

## Development of the Parasitic Stages of *Nematodirus abnormalis* in Experimentally Infected Sheep and Associated Pathology

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**ABSTRACT:** The development of *Nematodirus abnormalis* May, 1920 was examined in 10 worm-free lambs each infected with 60,000 larvae and killed 2-20 days after infection (DAI). Most worms developed in the anterior 4 m of the small intestine, with the third and fourth molts occurring 4-6 and 12-14 DAI respectively. A large proportion of larvae remained inhibited at the early fourth stage. Morphological development of the larvae including the genitalia and body ridges is described in detail. Pathological changes were associated with worms coiling around villi and distorting but not breaking the continuity of the epithelium. *N. abnormalis* is thought to be only mildly pathogenic for sheep.

*Nematodirus abnormalis* May, 1920 is a cosmopolitan parasite of sheep, goats, and other ruminants, but in spite of its possible economic importance, it has been little studied. Frequently it occurs in mixed infections with other species and is usually present as only a small proportion of the total number of *Nematodirus* (Brunsdon, 1961; Becklund and Walker, 1967). However, in sheep in South Australia, *N. abnormalis* is frequently the dominant species in mixed infections or may occur in monospecific infections (Beveridge and Ford, 1982). A similar situation occurs in other areas of the world with hot, dry Mediterranean type climates (Guralp and Oguz, 1967).

Aspects of development of the free-living stages of *N. abnormalis* have been studied by Onar (1975) and Neiman (1977), and adult morphology has been described in detail by Becklund and Walker (1967), Stringfellow (1968), Lichtenfels and Pilitt (1983), and Rossi (1983). In this paper we describe for the first time the development of the parasitic stages together with the pathological changes associated with infection by a single dose of *N. abnormalis* larvae.

### Materials and Methods

*Nematodirus abnormalis* was obtained from naturally infected sheep from Myrtle Springs Station, Leigh Creek, South Australia (30°27'S, 138°13'E). Two naturally infected sheep were killed and nematodes from the upper small intestine were collected in tissue culture medium (Medium 199, C.S.L. Melbourne, Australia) and held at 37°C for no more than 2-3 hr. Males were identified individually by the characteristic morphology of the spicule tip (Becklund and Walker, 1967), and males of *N. abnormalis* and all females present were surgically transplanted into the duodenum of a

lamb that had been raised worm-free. Feces from the recipient sheep were collected and cultured beginning 21 days after surgery. Feces were soaked in water, homogenized, and sieved to remove gross particulate material. The washings were allowed to sediment, the supernatant removed, and the residue mixed with a saturated sugar solution. The top layer of the flotation containing the eggs was removed and washed in a 44- $\mu$ m sieve to remove the sugar. The eggs were then cultured in water in large petri dishes at 25°C for up to 5 wk, until all the eggs had hatched. Larvae were concentrated using a Baermann technique and stored in water at 4°C until used. The isolate was passed through several generations in worm-free sheep prior to use in this experiment. Larvae used for experimental infections were fresh larvae, and were used within 4 wk of culturing.

Ten 12-wk-old Merino lambs, raised worm-free, were each infected with 60,000 *N. abnormalis* larvae by stomach tube. A single worm-free lamb served as a non-infected control, and was killed at the commencement of the experiment. The infected sheep were killed 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after infection (DAI). Sheep were killed using an intravenous barbiturate overdose, and the anterior small intestine was removed rapidly, freed of mesenteric attachments, and divided into six, 1-m-long sections using large artery forceps. Two small sections (4 cm) of tissue at the anterior end of each of the first four segments were separated with forceps and gently distended by injection of 10% buffered formol-saline or 2.5% glutaraldehyde in cacodylate buffer. As soon as the segments had been filled with fixative (within 3-10 min of the death of the sheep), the segments were opened, attached to small pieces of card, and placed in the appropriate fixative. Specimens fixed in glutaraldehyde were washed gently to remove debris, and after 4-6 hr were stored in cacodylate buffer with added sucrose.

The content of the six 1-m lengths and the remainder of the small intestine were washed out with normal saline, sedimented at room temperature, and the sediment from two of the sectors fixed with hot 70% ethanol for morphological study of the nematodes. The remainder were fixed with cold formol-saline. The number of nematodes in each segment of the intestine

**Table 1.** Distribution of *Nematodirus abnormalis* in 1-m segments of the small intestine of lambs experimentally infected with 60,000 larvae.

Time of killing (DAI)	Percentage of worm burden in each small intestine segment						
	Segment						Remainder
	1	2	3	4	5	6	
2	61.6*	28.0	5.6	0.9	0	0.9	2.8
4	21.5	55.4*	10.4	3.6	2.0	2.0	5.2
6	7.4	16.5	20.5	24.2*	21.5	7.1	2.7
8	3.3	9.5	15.2	5.4	12.8	27.2*	26.7
10	0	0	1.7	3.3	0	1.7	93.3*
12	0	0.6	29.0	39.3*	16.2	7.6	7.3
14	1.0	14.0	23.7	41.6*	17.2	2.5	0
16	0.4	13.1	41.2*	36.5	7.1	1.8	0
18	0	3.7	49.4*	19.2	6.57	13.4	7.7
20	8.8	35.1*	28.5	21.6	2.7	1.1	2.2
Total	10.4	17.6	22.5*	19.6	8.6	6.5	14.8
SE of mean	6.1	5.0	4.8	5.0	2.6	2.6	9.0

\* Mode.

was estimated by duplicate counts on 10% samples. Nematodes fixed in 70% ethanol were mounted in glycerine for morphological examination and a series of standard measurements made on 10 males and 10 females from each sheep using a micrometer eyepiece, or by drawing the worm with the aid of a drawing tube attached to the microscope, and obtaining the measurements from the drawing using a map measurer.

For quantitative studies on the growth of worms, both larvae and adults were selected for measurement. Prior to 6 DAI, the sex of larvae could not be determined, and measurements were made on 20 randomly selected larvae. At 6 and 8 DAI, 20 male and 20 female larvae were measured. From 12 DAI onwards, 20 male and 20 female adults were selected together with a number of early fourth-stage larvae and fourth-stage larvae close to the final molt. The measurements of each group of larvae were treated separately.

**Table 2.** Worm counts of Merino lambs experimentally infected with 60,000 larvae of *Nematodirus abnormalis* and killed 2–20 days after infection (DAI).

Time of killing (DAI)	Total no. worms	Per-cent established	Proportions of larval stage (%)			
			L <sub>3</sub>	Early L <sub>4</sub>	Late L <sub>4</sub>	L <sub>5</sub> adult
2	10,700	18	100	0	0	0
4	30,700	51	100	0	0	0
6	29,700	50	0	100	0	0
8	24,300	41	0	100	0	0
10	6,000	10	0	100	0	0
12	32,800	55	0	90	10	0
14	40,100	67	0	74	25	1
16	45,200	75	0	91	2	7
18	35,000	58	0	83	4	13
20	36,500	61	0	91	3	6

Drawings of genital organs were made with the aid of a drawing attachment to the microscope. Hand-cut sections of the nematodes were made in the esophageal, mid-body, and caudal regions and were examined in glycerine using Nomarski interference contrast to determine the pattern of the body ridges. Numbering of the body ridges follows the system of Lichtenfels and Pilitt (1983).

