Morphology of inhibited larvae of the bovine lungworm *Dictyocaulus viviparus*

G. von Samson-Himmelstjerna* and T. Schnieder

Institute of Parasitology, School of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

Abstract

Some morphological features of inhibited fourth stage (L4) and fourth moult larvae (4M) of the bovine lungworm *Dictyocaulus viviparus* are described. Inhibition was induced by maintaining the third stage larvae (L3) for 6 weeks at 4°C. Inhibited fourth stage (L4) and fourth moult larvae (4M) were collected by perfusion of the lungs of experimentally infected calves after necropsy at 15 and 68 days post infection (d.p.i.), respectively. Inhibited 4M, isolated at 68 d.p.i., were about ten times larger than inhibited L4 isolated at 15 d.p.i.

Introduction

It is well known that the inhibition of lungworm larvae may be induced by cooling of the larvae for several weeks at temperatures of about 4–7°C (Gupta & Gibbs, 1970; Pfeiffer & Supperer, 1980; Eysker *et al.*, 1992). Within a project dealing with the differences in gene expression between inhibited and uninhibited larval stages of *Dictyocaulus viviparus*, we isolated larval stages from the lungs of two calves. Currently, there is only very limited information about the morphology of parasitic lungworm larvae in cattle available. Gupta & Gibbs (1976) described some features of inhibited larvae isolated from naturally or experimentally infected calves. The only report on the morphology of uninhibited parasitic *D. viviparus* larvae described some anatomical features such as the morphology of the cephalic region and reproductive organs of larvae, which were isolated from the lungs of experimentally infected guinea pigs (Douvres & Lucker, 1958). The morphology of uninhibited *D. filaria* larvae from sheep was described in detail by Verster *et al.* (1971). Here we present information on the morphology of inhibited lungworm larvae isolated at different time intervals after infection.

Materials and methods

First stage larvae (L1) were collected from the faeces of experimentally infected calves and incubated in 20–30 ml tap water in Erlenmeyer flasks (250 ml) at room temperature for at least 5 days, until development to the third larval stage (L3) was completed. The *D. viviparus* strain used in this study has been passaged at the Institute of Parasitology in Hannover for about three years and was isolated on a farm in the coastal region of lower Saxony. Inhibited larvae were collected after incubation of 40,000 L3 for 6 weeks at 4°C and infection of one calf with 10,000 L3 and another one with 30,000 L3 followed by perfusion of the lungs (Eysker *et al.*, 1990) at 15 d.p.i. and 68 d.p.i., respectively. The faeces of the latter calf were examined for the shedding of larvae according to Baermann (1917). Larvae from the lungs of another calf infected with 5000 uninhibited L3 were also collected. All larvae were further processed for mRNA isolation directly after being microphotographed.

Results and Discussion

In the present study, morphological differences of inhibited lungworm larvae isolated at different intervals post-infection are documented for the first time. Inhibition was induced by cooling L3 larvae for 6 weeks at 4°C prior to infection. After the infection of two calves with inhibited larvae the lungs were perfused and larvae collected at 15 d.p.i. and 68 d.p.i. Shedding of larvae was recorded in the latter calf. At only one date 2 larvae per gram of faeces were found.

At each date of collection, simultaneous microphotograph

* Present address: Bayer AG, Business Group Animal Health, Institute for Parasitology, Agricultural Research Centre Monheim, D-51368 Leverkusen, Germany.
Fax: 02173 38 37 66
E-mail: georg.samson-himmelstjerna.gs@bayer-ag.de

https://doi.org/10.1017/S0022149X0070023X Published online by Cambridge University Press

and length estimation of ten larvae each were performed (table 1). All larvae isolated at 15 d.p.i. show genital primordia, whereas larvae isolated at 68 d.p.i. show a genital primordium and a sheath. However, a sheath is absent from larvae isolated at 15 d.p.i. Thus we consider the latter to be L4 stages. Males showed a bursa with bursa rays and spicules (fig. 1). A female (fig. 2) shows vulvar lips and a vulvar opening (fig. 3) located between 300 and 350 μm anterior to the posterior extremity (fig. 4). However, the opening of the vulva is not yet extended into the pars ejaculatrix or pars haustrix. In contrast, uninhibited L4 stages of *D. filaria* do already show a vulvar opening which extends to the pars ejaculatrix, although in general the development of *D. filaria* is slightly slower than that of *D. viviparus* (Verster *et al.*, 1971). This indicates the postponed development of inhibited *D. viviparus* larvae.

Table 1. Length (μm) of inhibited L4 and fourth moult (4M) stage larvae of *Dictyocaulus viviparus*.

