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Short communication

Gastrointestinal nematodes in grazing dairy cattle from small and medium-sized farms in southern Poland

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ABSTRACT

This study aimed to estimate the prevalence of gastrointestinal nematodes and the intensity of infection in grazing dairy cattle from small and medium-sized farms in southern Poland. The level of antibodies against *Ostertagia ostertagi* in the bulk tank milk (BTM) from the animals was also assessed. Rectal fecal samples collected from 361 cows on 20 farms were examined using Willis-Schlaaf flotation and the McMaster method. BTM samples were tested for the presence of *O. ostertagi* antibodies using ELISA. Multiplex PCR was used to identify the third-stage larvae (L3) of gastrointestinal nematodes derived from the culture of pooled fecal samples from sampled farms. Gastrointestinal nematode eggs were found in the samples from 18 of the 20 herds with a prevalence range from 20.4 to 94.5%. The average number of eggs excreted in the feces of the herds was 200 eggs per gram (EPG). Antibodies to *O. ostertagi* were found in 20 of the examined herds (100%), of which 6 had optical density ratios (ODR) greater than 0.5. PCR results showed the presence of three nematode species: *Ostertagia ostertagi*, *Cooperia oncophora* and *Oesophagostomum radiatum*.

1. Introduction

Gastrointestinal (GI) nematodes that cause substantial losses in dairy cattle breeding include members of the Trichostrongylidae (*Trichostrongylus, Haemonchus, Ostertagia, Cooperia*) and Molineidae (*Nematodirus*) families and *Oesophagostomum* species. Previous research confirmed that the negative effects of infection in dairy cows with subclinical symptoms included a decrease in milk yield of up to and even over 11% (Gross et al., 1999; Charlier et al., 2009).

The interpretation of fecal examination results has certain limitations. In order to distinguish gastrointestinal parasite species, it is necessary to conduct larval culture, perform morphometric analysis of the third larvae stage, or use PCR.

The *Ostertagia ostertagi* antibody levels in bulk tank milk samples (BTM) provide an important indicator of potential production losses in dairy herds. Potential loss of milk yield is already observed at antibody concentrations of 0.5 ODR (Charlier et al., 2007).

The greatest possibilities of infection with GI nematodes in Poland fall in late May and June and late August and

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September, as those are the periods with the most favorable weather conditions for infection. Small and medium-sized dairy farms have a significant share in the total milk production in Poland, but their parasitological status is unknown, so each examination is very important to fill this gap in our knowledge. Testing also has a huge impact on farmers' awareness. For them, the investment into deworming seems high, especially with preparations that have no withdrawal period, and there are no data for veterinarians to support the recommended prevention programs.

The main objective of this study was to investigate the prevalence and intensity of GI nematode invasion in dairy herds from small and medium-sized farms in southern Poland, to determine the *O. ostertagi*-specific antibody levels in BTM samples from the examined herds to estimate potential losses in production, and to identify the GI nematode species occurring in the studied herds.

2. Materials and methods

2.1. Study area and farms

Fecal examinations were conducted in October and November 2011, in 20 grazing dairy cattle herds: 12 from Lower Silesia and 8 from Lesser Poland (Galicia). The herd size ranged from 10 to 200 animals and their average age was about 5.5 years. The mean number of lactation periods across all the herds was 3.51 ± 0.124. The material consisted of 361 rectal fecal samples from lowland black-and-white and red Polish cows. The percentage of examined fecal samples from a given herd was between 19 and 73, depending on herd size. The grazing period had started at the end of April and ended in November 2011. The last anti-parasitic compounds had been given to cattle on 2 of the 20 farms 6 months prior to the study. The products used as the last treatment included ivermectin (Farm No. 19) and oxfendazole (Farm No. 20) (Table 1). The cattle on the other 18 farms had never been treated before sampling. The herds originated in areas characterized by moderate climates with maritime and continental features. The annual rainfall ranges from 550 to 700 mm, and July is the wettest month. The average temperature in Lower Silesia is −0.6 °C (30.92 °F) in January and 17.8 °C (64.04 °F) in July. The warmest month in Lesser Poland is July, with an average temperature of 14.5 °C (58.1 °F). January is the coldest month, with an average temperature of −9.4 °C (15.08 °F). The snow cover lasts 50 days on average.