Formalin-fixed tissues were trimmed longitudinally, embedded in wax, sectioned at a thickness of 6 µm, and the sections stained with hematoxylin and eosin. Tissues fixed in glutaraldehyde were sectioned similarly and stained to demonstrate alkaline phosphatase (Bancroft and Stevens, 1977), and larger pieces of tissue were dehydrated in graded ethanols, critical-point dried using CO<sub>2</sub>, mounted, and coated with a 200-nm layer of gold. Specimens were examined in a JEOL JSM-P15 scanning electron microscope (SEM).

## Results

### Distribution within the intestine

The majority of nematodes was recovered from the first 6 m of the small intestine (Table 1). There was considerable variation in parasite distribution between individual animals, with an apparent posterior migration prior to the final molt followed by an anterior migration. Because only one lamb was killed at each examination, the significance of this observation is uncertain and the results were combined. Most of the nematodes were recovered from meters 2–4 of the small intestine (Table 1). The higher mean percentage in the remainder compared with meter 6 was due to a single animal (10 DAI) in which the majority of its nematode burden was recovered from the lower small intestine.

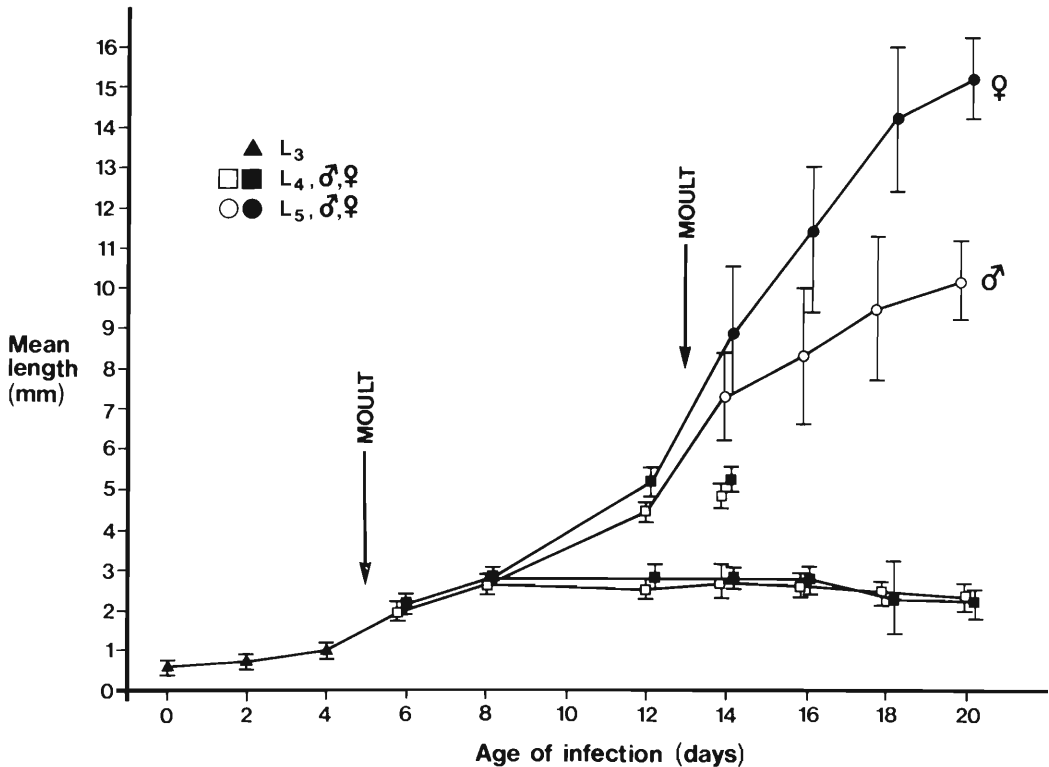


Figure 1. Growth of *Nematodirus abnormalis* in lambs experimentally infected with 60,000 larvae. Each point in main curve of development represents mean length of 20 larvae  $\pm$  SD.

#### Establishment and growth within the intestine

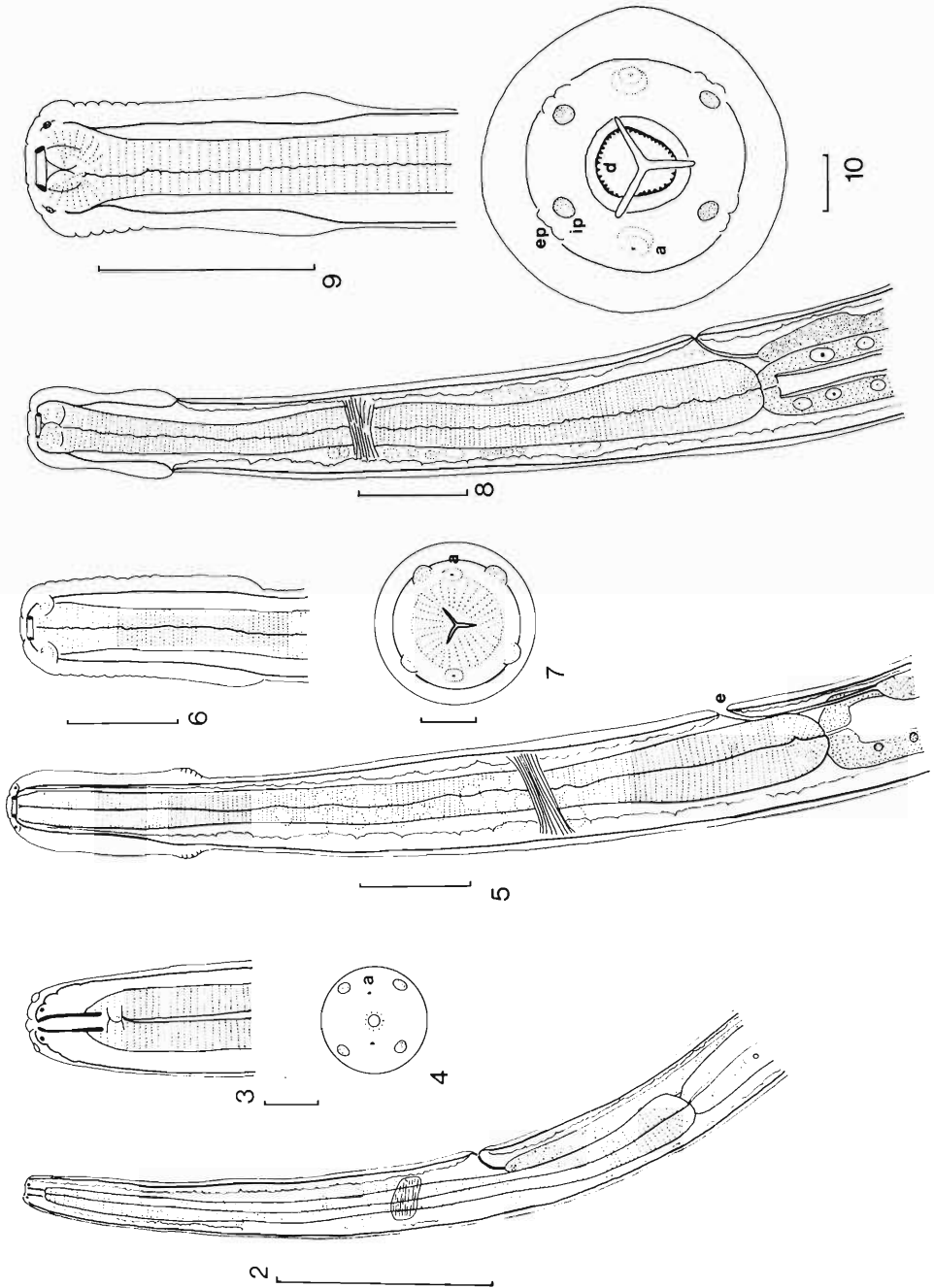
Between 10 and 75% (mean 49%) of larvae established and developed in the small intestine (Table 2). The single lamb with the lowest nematode burden (10 DAI) had the majority of its nematodes in the posterior region of the small intestine. Development within the small intestine was synchronous until after the third molt, with considerable variation (74–91% at 14–20 DAI) in the numbers of inhibited larvae occurring between individual sheep. At 12 and 14 DAI a large number of nematodes was found approaching the fourth molt (Table 2). Their percentage in the total count declined dramatically from 16 to 20 DAI as most developing nematodes became adults, however a small percentage was always present. It was not clear whether these were larvae that were developing more slowly, had been inhibited, and were resuming their development or, indeed, were inhibited prior to the final molt.

