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Use of fluorescent lectin binding to distinguish eggs of gastrointestinal nematode parasites of sheep

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ABSTRACT

The binding of a panel of 19 lectins to carbohydrates on the eggs of economically important nematode parasites of sheep has been assessed as the basis of a rapid test to distinguish parasite eggs, at least at the genus level. A total of six lectins can be used to identify eggs of six nematode parasites: peanut agglutinin (PNA) for *Haemonchus contortus*; *Lens culinaris* agglutinin (LCA) for *Teladorsagia* sp; *Aleuria aurantia* agglutinin (AAL) for *Trichostrongylus* sp; *Psophocarpus tetragonolobus*-II (PTLII) for *Nematodirus* sp; *Lotus tetragonolobus* lectin (LTL) for *Cooperia* sp and wheat germ agglutinin (WGA) for *Chabertia ovina*. For WGA, LCA and LTL, weak binding was also observed to *H. contortus* and *Teladorsagia* sp, *Trichostrongylus* sp and *C. ovina* eggs, respectively. Nematode eggs in two faecal samples were identically identified by both lectin binding and PCR, except for PCR identification of the eggs of *Nematodirus* sp, as these did not lyse. Lectins bound best to *H. contortus* eggs extracted from fresh faecal samples or after storage at room temperature or 4 °C for up to 24 h, but eggs stored at −20 °C or −80 °C did not bind PNA.

1. Introduction

Identification of the species of parasitic nematode infecting grazing ruminants can be important for choosing the best management of parasitism on individual farms. Adult worms and L3 can be readily identified from their morphological features, however, the eggs of many parasitic nematodes, particularly Strongyles, cannot be distinguished from one another based on size and morphology (Waghorn et al., 2006; Wilson et al., 2008; Bailey et al., 2009).

Although adult worms can be collected post-mortem and L3 occasionally from pasture samples, routinely, the parasites present in livestock are identified by culture for at least 7 days of faecal eggs to L3 (Bisset et al., 2014). Identification of larvae after culture requires skilled technicians and is laborious and time-consuming. Recently, accurate molecular-based methods have been developed to identify nematode species or genera (Roeber et al., 2012a,b; McNally et al., 2013; Bisset et al., 2014), but these also requires specialised equipment and skills. Development of a fast and inexpensive means of identifying the eggs in faecal samples is highly desirable. The present study explored the potential for developing a simple, rapid test to distinguish the eggs of economically important nematode parasites of sheep, based on the binding of specific lectins to carbohydrates on the egg surface.

Lectins are carbohydrate-binding proteins that are highly specific for monosaccharides or groups of sugars in a particular conformation within a large carbohydrate molecule. Binding of specific lectins to carbohydrates on the surface of eggs has been used to identify parasites of humans (Rao et al., 1987; Rao et al., 1989) and plants (Chen et al., 2001). There are reports of species differences in binding of lectins to the eggs of parasites of livestock, particularly that peanut agglutinin (PNA) binds only to *Haemonchus contortus* eggs and not to those of any other ruminant parasite (Palmer and McCombe, 1996; Colditz et al., 2002; Jurasek et al., 2010; Hillrichs et al., 2012). Binding of PNA is the basis of a commercial test offered by two laboratories in the USA to identify *H. contortus* in faeces, including eggs preserved in formalin. Hillrichs et al. (2012) further showed that *Lens culinaris* agglutinin (LCA) bound to eggs of *Teladorsagia circumcincta*, but not *H. contortus*, allowing the eggs of these two species to be distinguished using PNA and LCA.

In the present study, lectins specific for individual parasites eggs from six nematode parasites infecting sheep were identified from a panel of 19 lectins that covered a broad range of sugar specificities. The results of using lectin binding and PCR to identify parasite eggs to the species level were compared for eggs in two faecal samples

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Table 1
The carbohydrate-binding specificities and requirements for ions (0.1 mM Ca^{2+}, 0.01 mM Mn^{2+}) of the lectins tested for binding to nematode eggs.

Lectin		Carbohydrate binding structure	Metal ion
PNA	Peanut agglutinin	Gal (β-1,3) GalNAc	Ca^{2+} Mn^{2+}
LCA	*Lens culinaris* agglutinin	α-Man on N-acetylchitobiose cores containing α-Fuc	Ca^{2+} Mn^{2+}
AAL	*Aleuria aurantia* agglutinin	Fuc α-1,6-GlcNAc; Fuc (α-1,3) N-acetyllactosamine	Nil
PTLII	*Psophocarpus tetragonolobus-lectin* II	α-GalNAc	Nil
LTL	*Lotus tetragonolobus* lectin	α-1,2 Fuc; Fuc (α-1,3) GlcNAc	Ca^{2+}
WGA	Wheat germ agglutinin	Terminal GlcNAc or chitobiose	Ca^{2+}
PSA	*P. sativum* agglutinin	α-Man on N-acetylchitobiose cores containing α-Fuc	Ca^{2+} Mn^{2+}
BPL	*Bauhinia purpurea* lectin	Gal (β-1,3) GalNAc; α-GalNAc	Nil
ConA	ConcavalinA	α-Man; α-Glu	Ca^{2+} Mn^{2+}
DBA	*Dolichos biflorus* agglutinin	α-GalNAc	Ca^{2+}
RCA	*Ricinus communis* agglutinin	Terminal Gal or GalNAc	Nil
SBA	Soybean agglutinin	Terminal α/β-GalNAc or Gal	Ca^{2+}
PHA E + L	Phaseolus vulgaris agglutinin	Oligosaccharides; bi/tri-antennary Gal(β-1,4) GlcNAc (1,2) Man(α-1,6)	Ca^{2+}
BGSII	*Griffonia simplicifolia*-II lectin	α/β-GlcNac on non-reducing terminus	Ca^{2+}
UEAI	*Ulex europaeus* lectin	α-Fuc	Ca^{2+}
SNA	*Sambucus nigra* agglutinin	α-2,6 or 2,3 sialic acid on terminal Gal	Ca^{2+}
MPL	*Maclura pomifera* lectin	α-GalNAc	Nil
MAL	*Maackia amurensis* lectin-II	α-2,3-sialic acid	Nil
GSLI	*Griffonia simplicifolia*-I lectin	α-GalNAc; α-Gal	Ca^{2+}