Developmental stage	Days post infection	Range, ($\bar{x}$)	
		Male	Female
L4	15	800–893, (845)	786–1080, (906,8)
4M	68	8620–10850, (9710,3)	8333

In this study, the inhibited female and male L4 stages isolated at 15 d.p.i. demonstrated genital primordia. This is in contrast to the observations of uninhibited *D. viviparus* L4 isolated from guinea pigs (Douvres & Lucker, 1958). In the present study, the vulvar opening was present in the L4 stages but it was only recorded in L5 stages of uninhibited larvae isolated from the guinea pigs. Whereas in uninhibited male larvae from the lungs of

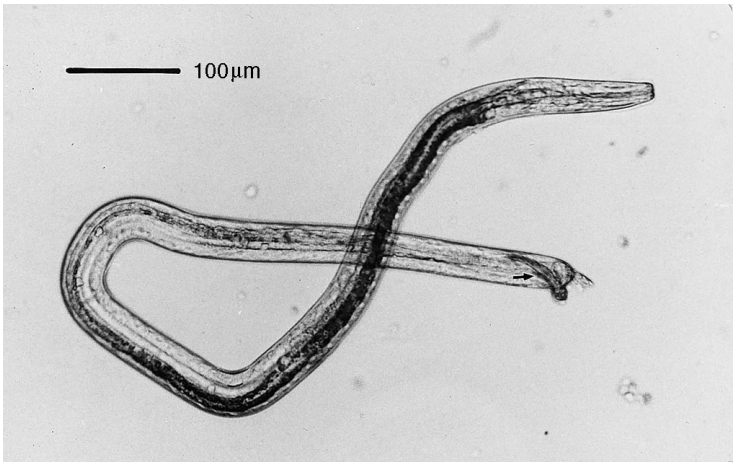

Fig. 1. Inhibited male fourth stage larva of *Dictyocaulus viviparus* isolated 15 days post infection showing spicules (arrowed). Scale bar = 100 μm.

Fig. 2. Inhibited female fourth stage larva of *Dictyocaulus viviparus* isolated 15 days post infection. Scale bar = 100 μm

https://doi.org/10.1017/S0022149X0070023X Published online by Cambridge University Press

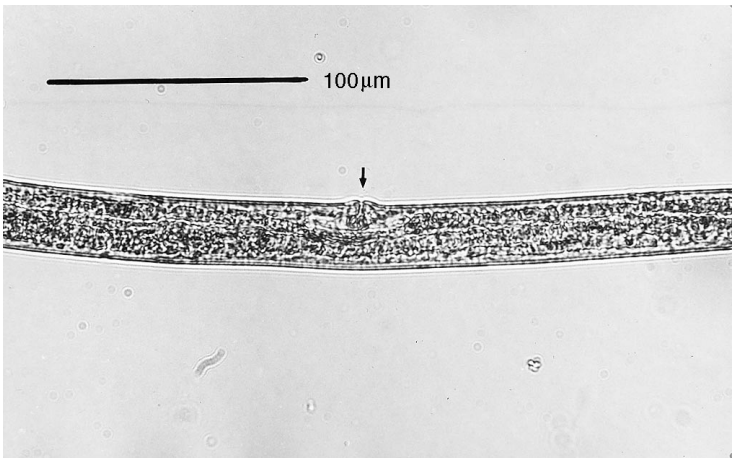

Fig. 3. Vulvar primordium (arrowed) of inhibited female fourth stage larva of *Dictyocaulus viviparus* isolated at 15 days post infection. Scale bar = 100 µm.

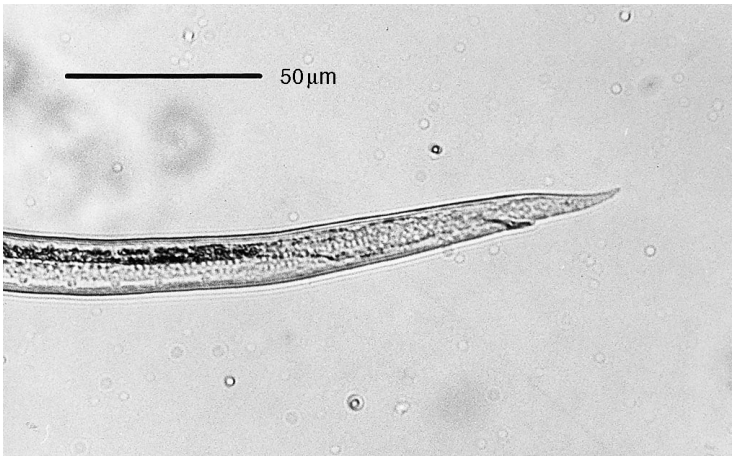

Fig. 4. Posterior end of inhibited female fourth stage larva of *Dictyocaulus viviparus* isolated at 15 days post infection. Scale bar = 100 µm.

