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Heligmosomoides polygyrus or Nematospiroides dubius?

J.M. Behnke, A.E. Keymer and J.W. Lewis

The intestinal trichostrongylid nematode of mice, Heligmosomoides polygyrus bakeri, is often referred to as Nematospiroides dubius. Here, Jerzy Behnke, Anne Keymer and John Lewis ask the question: which is correct?

To most biologists the use of Latin names to distinguish animals has a clear and very obvious purpose: the elimination of any possible ambiguities arising from the use of common names. However, the choice of Latin names is dependent on taxonomic criteria which, for the most part, are still based on morphological distinguishing features and which may change when new information becomes available. Occasionally taxonomic controversies touch parasite species of widespread interest, causing confusion in the associated literature as two or more names are used to describe the same organism. Already for those of us who teach the immunology of filarial worm infections, the taxonomic revision of *Dipetalonema viteae* to *Acanthocheilonema viteae*[1] causes headaches we could have done without.

A debate of more serious proportions concerns the intestinal trichostrongylid nematode of mice, *Heligmosomoides polygyrus bakeri* (or *Nematospiroides dubius*?). Even in the past year papers have been published which refer to the parasite by each of the two names without recognition of the alternative. The confusion has been exacerbated by the identification of subspecies and the realization that one of the most widely used isolates of this parasite was obtained from an incidental infection in an abnormal host. So, which name is correct? It is time that some consensus was arrived at.

Historic Background

The parasite was first described by Dujardin[2] in 1845 with four other nematodes from various rodents in France in a genus proposed as *Strongylus*. However, the descriptions were not sufficiently detailed to be accepted under the requirements of modern taxonomy. Baylis[3] believed that *Strongylus polygyrus*, as described by himself, was synonymous with *Heligmosomoides polygyrus* but Durette-Desset[4] was not convinced. Confusion was then generated by a number of authors who misinterpreted Dujardin or described parasites without due reference to his work. The genus *Heligmosomoides* was erected by Hall[5] to cater for a parasite described by Linstow in 1878 that Hall considered not to match the original description of *Strongylus polygyrus*[2].

The name *Heligmosomoides polygyrus* was first used by Boulenger[6] to describe worms isolated from *Microtus agrestis* (which is now known not to be susceptible to the species/strain derived from *Apodemus sylvaticus*[7]) and it is thus possible that the name *Heligmosomoides polygyrus* was originally coined for a quite different species of worm to that parasitizing *Apodemus sylvaticus*; *Microtus* spp in Europe are affected by many species of *Heligmosomum* and *Heligmosomoides*, all of which are superficially similar and can only be distinguished by detailed morphometric analysis[4,8,9]. Indeed, Durette-Desset[4] considered Boulenger's parasite (*Strongylus laevae* from *Microtus agrestis*) to be equivalent to *Heligmosomum laeve*[2], since revised to *Heligmosomoides laevis*[10,11].

In 1926 Baylis[3] reported a parasite recovered from *Apodemus sylvaticus* in Oxford. He believed this worm to differ from that described by Boulenger[6] and, because of the incomplete earlier descriptions, he named his parasite *Nematospiroides dubius* to avoid any further confusion! However, the story is further complicated by the description of a very similar worm from *Apodemus sylvaticus* and *Mus musculus* in the USSR by Schulz[12], which he named *Heligmosomoides skrjabini*. Baylis corresponded with Schulz and, after examining his specimens, later published an article[13] confirming that the two were indeed the same parasite, admitting that its features were consistent with those of the genus *Heligmosomoides*[5] but insisting that *Nematospiroides dubius* had priority because his paper was published on the first day of November and Schulz's not until later the same month.

In the following four decades the parasite changed names several times and considerable confusion was generated as to whether there was just one species common to field mice, house mice and voles, or whether several closely related species infected overlapping ranges of hosts. Moreover, the names *Heligmosomoides*, *Nematospiroides* and *Heligmosomum* (Railliet et Henry 1909) were abolished and reinstated in turn.

