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ORIGINAL PAPER

Use of fluorescent lectin binding to distinguish *Teladorsagia circumcincta* and *Haemonchus contortus* eggs, third-stage larvae and adult worms

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Abstract Lectin binding to carbohydrates on parasite surfaces has been investigated as a method of distinguishing adult worms, eggs and sheathed and exsheathed L3 of *Teladorsagia circumcincta* and *Haemonchus contortus*, economically important abomasal parasites in temperate climates. Both species were maintained as pure laboratory cultures of field isolates from New Zealand. Each of the four life cycle stages could be distinguished by the binding of at least one lectin: adult worms by *Sambucus nigra* agglutinin (SNA); eggs by peanut agglutinin (PNA), ConcavalinA and *Lens culinaris* agglutinin (LCA); exsheathed L3 by *Griffonia simplicifolia*-I lectin (GSL-I) and *Lotus tetragonolobus* lectin (LTL) and sheathed L3 by *Aleuria aurantia* lectin (AAL). The whole surface of both adult *T. circumcincta* and *H. contortus* strongly bound lectins specific for *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), mannose and fucose, but the two species could be distinguished by SNA binding only to *T. circumcincta*. Eggs could be distinguished by the binding of mannose-specific PNA to *H. contortus* and GalNAc-specific LCA and PSA to *T. circumcincta* eggs. GalNAc, GlcNAc and mannose lectins bound to the cuticle and over the excretory pores of a large proportion of sheathed L3 of both species, but only the *H. contortus* surface had exposed fucose or sialic acid complexes. The distinguishing lectin for sheathed L3 was AAL, which did not bind to *T. circumcincta*, but bound weakly to the head region of all fresh *H. contortus* and to 50–90% after 3 months storage. The cuticle of exsheathed L3 was unresponsive to all 19 lectins, and any binding was restricted to the head and tail regions. L3 exsheathed after 2–4 months storage could be distinguished by the binding of GSL-I and LTL to *H. contortus* but not to *T. circumcincta*. Lectin binding could be a useful adjunct in identifying L3, but lacked the consistency to be definitive, whereas it could be further developed as a practical method of distinguishing parasitic nematodes at other stages in the life cycle, particularly the eggs.

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Introduction

Identification of the species of parasitic nematodes contaminating pasture or established in ruminants can be an important consideration in the management of parasitism in grazing livestock. Adult worms can be readily identified, but collecting them requires euthanasia of the host. Morphological features cannot be used to distinguish eggs of all species parasitising sheep (Wilson et al. 2008; Bailey et al. 2009) and cattle (Waghorn et al. 2006); therefore, eggs are hatched in culture and the larvae are examined. Routine

identification of third-stage infective larvae (L3) is based on either morphological features or size (Lancaster and Hong 1987; McMurtry et al. 2000; van Wyk et al. 2004): *Haemonchus contortus* can be identified by a long, whip-like tail, whereas *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* are distinguished by length, the ranges of which can overlap for the two species (Lancaster and Hong 1987). A more precise method may be chemically distinguishing species by specific binding of fluorescently labelled lectins to carbohydrates on the L3 surface.

The nematode cuticle consists of collagens and cuticulins, overlaid by a lipid-rich epicuticle and an amorphous, strongly negatively charged surface coat, which is formed by oesophageal and secretory glands and secreted into the environment (Blaxter et al. 1992). The surface and secretions are highly immunogenic, due mainly to the presence of carbohydrates, rather than proteins (Dell et al. 1999; Haslam et al. 2001; Nyame et al. 2004). These antigens include CarLA, which is present on exsheathed L3 strongylid nematodes (Harrison et al. 2003a, b; Maass et al. 2007). The cuticle is a dynamic structure which is continuously replaced. To evade host immunity, antigens change at each moult (Lustigman et al. 1990; Blaxter et al. 1992; Raleigh et al. 1996), and there is active shedding of adherent antibodies (Smith et al. 1981; Politz and Philipp 1992; López de Mendoza et al. 1999), chemicals (López de Mendoza et al. 2000), cells (Badley et al. 1987; Spiegel and McClure 1995) and lectins (Page et al. 1992; Spiegel and McClure 1995). The rate of shedding is related to metabolism and movement and can be reduced by sodium azide or cooling (Smith et al. 1981; Maizels et al. 1984; López de Mendoza et al. 1999).