guinea pigs the spicules were first found in the L5 stage (Douvres & Lucker, 1958), here they appeared in all male larval stages. These differences in the morphology of *D. viviparus* larvae from the lungs of guinea pigs on the one hand and from lungs of calves on the other hand suggest that larval development is slightly different in the biological host (bovines) and abortive hosts (guinea pigs) as also discussed by Gupta & Gibbs (1976).

The larvae isolated at 68 d.p.i. were about ten times larger than those isolated at 15 d.p.i. All larvae isolated at 68 d.p.i. showed well developed genital primordia as well as a sheath. Among the ten larvae examined only one developed into a female. The males again possessed a bursa, bursa rays and spicules (fig. 5). The bursa of one male L5 isolated of the lungs of a calf infected with uninhibited L3 at 15 d.p.i. is shown in fig. 6.

According to the nomenclature used by Verster *et al.* (1971) the larvae isolated at 68 d.p.i. are 4M stages. These data suggest that inhibited larvae in the lungs of the cattle grow in size either continuously or at the end of the inhibition period, and during this process a change from L4 to 4M occurs. These results are in part different from the data reported by Inderbitzin (1976) and Pfeiffer (1976). These authors described the finding of inhibited L5 after infection with larvae which had been kept at 4°C for 8 weeks (Inderbitzin, 1976) or 7°C for 3 weeks (Pfeiffer, 1976). Larvae were isolated at 15, 35 and 60 d.p.i. (Inderbitzin, 1976) or at 35 d.p.i. (Pfeiffer, 1976). Both authors classified only those larvae as inhibited stages that were smaller than 5 mm and at the same time showed a genital primordium. They classified these stages as preadult L5 stages. No information about the occurrence of L4 or 4M stages was given in the above mentioned studies.

Gupta & Gibbs (1976) described the morphology of inhibited larvae isolated from experimentally and

https://doi.org/10.1017/S0022149X0070023X Published online by Cambridge University Press

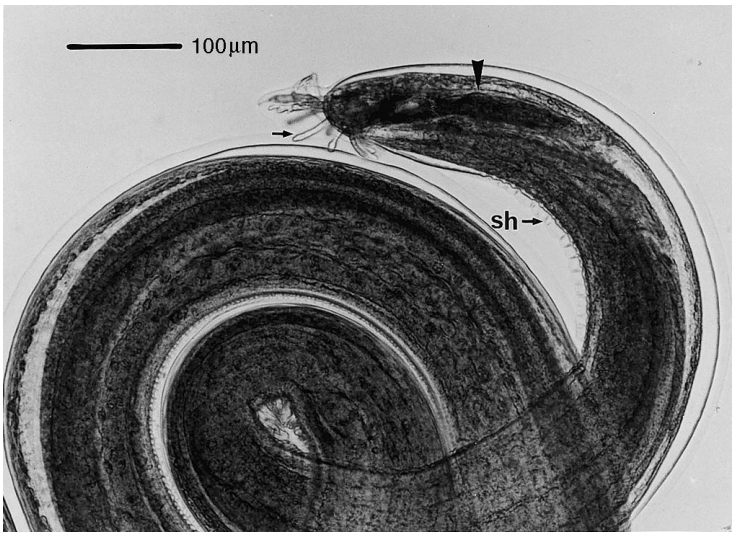

Fig. 5. Posterior end of ensheathed (sh, sheath) inhibited male fourth moult larva of *Dictyocaulus viviparus* isolated at 68 days post infection showing bursal rays (arrowed) and spicules (arrowhead). Scale bar = 100 μm.