2.2. Parasitological techniques (fecal egg counts, larval culture)

The fecal samples were examined using Willis-Schlaaf flotation with saturated NaCl solution (Pilarczyk et al., 2009). A modified version of the McMaster technique using saturated NaCl solution as the flotation fluid and a detection level of 50 gastrointestinal nematode eggs per gram of feces was performed on all individual fecal samples (Vadlejch et al., 2011). Pooled coprocultures obtained from the positive fecal samples from each herd were prepared and incubated at 24 °C in moist conditions for 14 days to obtain the infective third stage larvae (L3) that were harvested using the Baermann technique (Pilarczyk et al., 2009).

2.3. Molecular identification of gastrointestinal nematodes

The DNA of third-stage infective GI larvae collected from coprocultures was isolated using the Genomic Set AX STOOL Mini Kit (DNA-Gdańsk). Genotyping was conducted using multiplex PCR. The parameters of the reaction and peculiar starters were planned based on Zarlenga et al. (2001). Sequence data from internal (ITS) and external (ETS) transcribed spacers of the ribosomal DNA (rDNA) repeats and from the 3′-end of the small subunit rDNA and 5′-end of the large subunit rDNA were used to generate five primer sets that, when used simultaneously in multiplex PCR, produce a unique electrophoretic DNA banding pattern characterized by single DNA fragments for:

Ostertagia ostertagi (257 bp)
primer sequence forward 5′-TAAAAGTCGTAACAAGGTATCTGTGGT
reverse 5′-GTCTCAAGCTCAACCATAACCAACATGG;

Haemonchus placei (176 bp)
primer sequence forward 5′-CATTTTCGTCTTGGGCGATAT
reverse 5′-TGAGACCGCACGCGTTGATTCGAA;

Oesophagostomum radiatum (329 bp)
primer sequence forward 5′-GCAGAACCGTGACTATGGTC
reverse 5′-GACAAGGAGATCACGACATCAGCAT;

Trichostrongylus colubriformis (243 bp)
primer sequence forward
5′-CAGGGTCAGTGTCGAATGGTCATTGTCAAATA
reverse 5′-CAGGGTCAGTGGTTGCAATACAAATGATAATT;

Cooperia oncophora (151 bp)
primer sequence forward 5′-TCGATGAAGAGTTTTCGGTGTTC
reverse 5′-TTCACGCTCGCTCGTGACTTCA.

The PCR products (10 μl) were separated via 5% agarose gel electrophoresis.

2.4. Bulk-tank milk ELISAs

Bulk tank milk (BTM) samples of 30 ml were taken from the milk tank of each of the 20 herds after the morning milking, according to Polish standard *PN-A-86002:1999: Raw milk*. Milk samples were centrifuged at $16{,}000 \times g$ for 4 min and the fat fraction was removed. The BTM skim milk samples were tested for the presence of *O. ostertagi* antibodies using SVANOVIR *O. ostertagi*-Ab ELISA test (Svanova Biotech Ab, Uppsala, Sweden) according to the manufacturer's instructions. The optical density was measured at 492 nm and the test results were expressed as the optical density ratio (ODR). The ODR value was calculated for each herd according to the formula:

$$(OD_{sample} - OD_{negative\ control})/(OD_{positive\ control} - OD_{negative\ control})$$

2.5. Statistical analysis

The confidence limits (confidence level: 95%, $p < 0.05$) of infection incidence (%) were calculated according to the modified Wald method (Sauro, 2005). All of the statistical