Lectin binding has been used to identify human filaria (Rao et al. 1987, 1989) and hookworms (Kumar and Pritchard 1992) and plant parasitic nematodes (Chen et al. 2001). It is unlikely that exsheathing L3 would enhance the use of lectins to distinguish species, as there is little or no lectin binding to the surface of most exsheathed parasitic larvae (Bowman et al. 1988; Milner and Mack 1988; Hill et al. 1991; Kumar and Pritchard 1992, 1994; Joachim et al. 1999). Although not binding to the cuticle of exsheathed L3, lectins do bind to the amphids and phasmids of some parasites (Bowman et al. 1988) and may possibly be useful in distinguishing species. In contrast to larvae, many lectins bind strongly to adult nematodes (Bone and Bottjer 1985; Taylor et al. 1986) and eggs (Taylor et al. 1986; Palmer and McCombe 1996; Colditz et al. 2002). *H. contortus* eggs can be distinguished from those of other species by a positive reaction to peanut agglutinin (PNA) (Palmer and McCombe 1996; Colditz et al. 2002; Jurasek et al. 2010). Lectin binding may provide a more reliable method of identifying the eggs and other life cycle stages of other common sheep parasites.

In the present study, a panel of 19 lectins covering a broad range of sugar specificities was tested on eggs, sheathed and exsheathed L3 and adult *H. contortus* and *T. circumcincta*. Binding was enhanced by cooling or freezing the parasites to inhibit lectin shedding. Specific lectins were identified which could distinguish these two species of abomasal parasite at all four life cycle stages.

Materials and methods

Parasites

Laboratory strains of *T. circumcincta* and *H. contortus*, originally obtained as field isolates in New Zealand, were maintained by routine infection of nematode-free sheep and culture of L3 from faeces. Cultures were checked for purity from larval size and tail structure and adult worm size, colour and morphology. Individual parasites used for lectin binding were also examined. Sheathed L3 were cultured from faeces of sheep and stored in deionised water at 4°C for the former and at 10°C for the latter species. L3 stored for more than 3 months were Baermannised to remove inactive worms before suspending in 10 mM HEPES buffer pH 7.5. Lectin binding to L3 *H. contortus* was assessed after storage for 14 days (n=2) or 3 months (n=3) and to L3 *T. circumcincta* after 3 months (n=3).

L3 were exsheathed by exposure to CO_2: *H. contortus* after 2 or 4 months storage and *T. circumcincta* after 4 or 10 months. L3 were washed in phosphate-buffered saline (PBS), gassed with 100% CO_2 for 20 s and shaken at 100 strokes per minute at 40°C for 15 min, then gassed a second time for 2 min and shaken for 90 min. The percent exsheathed was assessed and, if less than 75%, the L3 were incubated for a further 5–12 h at 40°C.

Adult worms were recovered from abomasal contents 21 days after infection, using the technique of Simpson et al. (1999). Briefly, abomasal contents were mixed 2:1 with 3% agar and the solidified agar blocks incubated at 37°C in a saline bath. Clumps of parasites were removed from the saline soon after emergence and placed in PBS at 37°C until they were resuspended in HEPES buffer for lectin binding.

Eggs were collected from 70–100 adult worms after 48 h incubation in PBS at 37°C in 5% CO_2 in air. *H. contortus* eggs were 75–85 μm and *T. circumcincta* eggs 85–100-μm long. After manual removal of the worms, the tubes were centrifuged at 3,000×g for 1 min, and the concentrated eggs were resuspended in HEPES buffer.

Lectins

The binding characteristics of the 19 biotinylated lectins used (Vector Labs, Burlingame, USA) and their divalent cation

requirements are shown in Table 1. The lectins were reconstituted in 1-ml sterile Milli-Q® H_2O and stored in the dark at 4°C. Lectins were used in 10-mM HEPES buffer pH 7.5 in the presence of added 0.1 mM Ca^{2+} for 13 lectins (plus 0.01 mM Mn^{2+} for 4 lectins), as recommended by the supplier (Table 1). The specificity of lectin binding was confirmed by pre-incubation of 11 lectins which bound to *H. contortus* adult worms with recommended blocking sugars (Table 1). WGA binding was reduced and the other ten lectins no longer bound to the worms.

