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Research review paper

Advances in the diagnosis of key gastrointestinal nematode infections of livestock, with an emphasis on small ruminants

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ABSTRACT

Parasitic nematodes (roundworms) of livestock have major economic impact globally. In spite of the diseases caused by these nematodes and some advances in the design of new therapeutic agents (anthelmintics) and attempts to develop vaccines against some of them, there has been limited progress in the establishment of practical diagnostic techniques. The specific and sensitive diagnosis of gastrointestinal nematode infections of livestock underpins effective disease control, which is highly relevant now that anthelmintic resistance (AR) is a major problem. Traditional diagnostic techniques have major constraints, in terms of sensitivity and specificity. The purpose of this article is to provide a brief background on gastrointestinal nematodes (Strongylida) of livestock and their control; to summarize conventional methods used for the diagnosis and discuss their constraints; to review key molecular-diagnostic methods and recent progress in the development of advanced amplification-based and sequencing technologies, and their implications for epidemiological investigations and the control of parasitic diseases.

Contents

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1. Introduction

The phylum Nematoda (roundworms) includes many parasites that are of major socio-economic importance. For instance, grazing ruminants are usually parasitized by one or more nematodes (order Strongylida) which can cause parasitic gastroenteritis (PGE) (Taylor et al., 2007). Various species of strongylid nematodes can vary considerably in their pathogenicity, geographical distribution and susceptibility to anthelmintic drugs (Dobson et al., 1996). Mixed infections involving multiple genera and species are common, and usually have a greater impact on the host than monospecific infections. In addition, the species composition of the parasites present in a host animal can have an important relationship with the severity of infection (Wimmer et al., 2004). Depending on the number, species and burden of parasitic nematodes, common signs of PGE include reduced weight gain or weight loss, anorexia, diarrhea, reduced production and, in the case of blood-feeding species, anemia and edema, due to the loss of blood and/or plasma proteins (Kassai, 1999; Taylor et al., 2007). Therefore, the knowledge of the nematode species present in a particular geographical area, and their biology and epidemiology, have important implications for the control of PGE, particularly given the increasing problems of anthelmintic resistance (AR) in strongylid nematodes of livestock (Kaplan, 2004; Wolstenholme et al., 2004).

The accurate diagnosis of parasitic diseases and AR is central to these areas and the control of parasites. Traditional methods of diagnosis can be time-consuming to perform and have limitations, in terms of their specificity and sensitivity (Gasser, 2006). In particular, in the case of mixed infections, diagnosis can be laborious and time-consuming using techniques such as fecal egg counts (FEC) and larval culture and differentiation (MAFF, 1986). DNA techniques that rely on the amplification of nucleic acids, particularly those coupled to the polymerase chain reaction (PCR) (Saiki et al., 1988), are effective for the specific identification of parasites, and aid the diagnosis of infections from minute amounts of target template, if suitable genetic markers are employed. Such methods are likely to provide powerful alternative tools to traditional approaches, to underpin fundamental research into parasite epidemiology and to improve the control of parasitic diseases (Gasser, 2006). The purpose of this article was to: (i) concisely review the biology and significance of gastrointestinal strongylid nematodes of small ruminants; (ii) discuss salient aspects of parasite control and AR; (iii) review traditional methods for the diagnosis of strongylid infections and discuss their limitations; and (iv) summarize nucleic acid-based diagnostic techniques, emphasizing recent advances in the establishment of robotic PCR-based technology and its implications.

2. Gastrointestinal strongylid nematodes of small ruminants

2.1. Strongylids and their biology

The order Strongylida includes five superfamilies: the Diaphanocephaloidea, Ancylostomatoidea, Strongyloidea, Trichostrongyloidea and Metastrongyloidea. The Strongylida are characterized by the presence of a copulatory bursa in the male and are thus called bursate nematodes (Anderson, 2000). The first four of these superfamilies are monoxenous and predominantly live in the gastrointestinal tract of their vertebrate hosts (Fig. 1). Adult strongylid nematodes exist as females and males; the females produce relatively large numbers (depending on the species) of typically ovoid, strongylid eggs (70–150 μm), which are excreted in the feces into the external environment. The first-stage larva (L1) develops inside the egg to then hatch (within 1–2 days, depending on environmental conditions) and develops through to the second-stage larva (L2). Both the L1s and L2s feed on bacteria and other microorganisms in the external environment (feces). After the molts, the ensheathed third-stage larva (L3) develops (usually within 1–2 weeks, depending on species, temperature, humidity, pH and/or other factors). The cuticular sheath around the L3 prevents it from feeding but protects it from relatively harsh environmental conditions. After the L3 is ingested by the animal and passes through the stomach(s), it exsheaths (xL3) and (after a tissue phase) develops through to the fourth-stage larva (L4) and subsequently the adult at the predilection site in the alimentary tract. The time from the xL3 to the production of eggs by the adult female is usually 3–4 weeks.

2.2. Key nematodes and aspects of disease

Important gastrointestinal strongylid nematodes that infect small ruminants are listed in Table 1. Key nematodes responsible for disease in grazing sheep include *Haemonchus contortus*, *Teladorsagia circumcincta* and intestinal species of *Trichostrongylus* (Besier and Love, 2003). Sheep are usually infected with one or more nematodes, but the severity of disease can vary considerably (e.g., Donald et al., 1978). Disease is

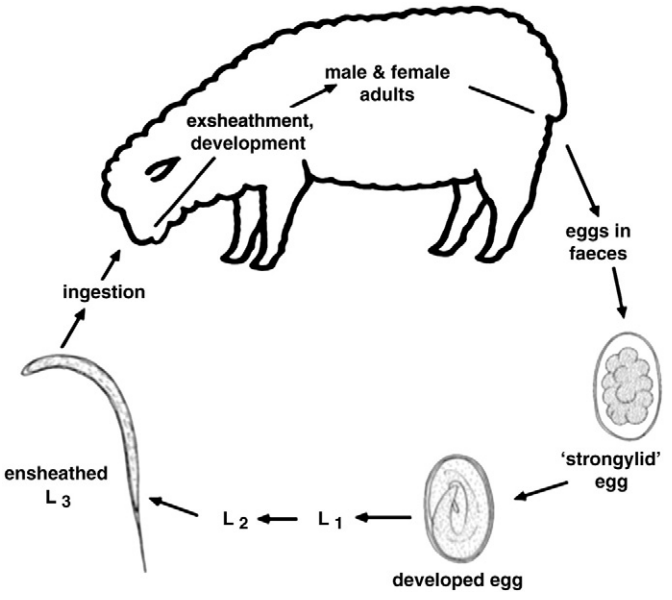

Fig. 1. Generalized life cycle representing key gastrointestinal strongylid nematodes of small ruminants (adapted from Demeler, 2005). First-, second- and third-stage larvae (L1, L2 and L3, respectively) are 'free-living' in the environment. The fourth larval (L4) and adult stages (dioecious) are 'parasitic' in the gastrointestinal tract of the host. Disease in the host animal is caused by the adult and/or L4 stages, and depends on the species of nematode; intensity of infection; species, age and immunological/health status of the host; host response against the parasite; stress and other environmental and management factors (Kassai, 1999; Taylor et al., 2007).

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