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Bovine and ovine rumen fluke in Ireland—Prevalence, risk factors and species identity based on passive veterinary surveillance and abattoir findings

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ABSTRACT

The prevalence of rumen fluke, the incidence of clinical paramphistomosis and the trematode's species identity were studied in cattle and sheep in the Republic of Ireland using passive veterinary surveillance (faecal examination and necropsy results; 2010–2013) and abattoir data. Based on faecal examination, the prevalence of rumen fluke was higher in cattle than in sheep. Rumen fluke prevalence in cattle and sheep fluctuated over the year and in most years (2011–2013), prevalence was higher in winter (December–February) than in summer (June–August). For 3 of 4 years studied, there was no correlation between monthly prevalence of rumen fluke and prevalence of liver fluke as estimated by faecal examination. At sample level, joint occurrence of rumen fluke and liver fluke was 1.1–2.0 times more common than would be expected under the assumption of independence. Based on necropsy data, a spike in deaths attributed to paramphistomosis was observed in 2012, when rainfall was unusually high. This spike in mortality was not accompanied by a spike in faecal prevalence, emphasizing that the incidence of disease, which is due to high burdens of juvenile rumen fluke in the gut, is not correlated with prevalence of infection, which is measured by faecal examination and reflects presence of adult fluke in the rumen. At slaughter, 52% of 518 cattle from 101 herds were positive for rumen fluke, compared to 14% of 158 sheep. Prevalence in cattle was higher than reported in most studies from mainland Europe and varied by animal category, age, sex, abattoir visit and location (county) of farm from which the animal was submitted for slaughter, but in multivariate analysis, only sampling month and county were significantly associated with detection of rumen fluke. The identity of rumen fluke in cattle and sheep was confirmed as *Calicophoron daubneyi*. Although *C. daubneyi* is thought to share an intermediate host snail with *Fasciola hepatica*, the differences in prevalence between host species and over time suggest that the epidemiology of *C. daubneyi* is distinct from that of *F. hepatica*. Further studies of the *C. daubneyi* life-cycle in ruminant hosts, intermediate snail hosts and the environment will be needed to gain a better understanding of modes of transmission and options for control of rumen fluke infection and disease.

1. Introduction

Rumen flukes or paramphistomes are trematode parasites that occur in ruminants almost worldwide, although clinical disease was believed to be confined mostly to tropical and subtropical regions (Horak 1971; Diaz et al., 2006; Taylor et al., 2007). Unlike the liver fluke, *Fasciola hepatica*, rumen fluke are not zoonotic and, in some tropical communities, they are actually harvested as a food source (Sarmah et al., 2014). Historically, rumen fluke were known to occur

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in occasional animals in the Republic of Ireland (ROI) and the United Kingdom (UK) but reported prevalence was very low (Kelly 1948; Willmott 1950). An apparent increase in prevalence was observed in recent years in ROI and the UK, with reports of clinical and subclinical infection in cattle and sheep since the late 2000s (Foster et al., 2008; Murphy et al., 2008; Mason et al., 2012). It was initially assumed that the rumen fluke occurring in livestock in ROI and UK was *Paramphistomum cervi* (Willmott, 1950). Gordon et al. (2013) recently examined a number of rumen flukes from Scottish sheep and cattle and found that all were *Calicophoron daubneyi*. This sample also included rumen flukes from an Irish-bred cow that had grazed in Scotland. Studies in Ireland have confirmed *C. daubneyi* as the most common, or even the only, rumen fluke species in cattle (Zintl et al., 2014).

There are various methods to detect the presence of rumen fluke. Passive surveillance in veterinary diagnostic laboratories is based on routine examination of faeces for rumen fluke eggs, and largely follows the same sedimentation procedure as for detection of liver fluke eggs (Gordon et al., 2012). This method allows for the rapid screening of large numbers of samples for the presence of adult rumen fluke (Zintl et al., 2014). Necropsy of animals that are presented after euthanasia or unexpected death may reveal the presence not only of adult rumen fluke in the reticulo-rumen, but also of juvenile fluke, which are responsible for clinical disease, in the intestine (Mason et al., 2012; Millar et al., 2012). Examination of the reticulo-rumen of animals at slaughter would be an effective way of assessing prevalence of adult rumen fluke in a population of healthy animals that are killed for food production (Arias et al., 2011; Gonzalez-Warleta et al., 2013; Malrait et al., 2015). In contrast to inspection of livers for diagnosis of liver fluke infection, detection of rumen fluke at slaughter does not take place routinely. Abattoir studies also allow for collection of risk factor data and specimens for species identification.