Lectin binding

All 19 lectins were tested on each parasite preparation. The metabolic activity of sheathed or exsheathed L3 was inhibited by freezing a suspension of larvae in PBS in liquid nitrogen for 1 min. After thawing, about 1,000 larvae per milliltre were suspended in PBS, washed by centrifuging at 3,000×*g* for 30 s and resuspended in 400 μl lectin binding buffer. About 1,500 eggs or three to five adult worms which had been chilled and straightened also were placed in 400-μl buffer. Cations and 20 μg/ml lectin were added, and the parasites were incubated for 1 h in the dark at room temperature. After washing three times in buffer, 1.5 μl of 1 mg/ml fluorescently tagged avidin (Alexa Fluor® 546, Invitrogen, USA) was added, and the tubes were again incubated in the dark for 1 h. After three washes in buffer, the parasites were resuspended in 50-μl buffer. Either a larval or egg suspension (15 μl) or single worms in buffer was placed on a microscope slide and covered with a cover slip. Samples were examined using either transmitted light or via a 540-nm excitation / 605-nm emission filter (Chroma Technology, Brattleboro, USA) using a Xenon lamp for fluorescence excitation. Photographs were captured with a DS-Fi1-U2 digital camera (Nikon, Tokyo, Japan). At least 20 worms or eggs were located under bright field illumination, then viewed and photographed under fluorescence excitation. Lectin binding intensity and location (head or tail region or cuticular surface) were recorded: + very weak fluorescence, ++ weak fluorescence, +++ moderate fluorescence, ++++ bright fluorescence.

Results

Eggs

H. contortus and *T. circumcincta* eggs could be distinguished by PNA, which bound only to *H. contortus* eggs,

Table 1 Carbohydrate-binding structures, blocking sugars and cation requirements of the lectins used to bind to abomasal parasites

Lectin		Carbohydrate-binding structures	Blocking sugar	Cation
DBA	*Dolichos biflorus* agglutinin	α-GalNAc	GalNAc[a]	Ca^{2+}
SBA	Soybean agglutinin	Terminal α/β-GalNAc or Gal	GalNAc[a]	Ca^{2+}
PNA	Peanut agglutinin	Gal (β-1,3) GalNAc	Gal	Ca^{2+} Mn^{2+}
GSL-I	*Griffonia simplicifolia*-I lectin	α-GalNAc; α-Gal	Gal/GalNAc[a]	Ca^{2+}
MPL	*Maclura pomifera* lectin	α-GalNAc	Gal[a]	
BPL	*Bauhinia purpurea* lectin	Gal (β-1,3) GalNAc; α-GalNAc	Lactose[a]	
PTL-II	*Psophocarpus tetragonolobus*-II	α-GalNAc	GalNAc	
RCA	*Ricinus communis* agglutinin	Terminal Gal or GalNAc	Gal or lactose[a]	
WGA	Wheat germ agglutinin	Terminal GlcNAc or chitobiose	GlcNAc or chitin hydrolysate[a]	Ca^{2+}
GSL-II	*Griffonia simplicifolia*-II lectin	α/β-GlcNAc on non-reducing terminus	GlcNAc[a]	Ca^{2+}
ConA	ConcavalinA	α-Man; α-Glu	α-Methylmannoside/α-methylglucoside[a]	Ca^{2+} Mn^{2+}
LCA	*Lens culinaris* agglutinin	α-Man on N-acetylchitobiose cores containing α-Fuc	α-Methylmannoside/α-methylglucoside	Ca^{2+} Mn^{2+}
PSA	*Pisum sativum* agglutinin	α-Man on N-acetylchitobiose cores containing α-Fuc	α-Methylmannoside/α-methylglucoside[a]	Ca^{2+} Mn^{2+}
UEA-I	*Ulex europaeus* agglutinin I	α-Fuc	Fuc	Ca^{2+}
LTL	*Lotus tetragonolobus* lectin	α-1,2 Fuc; Fuc (α-1,3) GlcNAc	Fuc	Ca^{2+}
AAL	*Aleuria aurantia* lectin	Fuc α-1,6-GlcNAc; Fuc (α-1,3) N-acetyllactosamine	Fuc[a]	
SNA	*Sambucus nigra* agglutinin	α-2,6 or 2,3 Sialic acid on terminal Gal	Lactose	Ca^{2+}
MAL	*Maackia amurensis* lectin-II	α-2,3-Sialic acid	Human glycophorin	
PHA E + L	*Phaseolus vulgaris* agglutinin	Oligosaccharides; bi-/tri-antennary Gal(β-1,4) GlcNAc (1,2) Man(α-1,6)	–	Ca^{2+}

[a] Blocking sugars tested with lectins on adult *H. contortus*

and by LCA, which bound only to *T. circumcincta* eggs (Table 2). These two lectins bound to the whole surface (Fig. 1a), whereas ConcavalinA (ConA) binding produced a bright outline on *T. circumcincta* eggs. PSA did not bind to *H. contortus* eggs, but bound to 90% of *T. circumcincta* eggs. Only MPL bound consistently to eggs of both species; many lectins did not bind at all, whilst small numbers of eggs were positive for BPL, SBA and WGA (Table 2).