Following infection, worms grew slowly until

4 DAI. The third molt occurred between 4 and 6 DAI without any obvious prior lethargus and the final molt occurred between 12 and 14 DAI, apparently not affecting the rapid rate of growth during this period. Larvae could be differentiated sexually from 6 DAI onwards. Lack of alcohol-fixed larvae 10 DAI precluded making measurements comparable with those made on the other nematodes. The inhibited larvae failed to increase in length, and were slightly shorter in the 18 and 20 DAI samples, when compared with earlier measurements. From 12 DAI onwards, female nematodes grew at a significantly greater rate than males (Fig. 2, Table 2). Patency was achieved 18 DAI.

#### Morphogenesis

Features of the morphogenesis are summarized below, under age of infection (DAI) and larvae stage ( $L_3$ ,  $L_4$ , or  $L_5$ ). Measurements (mm) of organs are presented as the range of 10 measurements followed by the mean in parentheses.

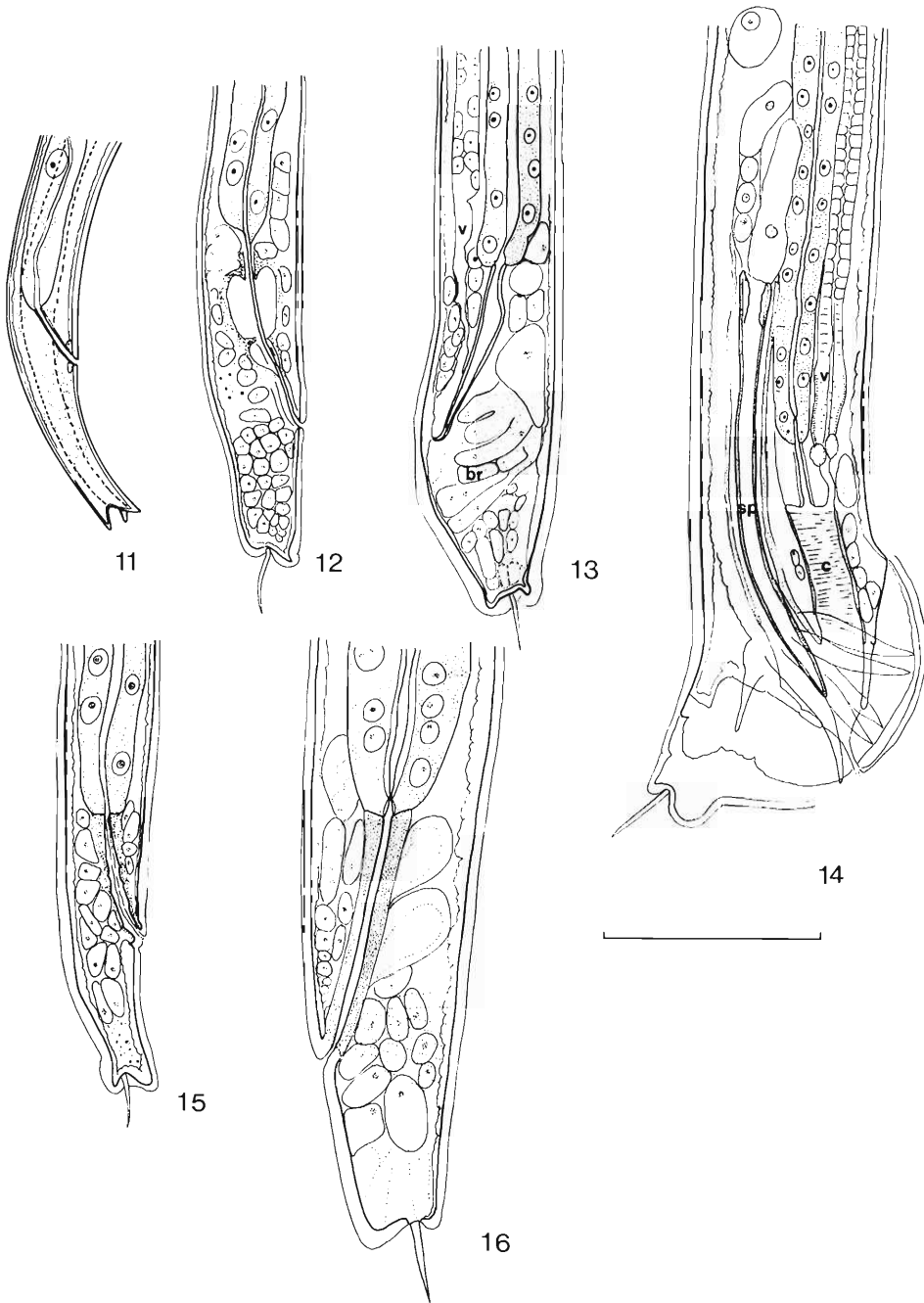


Figures 2-10. Anterior extremities of larval and adult stages of *Nematodirus abnormalis*. 2-4. Third larval stage. 2, 3. Lateral views. 4. En face view. 5-7. Fourth larval stage. 5, 6. Lateral views. 7. En face view. 8-10. Adult. 8, 9. Lateral views. 10. En face view. Legend: a, amphid; d, denticles; e, excretory pore; ep, external papilla; ip, internal papilla. Scale lines: Figures 2, 5, 8, 9, 0.1 mm; Figures 3, 4, 6, 7, 10, 0.01 mm.