The aim of this work was to describe the occurrence of rumen fluke in cattle and sheep from the ROI using three types of diagnostic information, i.e., passive surveillance data on prevalence (faecal examination) and incidence (necropsy) and inspection of the reticulo-rumen at slaughter. Risk factors for rumen fluke presence were examined in association with abattoir findings and species identity of rumen fluke specimens from cattle and sheep was determined using molecular methods.

2. Materials and methods

2.1. Veterinary surveillance

Prevalence data for the entire ROI were obtained from passive surveillance as conducted routinely by the Department of Agriculture Food and the Marine (DAFM) Veterinary Laboratory Service (VLS) using faecal examination. Fluke eggs in faeces were detected by a sedimentation method. Faeces (3 g) and cold tap water (42 ml) were mixed and strained through a clean sieve into a beaker. Part of the strained sample (12 ml) was decanted for other diagnostic tests and the remainder poured into a 20 ml universal bottle and allowed to sediment for 10 mins before supernatant was decanted. The deposit was washed repeatedly with water and 5 min sedimentation steps per wash until the deposit was free from as much debris as possible. Two drops of methylene blue were added before the sample was transferred to a petri dish and observed under a scan microscope for the presence of rumen fluke and liver fluke eggs. Liver fluke eggs are dark in colour due to imbibition of bile whereas rumen fluke eggs are pale in colour (Gordon et al., 2013). For the years 2006–2008, information on prevalence of trematode eggs in faeces, without differentiation between rumen fluke and liver fluke eggs, was obtained from the Annual Surveillance Report of the DAFM, both for cattle and for sheep (Anon., 2008). VLS began to separately report liver fluke and rumen fluke eggs halfway through 2009 and data from 2009 were not included in our analysis. For 2010 through 2013, rumen fluke- and liver fluke-specific results for bovine and ovine faecal samples were extracted from the databases of the DAFM's VLS. The number of clinical cases of larval paramphistomosis and fasciolosis diagnosed by the regional veterinary laboratories was also extracted from the VLS database. These results were based on routine diagnostic post mortems on unexpected deaths from any cause or on animals that were euthanized. As of 2009, diagnostic post mortems included inspection of the reticulo-rumen as well as the intestine for rumen fluke. Results are reported for 2010 through 2013, i.e., full calendar years with routine diagnostic investigation of the presence of rumen fluke.

2.2. Abattoir sampling

The prevalence of adult rumen fluke was measured during visits to a cattle abattoir in the south-east of Ireland on four occasions over a twelve-month period (April 2013, October 2013, January 2014 and March 2014) and a sheep abattoir (Irish Country Meats, Camolin, Co. Wexford) on a single occasion (March 2014). During each visit, all animals that were slaughtered were examined for presence of adult rumen fluke. For cattle, examination consisted of incising the dorsal surface of the reticulum and visually examining the mucosa, reticular groove and surface of the stomach contents for parasites. Examination of sheep was similar but the dorsal surface of both reticulum and rumen was opened. Presence or absence of rumen fluke was recorded. The intestine was not inspected in either species. The cattle stomachs, but not sheep stomachs, were individually identifiable and, by combining the databases of the factory with those of the DAFM, information could be extracted about the date of birth of cattle and the herd from which each animal was slaughtered. Based on age, sex and reproductive status, cattle were classed as young bull (intact male under 24 months), stock bull (intact male of 24 months or older), steer (castrated male), heifer (female that has not had calf) or cow (female that has had at least one calf).

2.3. Species identification

A convenience sample of adult rumen fluke specimens was collected from cattle or sheep at each abattoir visit. The number of specimens collected per visit depended on the number of positive animals detected. Specimens were collected at least 10 positive animals apart to minimise the chance of repeated sampling from a single batch of animals or a single herd or flock. In addition, adult or larval rumen fluke were collected from material submitted to the Kilkenny Regional Veterinary Laboratory for routine necropsy. Fluke specimens (2 fluke each from 25 cattle, and 2 fluke each from 11 sheep) were preserved in 70% methanol and sent to Moredun Research Institute. Species identity was determined using PCR amplification and DNA sequencing of a ~500 bp fragment of ITS-2 and its flanking regions using generic primers (Rinaldi et al., 2005), with subsequent sequencing of purified PCR amplicons as described by Gordon et al. (2013).

2.4. Statistical analysis

Abattoir data were examined for missing values, outliers and associations using Statistix 10 (Analytical Software, La Jolla, CA). Associations between independent variables were tested for significance using Chi-square tests (categorical variables) or *t*-tests (continuous variables). Overall prevalence of adult rumen fluke in cattle and sheep was calculated. For cattle, prevalence by county, age, type of animal and abattoir visit was also determined. The asso-