Adult worms

Adult *T. circumcincta* and *H. contortus* could be distinguished by SNA, which bound to *T. circumcincta*, but not to *H. contortus* (Table 2). PTL II, UEA-I and MAL did not bind to the cuticle of either species, although UEA-I bound to the mouth and anal pore of all *T. circumcincta*. AAL bound weakly to 10–30% of *T. circumcincta*, but more strongly to all *H. contortus*, whilst the reverse was the case with LTL (Table 2). The other 13 lectins all bound consistently to the entire cuticular surface and head of both species (Fig. 1c, d) with generally similar intensities, slightly more strongly to *H. contortus* than to *T. circumcincta*, except for ConA and PSA (Table 2).

Sheathed L3

The only lectin which could be used to distinguish 3 month-old sheathed L3 *H. contortus* and *T. circumcincta* with reasonable consistency was AAL, which bound only to the head region of *H. contortus* (Table 3). All fresh *H. contortus* bound AAL weakly (++), but there was weaker (+) and less than 100% binding (mean 80%) in some populations after 3 months storage. SNA also bound only to *H. contortus*, but its usefulness was limited, as binding was very weak, and with storage time, the binding site changed from the head to the cuticular surface. Few lectins bound to most or all individuals of a species and those that did were positive for both parasites. Binding was usually to the surface and/or head region (Fig. 2a) and only DBA bound to the tail of fresh L3 *T. circumcincta* in the region of the secretory pores and anus. Any surface binding generally was inconsistent, patchy and weak, except for MPL, which bound more consistently to a greater surface area. Storage had a marked effect on the location of lectin binding to sheathed L3 (Table 3).

Exsheathed L3

T. circumcincta and *H. contortus* L3, exsheathed after 2–4 months storage, could be distinguished by GSL-I binding to *H. contortus*, but not to *T. circumcincta* (Table 4). Binding intensity was assessed as either very weak (+) or weak (++). Lectins generally did not label the cuticle, except for weak binding to *T. circumcincta* by ConA to 35% of L3 exsheathed after 10 months storage and of DBA to 10% exsheathed after 3 (but not 10) months. PTL-II, UEA-I, MAL and PHA E+L did not bind to either species, whereas the other 16 lectins bound to one or both species to three structures in the head (mouth and sensory pores or

Table 2 Intensity of binding of lectins to eggs (n=2) and the cuticle of *H. contortus* and *T. circumcincta* adult worms (n=3). Percentages are shown when <100% of individual eggs or worms bound the lectin. Intensity was assessed as negative or on a scale from + to ++++

Lectin	*H. contortus* Adult worms	*T. circumcincta*	*H. contortus* Eggs	*T. circumcincta*
DBA	++	++	neg	neg
SBA	++++	++++	++ (20%)	++ (5%)
PNA	++++	+++	+++	neg
GSL-I	+++	++	neg	neg
MPL	+++	+++	++++	+++
BPL	++++	+++	neg	+++ (5%)
PTL-II	neg	neg	neg	neg
RCA	++++	+++	neg	neg
WGA	++++	++++	++ (20%)	++ (5%)
GSL-II	+++	++	neg	neg
ConA	+++	++++	neg	+++
LCA	++++	+++	neg	+++
PSA	+++	++++	neg	++ (90%)
UEA-I	neg	neg[a]	neg	neg
LTL	+ (10–30%)	++	neg	neg
AAL	+++	+ (10–30%)	neg	neg
SNA	neg	++	neg	neg
MAL	neg	neg	neg	neg
PHA E + L	+++	++	neg	neg