In 1968 the genus *Heligmosomoides*[5] was re-established by Durette-Desset[10] who distinguished *Heligmosomoides* from *Heligmosomum* on the basis that only the latter had oblique cuticular ridges on the dorsal side. In 1972 Durette-Desset and co-workers[14] discussed *Heligmosomoides polygyrus* (synonym: *Nematospiroides dubius*[3]) as the principal parasite of *Apodemus sylvaticus*. More recently Asakawa published comprehensive reviews of the genus *Heligmosomoides*[11] and *Heligmosomum*[9]. In the meantime British authors[15–19] continued to use *Nematospiroides dubius*[3] to describe the parasite infecting *Apodemus sylvaticus* in the UK.

Subspecies of *Heligmosomoides polygyrus*

The controversy would probably have disappeared in the scientific archives were it not for the isolation and successful maintenance in laboratory mice of a parasite conforming to the description of *Heligmosomoides polygyrus*. The life cycle is readily maintained in the laboratory and adult worms are long lived (eight months[8,20]) so that frequent passage is not necessary. Moreover, since the infective larvae can be kept in aqueous suspension at 4°C for many months[21], a single culture can provide thousands of larvae for months of subsequent research. All these attributes have led to the parasite being adopted as a popular laboratory model of intestinal trichostrongylid infection and a vast literature has grown about the worm. Unfortunately, laboratory workers confused by the continuing taxonomic revisions of the wild parasites have continued to use *Nematospiroides dubius*, *Heligmosomoides polygyrus* or both.

The first reported laboratory study was by Spurlock[22] who used larvae raised from wild *Mus musculus* caught on the Conway Ranch near Woodland, California. However, the present widely employed laboratory strain was originally isolated by Ehrenford[8] in 1950 from *Peromyscus maniculatus* also caught near Woodland. Forrester later called this isolate strain 50 (Refs 23,24). Spurlock provided larvae of this strain for various laboratories including the Wellcome Foundation in London, from where the parasite was distributed further. Interestingly, Ehrenford referred to this para-

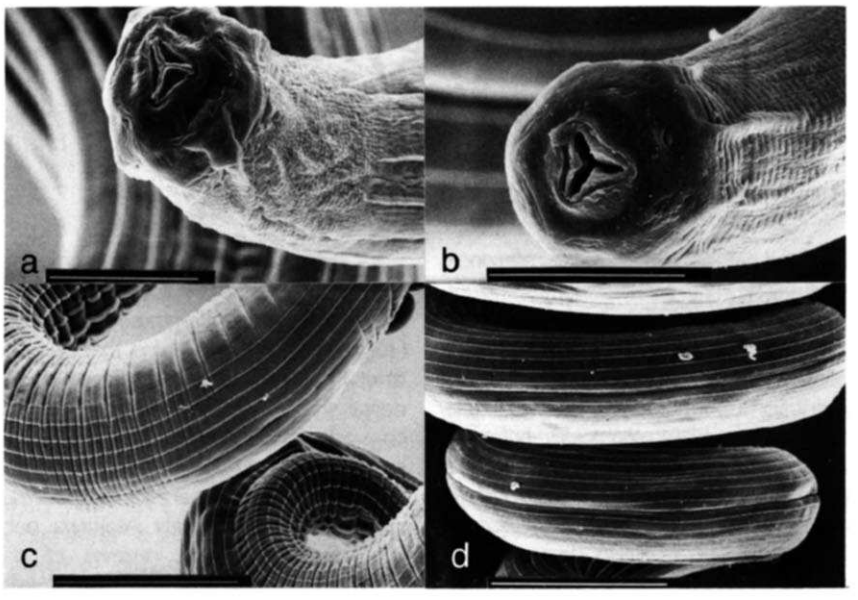

Fig. 1. Scanning electron micrographs comparing the anterior (a and b) and the midbody cuticle (c and d) of **Heligmosomoides polygyrus polygyrus** *(a and c) and* **Heligmosomoides polygyrus bakeri** *(b and d).* **H. p. polygyrus** *(c) has fewer cuticular ridges than does* **H. p. bakeri** *(d) but the cephalic ridges are more prominent (a versus b). Scale bars = 20 μm (a and b) and 100 μm (c and d).*