0 DAI: L<sub>3</sub>: Genital primordium oval, 0.016–0.030 (0.020) × 0.006–0.012 (0.010), 6–8 cells visible (Fig. 28).

2 DAI: L<sub>3</sub>: Cephalic vesicle absent (Fig. 2);

mouth opening circular; 2 amphids and 4 submedian papillae visible in en face view (Fig. 4); buccal capsule cylindrical, longer than wide (Fig. 3); esophagus filiform; excretory pore prom-



Figures 11-16. Caudal extremities of larval and adult stages of *Nematodirus abnormalis*. 11. L<sub>3</sub>, 4 DAI. 12. L<sub>4</sub>, 6 DAI, ♂, showing vacuolation around rectum indicating formation of bursa. 13. L<sub>4</sub>, 8 DAI, ♂, showing opening of vas deferens into future cloaca and vestigial bursal rays. 14. L<sub>4</sub>, 12 DAI, ♂, about to undergo final molt, showing spicule primordium with associated cells, and cloaca. 15. L<sub>4</sub>, 6 DAI, ♀. Note lack of vacuolation around rectum. 16. L<sub>5</sub>, 15 DAI, ♀. Legend: br, bursal rays; c, cloaca; sp, spicule; v, vas deferens. Scale: 0.1 mm.

inent, anterior to esophago-intestinal junction. Two body ridges visible in lateral view (Fig. 19); extend to within 0.08 from anterior end, replaced by single lateral ala (Fig. 17). Body ridges extend onto dorsal and ventral tail projections (Fig. 11). Slight enlargement of genital primordium evident,  $0.022\text{--}0.032$  (0.024)  $\times$   $0.008\text{--}0.014$  (0.012) with up to 14 cells visible (Fig. 29). Tail composed of three subequal triangular projections, two ventral, one dorsal (Fig. 11).

4 DAI:  $L_3$ : Just prior to molt, separation of cuticle evident at anterior and posterior ends; structures such as buccal ring and tail spike of  $L_4$  faintly visible within. Two types of anlagen present: majority oval or elongated, slight increase in cell number; cells larger, nuclei prominent, anlagen  $0.060\text{--}0.100$  (0.071)  $\times$   $0.012\text{--}0.016$  (0.014); some anlagen with prominent central body  $0.070\text{--}0.090$  (0.080)  $\times$   $0.014\text{--}0.018$  (0.016) and with tapering arms  $0.050\text{--}0.140$  (0.071) at either end (Figs. 30, 31).

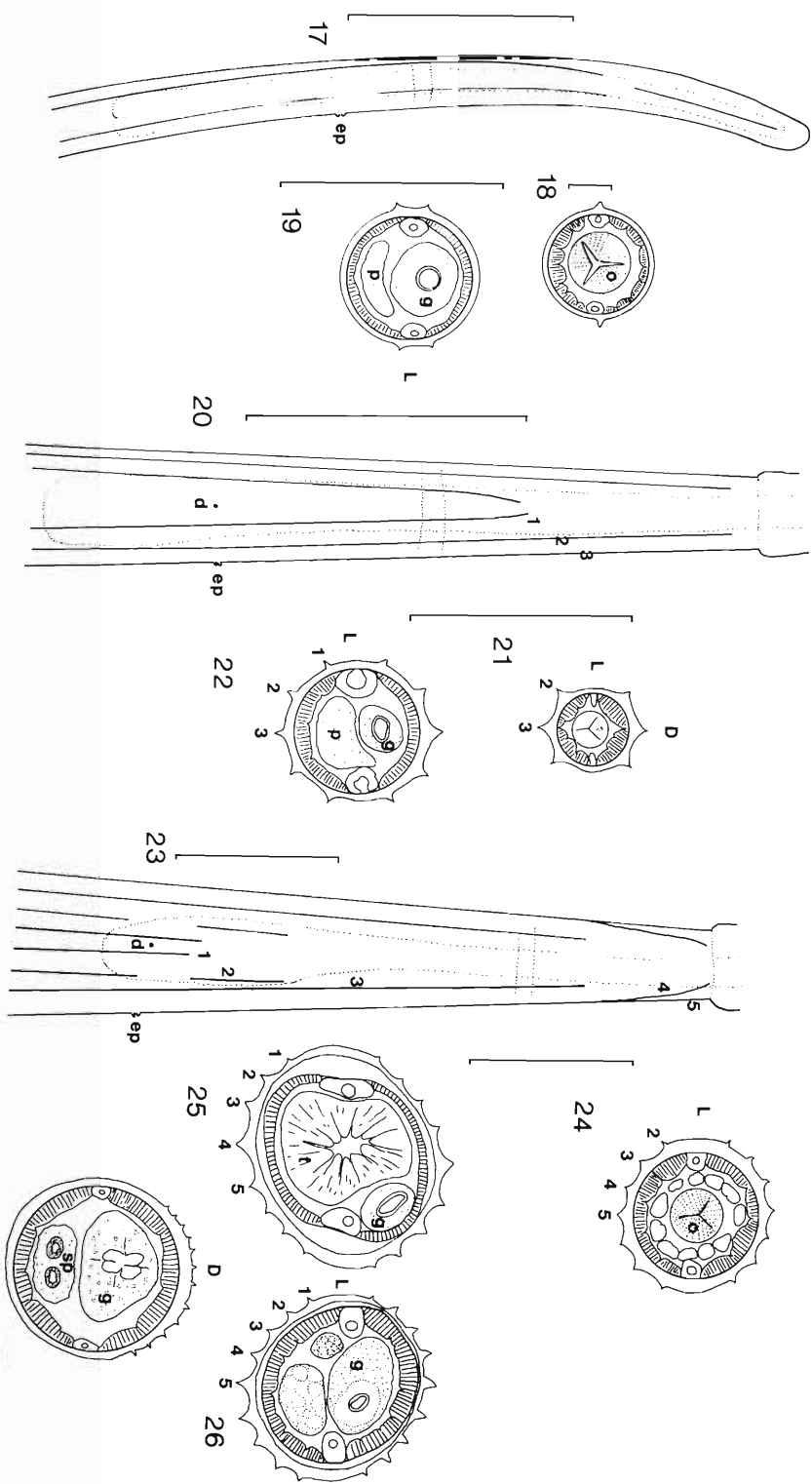
6 DAI:  $L_4$ : Cephalic vesicle present (Fig. 6); two amphids and four submedian papillae visible in en face view (Fig. 7); buccal capsule shallow, ring-like, peri-oral denticles absent (Fig. 6); esophagus claviform; excretory pore on slight eminence anterior to esophago-intestinal junction (Fig. 5). Five body ridges present on each side of body in mid-body region and posterior to it (Figs. 20, 22); ridges 1 and 5 terminate anterior to nerve ring; transverse sections anterior to termination show three ridges on each side (Fig. 21). Male anlage differentiated into elongate testis,  $0.13\text{--}0.36$  (0.25)  $\times$   $0.014\text{--}0.018$  (0.016), and narrow vas deferens  $0.090\text{--}0.160$  (0.120)  $\times$   $0.008\text{--}0.018$  (0.014); vas deferens terminates distally in two elongate cells, not reaching developing cloaca (Fig. 33). Female anlage differentiated into body of ovejector  $0.070\text{--}0.110$  (0.090)  $\times$   $0.016\text{--}0.028$  (0.021), formed of two parallel rows of cells; two vulval cells prominent on ventral aspects of ovejector; vaginae uterinae not differentiated (Fig. 32). Remainder of gonoduct undifferentiated; anterior branch,  $0.060\text{--}0.132$  (0.102) long, posterior branch reflexed,  $0.064\text{--}0.110$  (0.086) long. Tail of male and female with subtriangular dorsal and ventral lobes, and terminal spike (Fig. 12); area surrounding rectum and posterior to it occupied by large, prominent cells. Males distinguishable by slight swelling of posterior end and formation of cavity around rectum.