[a] ++ binding to mouth and anal pore

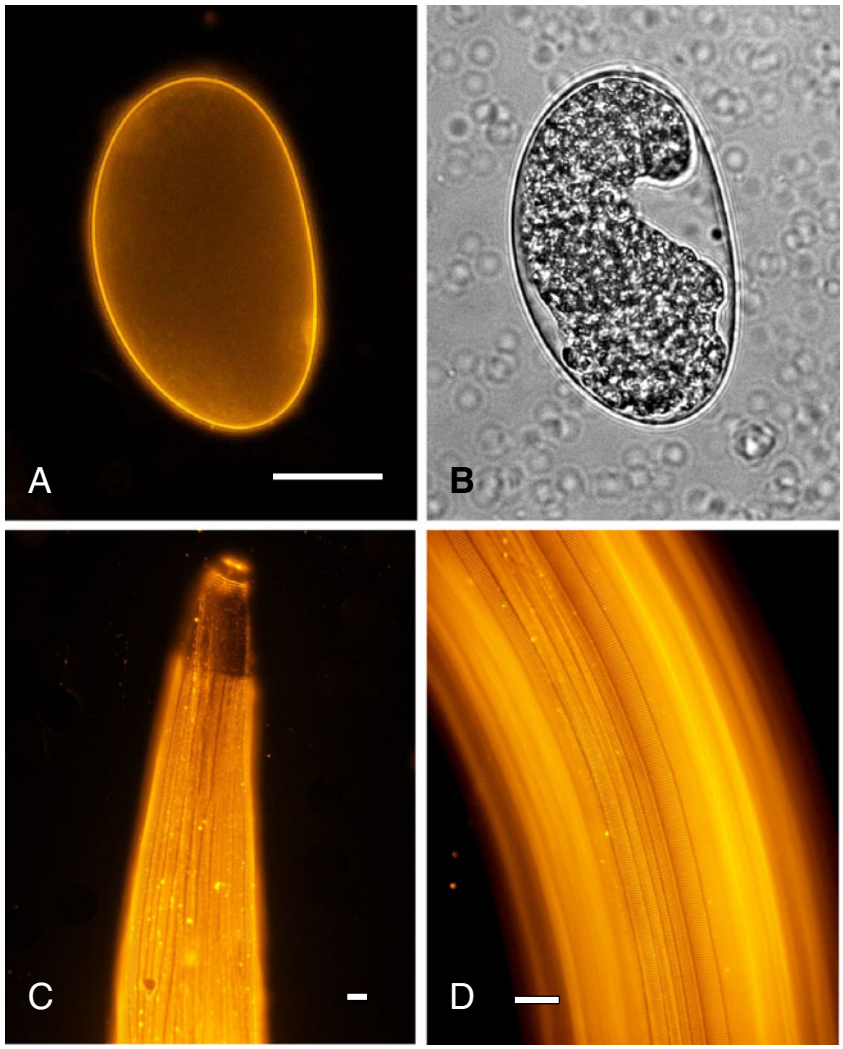

Fig. 1 Binding of streptavidin-labelled lectins to an *H. contortus* egg and *T. circumcincta* and *H. contortus* adult worms. **a** Fluorescence image of binding of peanut agglutinin (PNA) to an *H. contortus* egg with the corresponding bright field image (**b**). **c** Binding of wheat germ agglutinin (WGA) to the head and **d** *Pisum sativum* agglutinin (PSA) to the cuticle of an adult *T. circumcincta*. *Bar*=25 μm

amphids) or four positions on the tail in the region of secretory pores and anus. ConA bound to the new cuticle during the exsheathing process (Fig. 2c, d), but the cuticle rapidly ceased binding lectins. There was increased binding of some lectins with storage from 2 to 4 months for *H. contortus* and 3 to 10 months for *T. circumcincta*, but reduced or less consistent binding with other lectins.

Discussion

Each of the four life cycle stages of *H. contortus* and *T. circumcincta* could be distinguished by at least one lectin: adult worms by SNA; eggs by PNA, ConA and LCA; exsheathed L3 by GSL-I and sheathed L3 by AAL. Larvae were the most difficult to distinguish with lectins, as binding was weak and generally inconsistent and the structures bound changed and often became less intense with storage time of the larvae. Despite these limitations, lectin binding still could be useful when examining fresh cultures. Stage-specific lectin binding was marked, consistent with the changing expression of carbohydrates during the life cycle of many parasites, including *H. contortus*, *T. colubriformis* and *Ascaris suum* (Bone and Bottjer 1985; Hill et al. 1991). Changing antigens is considered to be a strategy for evasion of the host immune response (Maizels et al. 1993).