site as *Nematospiroides dubius*. It became apparent from subsequent work that *Peromyscus maniculatus* is an incidental host for the parasite in question. Forrester[23] examined 231 individuals from five localities in northern California and found no worms. In addition, *Peromyscus maniculatus* was refractory to laboratory infection, even when given whole body irradiation or transplanted adult worms[24] although susceptibility was enhanced following treatment with prednisolone[25]. It is thus very likely that Ehrenford established the most widely used strain of the parasite from an abnormal host and that the parasite was from the strain normally infecting *Mus musculus* in the USA. This strain (Strain 50) has been named *Heligmosomoides polygyrus bakeri* (Fig. 1 and Ref. 14).

American voles *Phenacomys intermedius* and *Phenacomys ungava* are also infected by a very similar parasite, now called *Heligmosomoides polygyrus americanus*[14], which can be distinguished from *Heligmosomoides polygyrus bakeri* by the difference in the arrangement of the dorsal ray and in the number of cuticular ridges and longer spicules[14]. Although similar to the other subspecies in most other morphological respects, *Heligmosomoides polygyrus americanus* was considered by Rausch and Rausch[26] to be a different species because it infects *Phenacomys intermedius* throughout the geographical range of this host, even host populations which have been isolated for some time. *Heligmosomoides polygyrus americanus* is therefore unlikely to have adapted to *Phenacomys intermedius* in recent historic times as suggested by Durette-Desset and colleagues[14]. Asakawa[11] considers *Heligmosomoides polygyrus americanus* to be a distant relative, more closely related to *Heligmosomoides johnsoni* and *Heligmosomoides hudsoni*, which parasitizes the American rodents *Phenacomys* and *Dicrostonyx*, respectively. Two other subspecies of *Heligmosomoides polygyrus* have also been recognized. The name *Heligmosomoides polygyrus polygyrus* has been ascribed to the normal parasite of *Apodemus sylvaticus* in Europe and *Heligmosomoides polygyrus corsicus* to similar worms from *Mus domesticus* in Corsica[4]. In this classification, *Heligmosomoides polygyrus polygyrus* is the original parasite described by Baylis[3] as *Nematospiroides dubius*[27].

British Species and Their Hosts

In the UK, the field mouse, *Apodemus sylvaticus*, is almost invariably parasitized by *Heligmosomoides polygyrus polygyrus* and, in some surveys, all of the animals studied have been reported to carry worms [6]. The parasite is still variously referred to as *Nematospiroides dubius*[15,18,19,28] or *Heligmosomoides polygyrus*[27,29] but British authors have given little attention to the possibility of there being other closely related species in the UK, particularly in voles[15,17,28]. Recently, Quinnell and colleagues[7] tried to infect both *Clethrionomys glareolus* and *Microtus agrestis* with the laboratory mouse maintained strain (*Heligmosomoides polygyrus bakeri*) and with recent isolates of *Heligmosomoides polygyrus polygyrus* from *Apodemus sylvaticus*. No adult worms were recovered from any of the voles and eggs were detected in the faeces on only one occasion when the voles had been treated with the immunosuppressive agent cortisone. Even in this case faecal examinations were negative from day 14 onwards. Therefore, it appears likely that reports of *Heligmosomoides polygyrus* from voles in the UK may be misidentifications[15,17,29]. The most promising candidate is *Heligmosomoides glareoli*, originally described by Baylis[30] from *Clethrionomys glareolus* in Oxford. *Heligmosomoides glareoli* has been recorded from *Clethrionomys glareolus* in France[31] but European voles are affected additionally by species whose taxonomy is still not totally resolved[4,9,11] and which also need to be considered.

Conclusions

These confusions would probably have gone unnoticed were it not for the fact that the worm is an extremely popular laboratory model. It is a continuing source of irritation that there is still no consensus as to what the parasite should be called, at least as adjudged by the reports from experimental parasitologists. It is clear from the confusion surrounding the original descriptions that it is possible to argue *ad nauseam* in favour of any of the names proposed. It is our view that the seminal paper by Durette-Desset and colleagues[14] should be considered the last word for the laboratory mouse-maintained parasite and that all worms derived from the original isolate in California, USA[8] should be called *Heligmosomoides polygyrus bakeri*. We suggest that the usage of *Nematospiroides dubius* be abandoned totally. The common parasite of *Apodemus sylvaticus* in Europe should be referred to as *Heligmosomoides polygyrus polygyrus*.