8 DAI:  $L_4$ : Testis greatly elongated,  $0.52\text{--}0.62$  (0.57)  $\times$   $0.012\text{--}0.020$  (0.016); vas deferens  $0.32\text{--}$

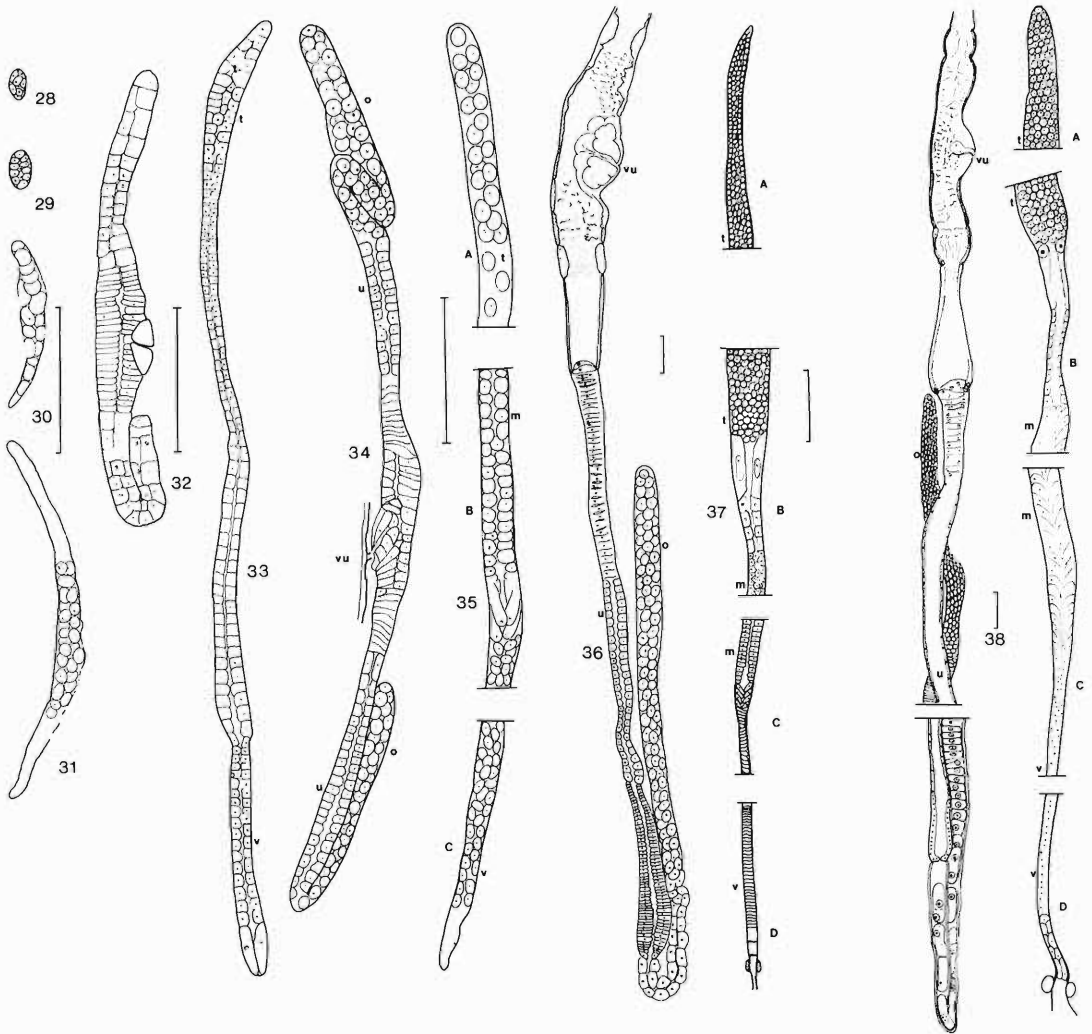
$0.52$  (0.39)  $\times$   $0.008\text{--}0.016$  (0.011) separated from testis by series of elongate, radially arranged cells; vas deferens enters developing cloaca (Fig. 35). Female genitalia: Ovejector  $0.072\text{--}0.110$  (0.090)  $\times$   $0.016\text{--}0.028$  (0.021); vulva composed of two rows of four cells; vaginae uterinae differentiated from ovejector as series of elongate, transverse cells; uteri short, lined with columnar cells; ovary distinct from uterus; anterior ovary and uterus flexed; posterior ovary and uterus reflexed,  $0.25\text{--}0.32$  (0.30) (Fig. 34). Male tail swollen, primordial bursa present with vestigial bursal rays present in some specimens (Fig. 13).

12 DAI:  $L_4$ : Larvae just about to enter final molt. Major components of testis fully developed; germinative zone  $0.68\text{--}1.28$  (0.94) long, filled with germinative cells, separated from maturation zone by two elongate cells and two short rows of large cuboidal cells; maturation zone  $0.32\text{--}0.56$  (0.42) long; vas deferens  $0.68\text{--}1.16$  (0.96) long (Fig. 37). Elements of ovejector differentiated; infundibulum  $0.24\text{--}0.38$  (0.31) long; vulva a dense mass of cells around genital opening; sphincters of infundibulum partly formed; vaginae uterinae  $0.10\text{--}0.18$  (0.12) long; posterior uterus  $0.53\text{--}0.70$  (0.68) long; oviduct differentiated; ovary  $0.38\text{--}0.72$  (0.50) long, reflexed, anterior branch reaches vagina uterina (Fig. 36). Tail of male greatly swollen (Fig. 14); lobes and rays of bursa fully developed; cloaca present with openings to intestine and vas deferens; spicule primordium present, short, poorly sclerotized; large cells anterior to tip of spicule move anteriorly as spicule elongates.