Eggs

H. contortus and *T. circumcincta* eggs could be distinguished by GalNAc- and mannose-specific lectins, respectively: PNA bound only to *H. contortus* and LCA only to *T. circumcincta* eggs (and PSA to 90% of eggs). PNA previously has been recognised as a specific lectin for *H. contortus* eggs (Palmer and McCombe 1996; Colditz et al. 2002; Jurasek et al. 2010) and has been used to distinguish them from eggs of other strongyles in faecal samples (Jurasek et al. 2010). As in the present study, Palmer and McCombe (1996) and Jurasek et al. (2010) observed binding of PNA only to *H. contortus* eggs using fluores-

Table 3 Intensity of binding of lectins to *H. contortus* and *T. circumcincta* sheathed L3 stored for 14 days (n=2) or 3 months (n=3)

Lectin	*H. contortus* 14 days	*H. contortus* 3 months	*T. circumcincta* 3 months
DBA	H ++ T ++	H+(50–80%) S+(0–30%)	H ++ (0–40%)
SBA	S +	S+(0–10%)	S+(30–70%) H+(20–50%)
PNA	S + H+(80–100%)	S+(10–60%)	S+(0–50%) H+(0–50%)
GSL-I	S ++ (90–100%) H+(30%)	S +	S+(30–90%)
MPL	H ++ S ++ (60%)	S + H+(0–20%)	S ++ (90–100%) H+(70%)
BPL	H + S+(40%)	H+(50%)	S+(0–10%)
PTL-II	S ++ (30%)	S+(10–50%)	neg
RCA	neg	S+(30%)	S ++ (40–60%)
WGA	H+(90–100%)	S ++ (90–100%)	S ++ (60–90%) H ++ (60–80%)
GSL-II	S+(20%)	S+(60–100%)	S+(0–10%)
ConA	S ++	S +	S ++ (40–80%) H ++ (30%)
LCA	S ++ (90–100%) H+(80–90%)	S+(80–100%) H+(80–100%)	S+(0–80%)
PSA	S+(70–100%)	S+(10–50%)	neg
UEA-I	S ++ (60%)	neg	neg
LTL	neg	H+(10–70%)	neg
AAL	H ++	H+(50–100%)	neg
SNA	H +	S+(90%)	neg
MAL	neg	neg	neg
PHA E + L	S +	neg	neg

Percentages are shown when <100% of L3 bound the lectin. The binding site is indicated as *S* surface, *H* head region, *T* tail region. Intensity was assessed as negative or on a scale from + to ++++

cence microscopy, and Colditz et al. (2002) reported weak binding to *T. circumcincta* eggs using flow cytometry. None of these other studies included LCA or PSA, which bound only to *T. circumcincta* eggs.

The distinguishing lectins for *T. circumcincta* eggs (ConA, LCA and PSA) all bind mannose residues, the latter two specifically to mannose in core-fucosylated N-glycans (Tateno et al. 2009), whereas the specific lectin for *H. contortus* eggs (PNA) recognises Gal (β-1,3)GalNAc structures. A few *T. circumcincta* eggs bound BPL (Gal (β-1,3)GalNAc- or α-GalNAc specific). In contrast to species-specific structures, there appear to be shared epitopes, as all eggs of both species bound MPL (α-GalNAc) and a small proportion also were recognised by SBA (terminal α/β-GalNAc or Gal) and WGA (GlcNAc). Neither species bound sialic acid- or fucose-specific lectins. In the present study, LCA did not visibly bind to *H. contortus* eggs, although it can bind P-glycoproteins (Kerboeuf et al. 2002) located in lipid rafts (Riou et al. 2010). As this binding could not be detected microscopically, but only by flow cytometry (Kerboeuf et al. 2002), the use of LCA or the other two mannose-binding lectins could still be used to distinguish *T. circumcincta* eggs.

Adult worms

Adult *T. circumcincta* and *H. contortus* could be distinguished by binding of SNA only to *T. circumcincta*. The ligand was most likely α-2,6 linked sialic acid on terminal galactose, as MAL does not bind to 2,3 sialic acids, the other structures recognised by SNA. There was intense binding to the cuticle and head of all adult worms by lectins with a range of carbohydrate specificities. Exceptions were the fucose-binding lectins AAL and LTL: AAL bound to all *H. contortus* adult worms but only to 10–30% of *T. circumcincta*, while the reverse was true for LTL (Table 2).

Lectin binding to adult *H. contortus* has been reported by Bone and Bottjer (1985), but there appear to be no similar studies on *T. circumcincta*. Some of the 19 lectins used in the present study were also used by Bone and Bottjer (1985), although many lectins were not included in both experiments. There was strong binding of some lectins with affinities for