The taxonomic position of the other *Heligmosomoides* spp still poses unresolved questions. It is likely that a species complex exists without clear dividing lines between some of the organisms involved. Durette-Desset and colleagues[4] suggested that the parasites subspeciated in recent historic times (see also Asakawa[9,11]). If this was indeed the case, the whole genus is probably subject to intense selection pressure imposed by the various hosts and their ecology. In view of the complex taxonomy of this group of nematodes, few of the field reports can be taken at face value.

As far as British rodents are concerned, the relationships of parasites in voles, field mice and house mice need to be reconsidered in the light of detailed descriptions of American and European species. Although the *Heligmosomoides* and *Heligmosomum* species affecting voles in Europe are distinct, there is still work to be completed on the species affecting voles in the UK. It is time that the common species, subspecies and strains were isolated and subjected to isoenzyme and DNA sequence analysis to establish accurately their phylogenetic relationships.

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Critical Stages in the Development of *Plasmodium* in Mosquitoes

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One tool for the control of malaria that may become available to future generations of public health workers is the introduction of genes into the Anopheline vector populations that will render the mosquitoes refractory to Plasmodium. *Insights from basic research that could transform this idea into a technical reality are presently lacking. In this review, Alon Warburg and Louis Miller focus on one crucial area of research: the identification of potentially vulnerable points in the developmental cycle of* Plasmodium *in mosquitoes.*

It may be argued that, due to selective pressure, *Plasmodium* spp would overcome any barrier genetically engineered into vector mosquitoes. However, the adaptive versatility of malaria parasites is not limitless. For example, mammalian malarias are transmitted by Anopheline mosquitoes, and avian malarias by Culicine mosquitoes. After persistent mutual exposure, why have mammalian malaria parasites never adapted to the coindigenous Culicine mosquitoes? Recent evidence suggests that *P. falciparum*, a major human malaria parasite, is phylogenetically closer to avian *Plasmodium* spp than to other human or primate malarias[1]. It seems likely that an avian malaria parasite, at some point in the past, made the transition from bird to human. Why have Culicine mosquitoes lost their ability to transmit *P. falciparum*? It would appear that a fundamental difference exists between Anopheline and Culicine mosquitoes that restricts their vectorial capacity to mammalian and avian parasites, respectively (Table 1).

The successful completion of the sporogonic development of malaria parasites in the midgut, haemocoel and salivary glands of the mosquito vectors depends on their ability to overcome a series of barriers. In the midgut, gametocytes transform into gametes that fertilize to produce motile zygotes or ookinetes. Ookinetes then cross the peritrophic membrane, a process probably made possible by specific recognition and penetration mechanisms. Attachment to the midgut epithelium and passage through it may also depend on receptor-mediated recognition and invasion. Survival of oocysts in the mosquito haemocoel is made possible by their ability to evade haemolymph-mediated immune reactions and the availability of essential nutritive factors. Sporozoites released into the haemocoel must locate, recognize and penetrate the salivary glands. They survive within the acinar cells of the glands from where they exit into the salivary duct and are injected with the saliva into the vertebrate host during subsequent feedings[2].

Development in the Midgut

Gametogenesis is triggered by slightly alkaline conditions (pH ~8.0) and a reduction in temperature from that of the vertebrate host[3]. A mosquito-derived molecule stimulates exflagellation[4] and, at least in some mosquito species, digestive enzyme activity may influence the ability of ookinetes to penetrate the gut wall[5]. Other than that, very little definitive information exists about the possible role of extrinsic factors in the development of gametes and ookinetes in the lumen of the midgut. However, these developmental stages have been the focus of intensive efforts to develop transmission-blocking vaccines[6]. Such vaccines stimulate the production of antibodies that recognize surface antigens on gametes and