14 DAI:  $L_5$ : Cephalic vesicle present (Fig. 9); two amphids, four internal, four external cephalic papillae visible in en face view (Fig. 10); peri-oral denticles present; esophagus claviform; excretory pore just anterior to esophago-intestinal junction (Fig. 8). Nine body ridges present on each side of body in mid-body region (Figs. 25, 26); ridges 1 and 9 terminate anterior to deirid (Fig. 24); ridges 2 and 8 interrupted before termination in mid-esophageal region (Fig. 23); ridges 3 and 7 terminate posterior to cephalic vesicle; posterior end of male with 12 dorsal ridges, no ventral ridges (Fig. 27). Testis fully formed; germinative zone  $1.67\text{--}2.86$  (2.02) long; maturation zone  $0.60\text{--}0.82$  (0.73) long; vas deferens patent,  $1.28\text{--}2.16$  (1.68) long (Fig. 39). Infundibulum, vulva, sphincters fully developed; infundibulum  $0.35\text{--}0.59$  (0.50) long; posterior uterus almost entirely patent  $0.85\text{--}2.40$  (1.74) long; ovary reflexed,  $0.76\text{--}2.40$  (1.71) long, ex-



Figures 17-27. Body ridges of larval and adult stages of *Nematodinus abnormalis*. 17-19. L<sub>1</sub>-L<sub>3</sub>. 17. Lateral view of anterior end. 18. Transverse section in anterior esophageal region. 19. Transverse section in mid-body region. 20-22. L<sub>4</sub>. 20. Lateral view of anterior end. 21. Transverse section in anterior esophageal region. 22. Transverse section in mid-esophageal region. 23-27. Adult. 23. Lateral view of anterior end. 24. Transverse section in mid-esophageal region. 25. Mid-body section of male. 26. Mid-body section of female. 27. Transverse section of posterior extremity of male. Numbering of ridges following Lichtenfels and Pfitt (1983). Legend: d, detritid; ep, excretory pore; g, gut; o, esophagus; p, excretory gland; sp, spicule; t, testis; D, dorsal; L, lateral; V, ventral. Scale lines: Figure 18, 0.01 mm; Figures 17, 19-27, 0.1 mm.



Figures 28-39. Development of the genital organs of the larval and adult stages of *Nematodirus abnormalis*. 28. Genital primordium, infective larvae. 29. Genital primordium,  $L_3$ , 2 DAI. 30. Genital primordium,  $L_3$ ,  $\delta$ , 4 DAI. 31. Genital primordium,  $L_3$ ,  $\varphi$ , 4 DAI. 32. Genital system,  $L_4$ ,  $\varphi$ , 6 DAI. 33. Genital system,  $L_4$ ,  $\delta$ , 6 DAI. 34. Genital system,  $L_4$ ,  $\varphi$ , 8 DAI. 35. Genital system,  $L_4$ ,  $\delta$ , 8 DAI; (a) anterior extremity of testis, (b) junction of maturation zone with vas deferens, (c) vas deferens. 36. Posterior ramus of genital system,  $L_4$ ,  $\varphi$ , 12 DAI. 37. Genital system,  $L_4$ ,  $\delta$ , 12 DAI; (a) anterior extremity of testis, (b) junction of testis with maturation zone, (c) junction of maturation zone with vas deferens, (d) posterior region of vas deferens. 38. Posterior ramus of genital system, adult,  $\varphi$ , 14 DAI. 39. Genital system, adult,  $\delta$ , 14 DAI; (a) anterior extremity of testis, (b) junction of testis with maturation zone, (c) junction of maturation zone with vas deferens, (d) posterior region of vas deferens. Legend: m, maturation zone; o, ovary; t, testis; u, uterus; v, vas deferens; vu, vulva. Scale line: 0.1 mm.

tending to vagina uterina (Fig. 38). Spicule clear to pale yellow in color, incompletely sclerotized; bursa with inconspicuous internal bosses.

16 DAI:  $L_5$ : Testis: Germinative zone of increased length, 2.56-5.36 (3.98) long; maturation zone 0.75-1.36 (1.01) long; amoeboid sperm present in vas deferens, vas deferens 1.92-3.72 (2.69) long. Sperm present in uteri of females;

some females with partially developed eggs in uteri; infundibulum 0.50-0.58 (0.55) long; posterior uterus longer, 2.00-3.36 (2.56) long; ovary also of greater length, 3.84-5.92 (4.68) long, extending anterior to infundibulum. Spicules fully sclerotized, dark brown in color; bosses on bursa distinct.

18 DAI:  $L_5$ : Main changes involve increases



**Table 3.** Measurements (mm) of *Nematodirus abnormalis* from experimentally infected lambs (means of 10 measurements  $\pm$  SD).

Time after infection (days)	Stage of development	Sex of nematodes	Total length	Length of esophagus	Length from excretory pore to anterior end	Tail	Length from vulva to posterior end
2	L <sub>3</sub>	—	0.66 $\pm$ 0.14	0.18 $\pm$ 0.03	0.12 $\pm$ 0.01	0.05 $\pm$ 0.02	—
4	L <sub>3</sub>	—	0.98 $\pm$ 0.10	0.23 $\pm$ 0.02	0.15 $\pm$ 0.02	0.05 $\pm$ 0.03	—
6	L <sub>4</sub>	♂	2.04 $\pm$ 0.17	0.27 $\pm$ 0.02	0.21 $\pm$ 0.03	0.05 $\pm$ 0.01	—
		♀	2.13 $\pm$ 0.10	0.24 $\pm$ 0.09	0.22 $\pm$ 0.03	0.05 $\pm$ 0.01	0.4 $\pm$ 0.03
8	L <sub>4</sub>	♂	2.75 $\pm$ 0.23	0.29 $\pm$ 0.02	0.23 $\pm$ 0.02	0.06 $\pm$ 0.02	—
		♀	2.81 $\pm$ 0.16	0.28 $\pm$ 0.03	0.28 $\pm$ 0.03	0.05 $\pm$ 0.01	0.52 $\pm$ 0.06
12	L <sub>4</sub>	♂	4.38 $\pm$ 0.18	0.30 $\pm$ 0.02	0.30 $\pm$ 0.01	—	—
		♀	5.25 $\pm$ 0.44	0.32 $\pm$ 0.02	0.31 $\pm$ 0.01	0.60 $\pm$ 0.02	1.13 $\pm$ 0.08
14	L <sub>5</sub>	♂	6.94 $\pm$ 0.98	0.38 $\pm$ 0.02	0.33 $\pm$ 0.04	—	—
		♀	8.55 $\pm$ 1.40	0.40 $\pm$ 0.03	0.34 $\pm$ 0.02	0.06 $\pm$ 0.01	2.35 $\pm$ 0.55
16	L <sub>5</sub>	♂	8.9 $\pm$ 0.85	0.36 $\pm$ 0.02	0.36 $\pm$ 0.05	—	—
		♀	11.5 $\pm$ 1.67	0.39 $\pm$ 0.02	0.41 $\pm$ 0.04	0.07 $\pm$ 0.01	3.51 $\pm$ 0.82
18	L <sub>5</sub>	♂	9.5 $\pm$ 1.79	0.39 $\pm$ 0.02	0.38 $\pm$ 0.03	—	—
		♀	13.6 $\pm$ 2.04	0.42 $\pm$ 0.04	0.37 $\pm$ 0.03	0.06 $\pm$ 0.01	4.78 $\pm$ 0.85
20	L <sub>5</sub>	♂	9.4 $\pm$ 0.83	0.38 $\pm$ 0.01	0.39 $\pm$ 0.03	—	—
		♀	15.5 $\pm$ 0.52	0.42 $\pm$ 0.02	0.38 $\pm$ 0.03	0.07 $\pm$ 0.01	5.64 $\pm$ 0.69

in size of most components of genital system. Germinative zone of testis 4.16–5.60 (4.89); maturation zone 0.96–1.68 (1.28) long; vas deferens 1.92–2.56 (2.26) long; infundibulum 0.60–0.72 (0.68) long; posterior uterus 2.72–4.00 (3.18) long; ovary 8.16–12.4 (10.3) long; females fully gravid.