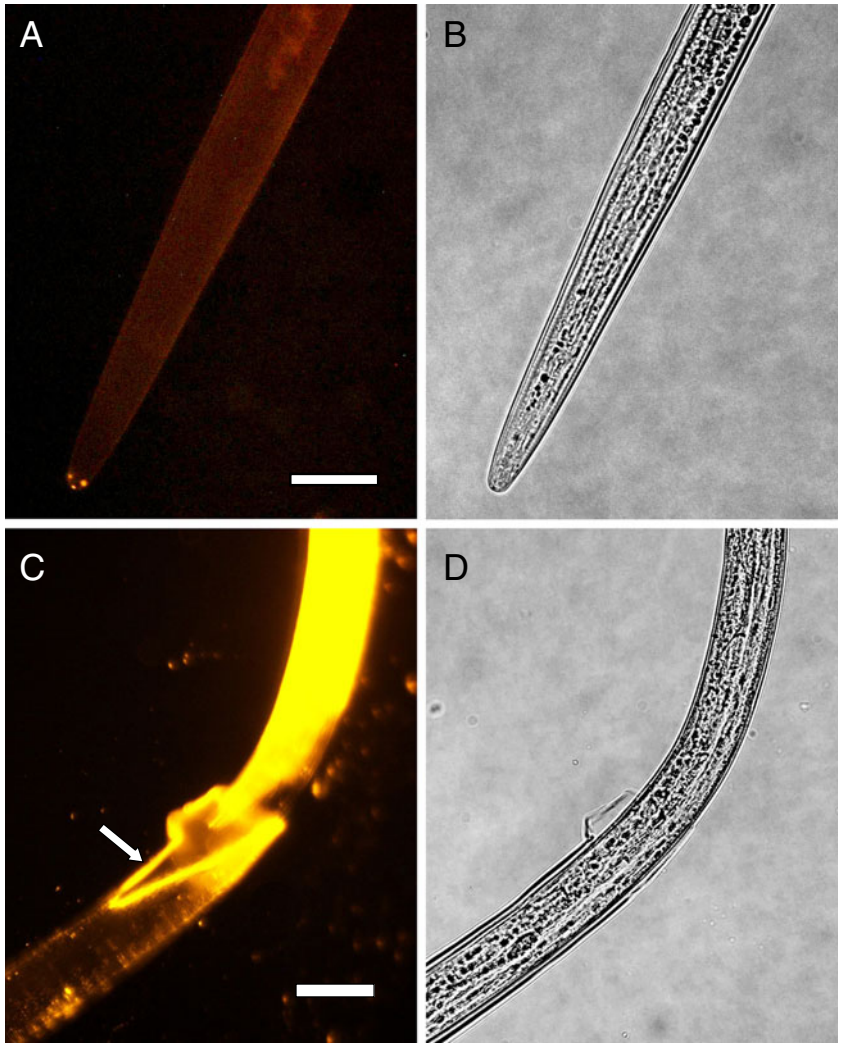

Fig. 2 Binding of streptavidin-labelled lectins to *H. contortus* and *T. circumcincta* L3. **a** Fluorescence image and **b** bright field image of binding of *Aleuria aurantia* lectin (AAL) to the head region of a sheathed L3 *H contortus*; **c** fluorescence image and **d** bright field image of binding of ConcavalinA to the cuticle of an L3 *T. circumcincta* during the process of exsheathing (the *arrows* indicate the opening of the sheath). *Bar*=25 μm

GalNAc, Gal and GlcNAc to adult *T. circumcincta* (Table 2) and also to *H. contortus* in both studies; however, PTL, which also recognises some α-GalNAc-containing structures, did not bind to either worm. Bone and Bottjer (1985) did not detect mannose on *H. contortus*, whereas ConA, LCA and PSA all bound strongly to both species in the present study. Whilst these contrasting results may be caused by the strain of parasite, it also could be caused by the differing in vitro conditions in the two studies, such as length of incubation with lectins, different buffers and cation concentrations and whether there was inhibition of lectin shedding. Fucose was present on both worms (positive for LTL and AAL) (Table 2), although not detectable in either study by UEA-I, which was the only fucose-specific lectin used by Bone and Bottjer (1985). In neither study, did *H. contortus* bind sialic acid-specific lectins: SNA and MAL in the present study (Table 2) and *Limulus polyphemus* agglutinin (LPA) in Bone and Bottjer (1985). In contrast, adult *T. circumcincta* bound SNA, the differentiating lectin for adult worms of the two species.