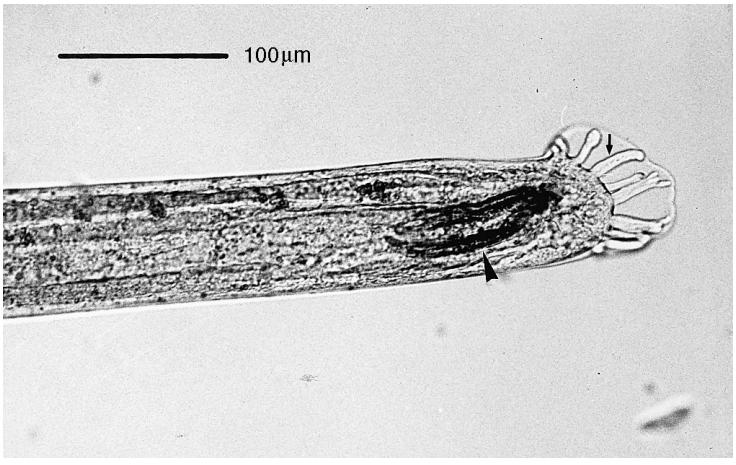

Fig. 6. Uninhibited male fifth stage larva of *Dictyocaulus viviparus* isolated at 15 days post infection showing bursal rays (arrowed) and spicules (arrowhead). Scale bar = 100 μm.

naturally infected calves. The age of the inhibited larvae was not clearly defined, however, it was stated that the calves were held at least four weeks under parasite-free conditions before larvae were isolated. The inhibited larvae ranged in length from 0.9 to 2.7 mm and that the genital systems of all larvae were well developed to a similar extent. As in the present study, the data of Gupta & Gibbs (1976) indicate that during inhibition the larvae undergo a postponed development during which they grow in size and that inhibition of *D. viviparus* occurs at the L4 and 4M stage as well as and not only at the L5 stage. This is also in accordance with other parasitic nematodes such as *Ostertagia ostertagi* (Armour *et al.*, 1969) and *Haemonchus contortus* (Blitz & Gibbs 1972), where inhibition already occurs at the fourth larval stage. Since these data are based only on a limited number of worms, further investigations on the morphology of inhibited stages of *D. viviparus* are necessary.

References

Armour, J., Jennings, F.W. & Urquhart, G.M. (1969) Inhibition of *Ostertagia ostertagi* at the early fourth larval stage-I. The seasonal incidence. *Research in Veterinary Science* **10,** 232–237.

Baermann, G. (1917) Eine einfache Methode zur Auffindung von Ankylostomum-(Nematoden)-Larven in Erdproben. *Tijdschrift voor Diergeneeskunde* **57,** 131–137.

https://doi.org/10.1017/S0022149X0070023X Published online by Cambridge University Press

Blitz, N.M. & Gibbs, H.C. (1972) Studies on the arrested development of *Haemonchus contortus* in sheep-I. The induction of arrested development. *International Journal for Parasitology* **2,** 5–12.

Douvres, F.W. & Lucker, J.T. (1958) The morphogenesis of the parasitic stages of the cattle lungworm, *Dictyocaulus viviparus*, in experimental infected guinea pigs. *Journal of Parasitology* **44,** 28–29.

Eysker, M., Boersema, J.M. & Hendrikx, W.M.L. (1990) Recovery of different stages of *Dictyocaulus viviparus* from cattle lungs by a combination of a perfusion and a Baermann technique. *Research in Veterinary Science* **49**, 373–374.

Eysker, M., Boersema, J.H., Cornelissen, J.B.W.J., Kooyman, F.N.J. & De Leeuw, W.A. (1992) *Dictyocaulus viviparus* in calves: effect of rotational grazing on the development of infections. *Veterinary Parasitology* **41,** 127–135.

Gupta, R.P. & Gibbs, H.C. (1970) Epidemiological investigations on *Dictyocaulus viviparus* (Bloch, 1782) infection in cattle. *Canadian Veterinary Journal* **11,** 149–156.

Gupta, R.P. & Gibbs, H.C. (1976) A note on the morphogenesis of inhibited stages of *Dictyocaulus viviparus* (Bloch 1782). *Haryana Agricultural University Journal of Research* **6,** 205–207.

Inderbitzin, F. (1976) Experimentell erzeugte Entwicklungshemmung von *Dictyocaulus viviparus* des Rindes. PhD thesis, University of Zürich.

Pfeiffer, H. (1976) Zur verzögerten Entwicklung des Rinderlungenwurmes, *Dictyocaulus viviparus*. *Wiener Tierärztliche Monatsschrift* **63,** 54–55.

Pfeiffer, H. & Supperer, R. (1980) Die Dictyocaulose des Rindes. *Berliner und Münchener Tierärztliche Wochenschrift* **93,** 365–370.

Verster, A., Collins, H.M. & Anderson, P.J.S. (1971). Studies on the *Dictyocaulus filaria* IV. The morphogenesis of the parasitic stages in lambs. *Onderstepoort Journal of Veterinary Research* **38,** 199–206.

(Accepted 20 August 1998)

https://doi.org/10.1017/S0022149X0070023X Published online by Cambridge University Press