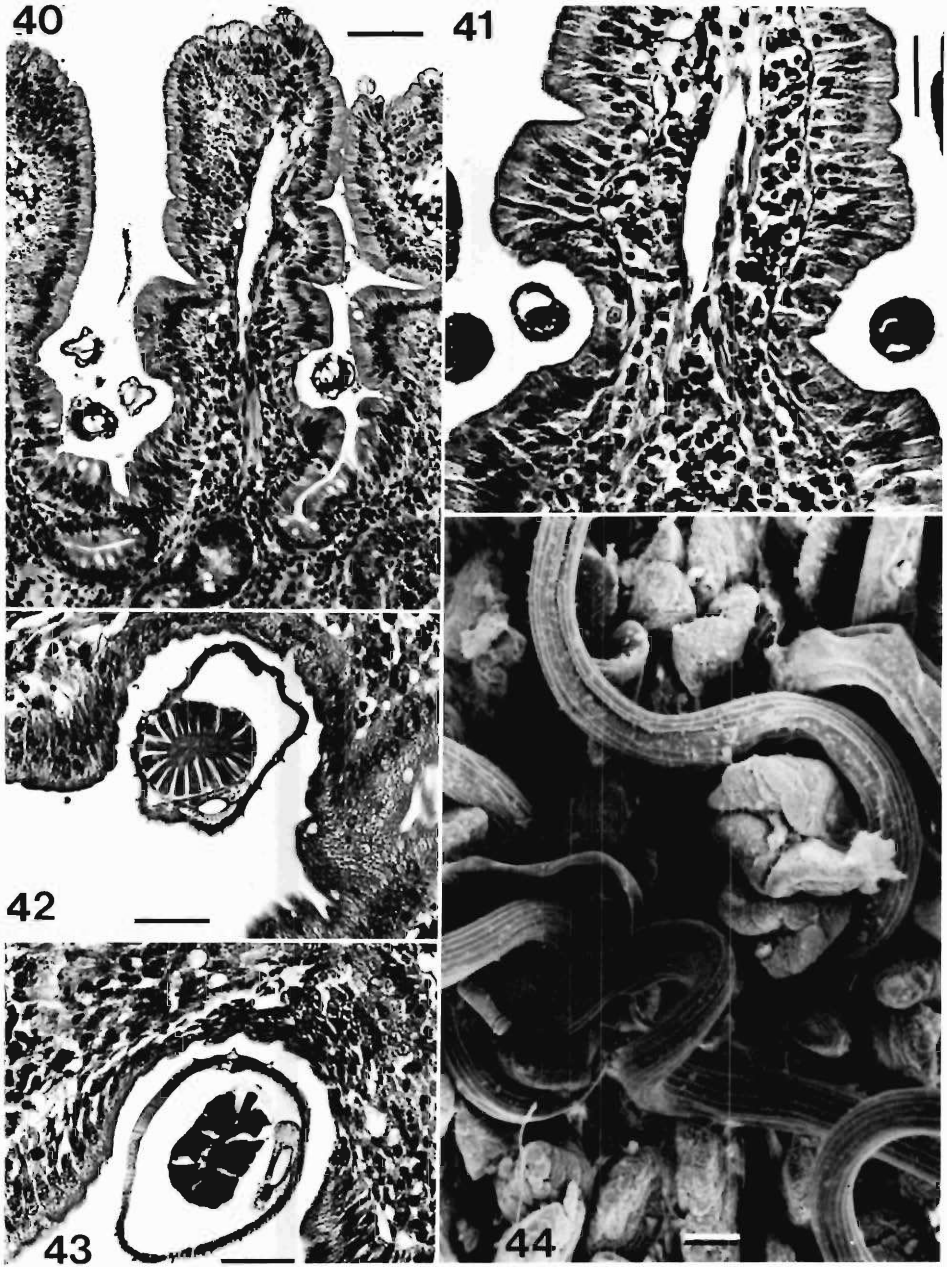
20 DAI: L<sub>5</sub>: Morphological changes limited to changes in size of genital organs; germinative zone of testis 3.44–6.00 (4.96) long; maturation zone 1.20–2.24 (1.45) long; vas deferens 1.92–2.40 (2.12) long. Infundibulum 0.53–0.73 (0.59) long; posterior uterus 2.56–3.52 (3.12) long; ovary 10.4–13.4 (11.8) long.

### Pathology

SCANNING ELECTRON MICROSCOPY: Worms were found lying coiled or uncoiled on the surface of the intestine, with some worms lying between villi towards their bases. Some worms were coiled spirally around villi causing obvious compression. A small number of nematodes had their anterior ends deeply buried in crypts. There was no evidence of villous atrophy.

HISTOLOGY: Histological changes were related to the position of nematodes. Nematodes were frequently seen coiled around a single villus (Fig. 40), resulting in compression of the epithelium at the site of attachment and a noticeable indentation in the side of the villus. In the case of

third-stage larvae, 4 DAI, the compression of the villus was out of all proportion with the diameter of the larvae (Fig. 41). The epithelial cells were reduced to a cuboidal form but there was little loss of the brush border. In infections with adult worms (Figs. 42, 43) there was similar compression of villi. The epithelium was reduced to a cuboidal form (Fig. 42) or even to a squamous epithelium (Fig. 43) with, occasionally, a break in the continuity of the epithelium. The body ridges of the nematodes made marked indentations into the epithelium (Fig. 43) and there was a complete loss of brush borders. A mild infiltration of mononuclear cells was evident in the lamina propria, and eosinophils were more prominent than in the control. In situations where the nematode body lay adjacent to the epithelium but was not constricting it (in crypts), there were no obvious changes in the epithelium. In a few instances where nematode heads were found deeply buried in a crypt, there was a marked accumulation of mononuclear cells in the lamina propria, but no obvious penetration of the epithelium by the parasite. There was some variation in villus height between individual sheep, but no marked villus atrophy was detected in infected sheep. The villi of sheep killed 16–20 DAI were marginally shorter than those in sheep killed 2–6 DAI, but were no shorter than the control. No differences were noted in the alkaline



Figures 40-44. Histological changes associated with infection of *Nematodirus abnormalis* in lambs. 40. Villus with fourth-stage larva (18 DAI) coiled around base, causing indentation of epithelium. 41. Third-stage larvae (4 DAI) coiled around villus; note extent of indentation. 42. Adult male coiled around villus causing reduction of columnar epithelium to cuboidal. 43. Adult male coiled around villus reducing epithelium to squamous layer; note indentations in epithelium produced by body ridges. 44. Scanning electron micrograph of adult *N. abnormalis* (16 DAI) coiling around tips of villi. Scale bars: 0.1 mm.

phosphatase activity in the mucosa of parasitized sheep.