Larvae

Despite weaker binding than by adult worms, both sheathed and exsheathed L3 could be distinguished by specific lectins: exsheathed L3 by GSL-I and sheathed L3 by AAL. Sheathed *H. contortus* and *T. circumcincta* L3 bound many lectins to the cuticle as well as specialised areas in the head and tail regions, presumably due to secretions from the excretory pores, as is also the case for sheathed *Toxocara canis* (Bowman et al. 1988) and *T. colubriformis* (Milner and Mack 1988). The use of lectins to distinguish sheathed L3 was limited by the specific lectin binding only to the head region and the changing lectin binding patterns with prolonged storage of L3 (Table 3). Inconsistency of lectin binding with age of larvae could present difficulties in using this technique to distinguish larvae on pasture. All lectins which bound to the cuticle recognised both species, although none bound to all *T. circumcincta*. Sheathed L3 of the two species could be distinguished by AAL, which did

Table 4 Intensity of binding of lectins to *H. contortus* and *T. circumcincta* exsheathed L3 (n=3) after storage for 2 or 4 months and 3 and 10 months, respectively

Lectin	*H. contortus*		*T. circumcincta*	
	2 months	4 months	3 months	10 months
DBA	H, T ++ (90–100%)	H ++	H ++ (90%) T+(90%) S+(10%)	H+(20%)
SBA	H, T ++ (70–100%)	H+(70–100%)	H, T ++	neg
PNA	H+(50%)	neg	H ++ (20–80%)	H+(50%)
GSL-I	H, T ++ (80–90%)	H, T ++	neg	neg
MPL	H, T ++	H ++	H, T ++	H, ++ T +
BPL	H, T ++	H, T ++	H, T ++	H, T ++
PTL-II	neg	neg	neg	neg
RCA	H, T ++	H ++ T ++ (50%)	H, T ++	H+(10%)
WGA	H, T ++	H, T +	H, T ++	H, T ++
GSL-II	H+(50–70%) T ++ (50–70%)	H +	H, T ++	H ++ (20%)
ConA	H, T ++ (80–90%)	H ++ (50%) T ++ (80–90%)	H ++	H ++ S+(10–40%)
LCA	H+(50–70%)	H ++ T +	H ++ (80%) T+(50%)	neg
PSA	neg	H, T +	neg	H+(60%) T+(20%)
UEA-I	neg	neg	neg	neg
LTL	H+(30–50%)	H ++	neg	neg
AAL	T +	H ++	H ++ (90%)	H ++
SNA	neg	neg	H, T +	neg
MAL	neg	neg	neg	neg
PHA E + L	neg	neg	neg	neg

Percentages are shown when <100% of L3 bound the lectin. The binding site is indicated as *S* surface, *H* head region, *T* tail region. Intensity was assessed as negative or on a scale from + to ++++

not bind to *T. circumcincta*, but bound weakly to the head region of all fresh *H. contortus* and to about 80% after 3 months storage. SNA could be used as an adjunct, despite the site of binding changing from the head at 14 days to the surface of nearly all *H. contortus* after 3 months, as in neither case did it bind to any *T. circumcincta*. The specificities of the lectins used indicated that GalNAc, GlcNAc and mannose were present on sheathed L3 of both species, but only the *H. contortus* surface had exposed fucose or sialic acid complexes.

In contrast to sheathed larvae, the cuticle of exsheathed L3 did not bind any of the 19 lectins tested, but there was relatively consistent, weak binding in the head and tail regions. GSL-I bound only to *H. contortus* and could be used to distinguish them from *T. circumcincta*. Although the cuticles of exsheathed parasitic larvae typically do not bind lectins (Milner and Mack 1988; Kumar and Pritchard 1992, 1994; Joachim et al. 1999), they contain very antigenic carbohydrates, such as Gal-rich CarLA to which immune sheep produce mucosal antibodies (Harrison et al. 2003a, b, 2008; Maass et al. 2007). One *H. contortus* L3 observed in the process of exsheathing (Fig. 2c) bound ConA to the newly exposed cuticle, perhaps to secretions which are subsequently lost. A PAS-positive substance is secreted between the *H. contortus* L2 and L3 cuticles prior to exsheathing and accumulates particularly in the large spaces at the head and tail and where the body bends (Bird 1990). The carbohydrates on the surface after exsheathing may be unusual sugars or polymers which lectins do not recognise.

Conclusions

Lectin binding can be used to distinguish each of the life cycle stages of *H. contortus* and *T. circumcincta* and may be developed into a useful diagnostic tool. This technique would be most useful for eggs and larvae, as the species of adult worms can be determined morphologically. It was hoped that lectins might be used to identify larval species on pasture; however, the general reduction in lectin binding in stored L3 also may be a limitation in applying this technique to field samples. There could be a role in

examining fresh L3 cultures for contamination by *H. contortus* or as an adjunct to morphological identification of similar species. The most promising application could be in rapid identification of eggs in faecal samples by avoiding the necessity for egg hatching, provided the specificity of the lectins for laboratory strains of parasites can be verified in local field isolates.

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