from field-infected sheep. In addition, the effects of some storage conditions on PNA binding to *H. contortus* eggs were examined.

2. Materials and methods

2.1. Lectin binding to nematode eggs

Eggs were laid *in vitro* by adult *H. contortus, Teladorsagia* sp, intestinal *Trichostrongylus* sp, *Nematodirus* sp, *Cooperia* sp and *Chabertia ovina,* which were obtained from the gastrointestinal tracts of field-infected sheep.

2.1.1. Collection of eggs

Adult worms were collected from the abomasum and small and large intestines of gastrointestinal tracts of sheep killed at a local abattoir. The worms were identified microscopically at the genus/species level and 40–50 adult females of each parasite were placed in petri dishes in 10 mM HEPES buffer pH 7.5 to lay eggs during a 24 h incubation period at 37 °C. After manual removal of the worms, the buffer containing the eggs was centrifuged at 4000 × *g* for 1 min, the eggs were re-suspended in 6 ml of fresh HEPES buffer and 300 μl aliquots, each containing about 1500 eggs, were placed in tubes for lectin binding.

2.1.2. Binding of lectin to eggs

The panel of 19 lectins (Vector Laboratories, USA) covering a broad range of sugar specificities, and their bivalent metal ion requirements, are shown in Table 1. A stock solution (2%) of each lectin was prepared by reconstituting in 1 ml sterile Milli-Q® water and storing in the dark at 4 °C.

Each of the 19 lectins was tested separately on eggs of each parasite genus/species. Stock solution (1.2–12 μl) of lectin was added to the 300 μl aliquot of eggs suspended in buffer containing Na azide (0.08%) to make a final 0.002% lectin concentration. The tubes were incubated for 1 h in the dark at room temperature. The eggs were then washed three times in HEPES buffer, 1.5 μl of 1 mg/ml strepavidin-Alexa Fluor® 546 (Invitrogen, USA) was added and the tubes were incubated again in the dark for a further 1 h at room temperature. After three washes in buffer, the eggs were suspended in 50 μl buffer and 15 μl was placed on a microscope slide and covered with a cover slip. Eggs were examined using a Xenon lamp for fluorescence excitation and 540 nm excitation and 605 nm emission filters (Chroma Technology, USA). If there were no eggs visible by fluorescence, the presence of unstained eggs was confirmed under bright field illumination. Images were captured with a DS-Fi1-U2 digital camera (Nikon, Japan).

The intensity of fluorescence produced by lectin binding was recorded as: ++++ bright; +++ moderate; ++ weak or + very weak, as illustrated in Fig. 1. Fluorescence intensity of ++++ was assigned when the whole egg surface or the circumference was brightly stained; +++ when the whole circumference was stained; ++ staining was at the poles of the egg and + stainingwas very weak in small areas, particularly at the poles.

2.2. Effect of storage on lectin binding to contortus eggs

The binding of PNA to eggs collected from a laboratory strain of *H. contortus* was examined after storage of faecal samples or eggs isolated from faeces. The faeces were collected in faecal bags, 18–20 days after infection with L3 *H. contortus* of sheep housed indoors (Grasslands Animal Ethics # 13622).

2.2.1. Storage of faeces

Faecal samples, each of about 50 g, were placed in sealable plastic bags, the air was removed and the faeces were stored at:

(1) room temperature for 0, 24, 48 or 72 h;

(2) room temperature with saturated NaCl solution covering the faeces for 0, 24, 48 or 72 h;

(3) 4 °C with or without saturated NaCl solution for 24, 48 or 72 h or

(4) −20 °C for 24, 48 or 72 h.

2.2.2. Isolation of eggs from faeces

About 50 g faeces were suspended in 100 ml 13% NaCl and vortexed until a pourable slurry formed. This was poured through a coarse sieve, collected in a centrifuge bottle and centrifuged at 3600 × *g* for 5 min. The supernatant was poured into a clean centrifuge bottle and an equal or greater volume of Milli Q® water was added and centrifuged at 3600 × *g* for 5 min. The supernatant was aspirated, the pellet was disrupted and suspended in 45 ml of 13% NaCl, then centrifuged at 3600 × *g* for 5 min. These two steps were repeated twice and the pellet was re-suspended in 10 ml of Milli Q® water and the eggs counted to ensure 1000–1500 eggs were available for each assay tube.

2.2.3. Storage of eggs

500–1000 eggs extracted from fresh faeces were stored in 5 ml sterile water at:

(1) room temperature or 4 °C for 0, 24, 48 or 72 h;