### Discussion

The distribution of larval and adult *N. abnormalis* in the anterior portion of the small intestine of experimentally infected sheep compares favorably with earlier studies on *N. filicollis* and *N. battus* by Thomas (1959b) in which most nematodes were recovered from the anterior 20 ft of the small intestine, the mode being in the 10–15-ft sector. Detailed quantitative investigations of distribution during development have been made only on *N. battus* in rabbits (Gallie, 1972), and in this study nematodes were distributed throughout the small intestine and were thought to select sites for attachment and undergo development at that site. Our data suggest that *N. abnormalis* larvae attach in the first few meters of the small intestine and that during development there is a general posterior migration of nematodes, followed after the final molt by an anterior migration. Because only a single lamb was killed at each time interval, these results must be treated with some caution and the phenomenon requires further study. The combined results indicate that the majority of nematodes occur in the first few meters of the small intestine, and this result agrees with data published for other trichostrongyloid nematodes in sheep (Barker, 1974; Taylor and Kilpatrick, 1980; Beveridge and Barker, 1983).

The general pattern of development of *N. abnormalis* in sheep was similar to that described for *N. battus* in rabbits, except that the development of *N. abnormalis* was more synchronous. The first molt occurred between 4 and 6 DAI in *N. abnormalis*, whereas Gallie (1972) noted the presence of some fourth-stage larvae of *N. battus* as early as 4 DAI. Likewise, *N. abnormalis* underwent the final molt between 12 and 14 DAI, whereas *N. battus* molted over a period of 10–14 DAI. With *N. abnormalis*, the percentage of late fourth-stage larvae reached a maximum of 25% 14 DAI. In spite of minor differences, the growth patterns of the two species are quite similar, with a percentage of early fourth-stage larvae remaining inhibited. In lambs killed 16, 18, and 20 DAI, there were two obvious populations of nematodes, one of developing adults, and a second of small inhibited larvae, with only a few larvae in the late fourth and early fifth stages.

A striking difference between this and earlier studies is the high proportion of nematodes that remained inhibited. In the lambs killed 16–20 DAI, only 6–13% of the nematodes were adult, with 74–91% remaining as inhibited at the fourth stage. By contrast, in Gallie's (1972) studies on *N. battus* less than 10% of the nematodes became inhibited. However, Thomas (1959a) reported that a lamb infected with *N. filicollis* and slaughtered 15 DAI contained mainly immature nematodes, with only 25% of adults present, a level approaching that seen in our study.

The mechanisms responsible for larval inhibition in trichostrongyloid nematodes are not clear, but work with certain species has shown that exposure of the infective larvae to cold may increase the percentage that became inhibited (Armour et al., 1969; Hutchinson et al., 1972; Michel et al., 1975). The larvae used in this experiment were stored at 4°C prior to use, and this treatment may have affected their subsequent development.

The morphogenesis of *Nematodirus* spp. has not previously been described in detail as early studies utilized few hosts and were little concerned with the detail of morphological development. A number of features of the development of *N. abnormalis* are of interest. In the male, the essential features of the genital system are formed prior to the final molt. Sperm are first present in the vas deferens 16 DAI, yet the germinative zone continues to increase from a mean length of 3.98–4.96 20 DAI, whereas the vas deferens and maturation zones remain at approximately the same size. Similarly, the posterior ovary elongates in the same period from a mean length of 4.68 at 16 DAI when partially formed eggs are visible in the uteri to a mean of 11.8 long 20 DAI when fully gravid. In the case of the female genitalia, only the posterior uterus and ovary were clearly visible throughout their development and consequently they are the only parts of the genitalia described. The spicule of the male is first visible 12 DAI, though poorly sclerotized, and continues to increase in length until 18 DAI. The spicule apparently grows from its anterior, knobbed extremity, and several large, prominent cells associated with the anterior end of the spicule move anteriorly as the spicule elongates, suggesting a direct connection with spicule formation.

The body ridges of adult *Nematodirus* spp. have been studied in detail by Durette-Desset

(1979), Rossi (1983), and particularly by Lichtenfels and Pilitt (1983). Limited observations on the morphology of ridges in the fourth-stage larva have been made by Durette-Desset (1979) and Rossi (1983), but the morphogenesis of the ridges has not previously been followed through all parasitic larval stages. The fourth larval stage of *N. abnormalis* has only five ridges on each side of the body compared with nine in the adult, and in this respect resembles *N. spathiger* (Durette-Desset, 1979). However, just as ridges 1 and 2 terminate anterior to the excretory pore in the adult, leaving five ridges that continue to the cephalic vesicle, so in the fourth stage, ridge 1 terminates anterior to the nerve ring, with only ridges 2 and 3 continuing to the cephalic vesicle. In the third larval stage only two lateral ridges are present, and anterior to the nerve ring these terminate and are replaced by a single lateral ridge. Thus, through the parasitic larval stages, there is a regular increase in the number of body ridges (2–5–9), but always with a major discontinuity in the esophageal region. The recent use of body ridges in a cladistic analysis of certain *Nematodirus* spp. by Lichtenfels and Pilitt (1983) is therefore supported by the present examination of the ontogenesis of ridges in *N. abnormalis*, since both *N. abnormalis* and *N. spathiger*, which form a monophyletic pair in their cladistic analysis, have similar body ridge patterns in the fourth larval stage.

Only limited conclusions can be made about the pathogenicity of *N. abnormalis* since a single lamb was killed at each time interval and the number of nematodes established in these lambs was not high. However, the basic pathogenetic mechanisms appeared to be coiling around villi, deformation of the epithelium, but lack of penetration into the lamina propria.

By contrast, *N. battus*, the most pathogenic species known from sheep, penetrates the lamina propria as far as the muscularis mucosae in both sheep and rabbits (Thomas, 1959a; Gallie, 1972, 1973) during the fourth larval stage and is responsible for diarrhea and weight loss prior to patency. *N. spathiger*, which causes severe diarrhea in lambs (Kates and Turner, 1953; Seghetti and Senger, 1958), also undergoes an extensive migration into the lamina propria during the fourth larval stage (Kates and Turner, 1953). Data on *N. flicollis* are too scanty for comparison (Thomas, 1959a). *N. helveticus*, a parasite of cattle, is reported to burrow deeply into the in-

testinal mucosa causing severe pathological changes (Herlich, 1954; Samizadeh-Yazd and Todd, 1978).

The experimental lambs used in this experiment exhibited no clinical symptoms, no diarrhea, and no obvious weight losses. Effects on epithelium ultrastructure were not investigated, but no alteration in mucosal alkaline phosphatase activity was detected. *N. battus* by contrast causes a marked reduction in the activity of this enzyme in infected lambs (Coop et al., 1973). These observations coupled with the failure of the larval stages to penetrate the epithelium suggest that *N. abnormalis* will prove to be only mildly pathogenic in sheep.

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