical *T. spiralis* transmission in guinea pigs and ferrets (Webster & Kapel, unpublished observations). One way to test the existence of vertical transmission in the pig model could be to infect sows experimentally during gestation with *T. canis*, which is known for its transplacental transmission, to elucidate whether it is the placenta that prevents ascarid larvae from infecting the foetus, or whether perhaps differences in migratory route are responsible.

In conclusion, there are many suitable animal models available for the study of human host–parasite relationships. The choice of host animal will depend on the parasite studied and the questions asked, but a high degree of similarity between the model animal and the human target is vital. Validation of animal model results using mathematical modelling is an option worth considering and has

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been attempted successfully with the pig model. Recent results advocate the use of the pig-parasite model to study nutrition–parasite interactions, treatment and control strategies and helminth–bacteria interactions. Finally, even though a model is an artificial picture of the natural situation, and there are areas of research where pigs may not be the optimal model, current studies suggest a significant role for the pig model in the study of human nematode infections.

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