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Serological evidence of *Ostertagia ostertagi* infection in dairy cows does not impact the efficacy of rabies vaccination during the housing period

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ABSTRACT

Nematode infections modulate the immune reaction of humans and livestock and may impair immune responses to non-parasitic antigens such as those present in vaccines. In this study, the relationship between antibodies directed against *Ostertagia ostertagi*, the economically most important nematode infection of cattle in temperate regions, and the magnitude and the kinetics of the antibody response to rabies vaccination was investigated in a commercial dairy herd of 46 cows. During the stabling period, all animals received a single intramuscular administration with a commercial inactivated rabies vaccine (Rabisin®, Merial). The serum antibody levels against *O. ostertagi* on day 0 were compared with anti-rabies IgM, IgA, IgG1, IgG2 and virus-neutralizing antibodies on days 0, 7, 14 and 21 after vaccination. In addition, to explore the potential effect of newly acquired *O. ostertagi* infections, the kinetics of the *O. ostertagi* antibody levels during the first 2 months after turnout on pasture were compared with concurrent changes in the rabies antibodies. During the stabling period the *O. ostertagi* antibody level tended to be positively associated with the magnitude, rate of increase and rate of decrease of the rabies antibodies. However, none of these associations were significant ($P > 0.05$). Over the first 2 months at pasture, an increase in *O. ostertagi* antibody level tended to be associated with a decrease in rabies IgG2 and IgM, but again these associations lacked statistical significance ($P > 0.20$). We conclude that the *O. ostertagi* antibody level in adult cattle over the housing period has no significant association with the antibody response to rabies vaccination. We recommend that future studies aiming to assess the relationship of nematode infections with humoral immune responses to vaccines are conducted on a larger scale and focus on the summer period when cattle are exposed continuously to nematode challenge from the pasture and hence are actively responding immunologically to nematode antigen exposure.

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1. Introduction

Gastrointestinal (GI) nematodes of cattle are an important cause of morbidity and economic losses throughout the world. The most abundant and pathogenic species in adult cattle in temperate climate regions is *Ostertagia ostertagi*. The economic losses are caused by reductions in weight gain, carcass quality, milk production and fertility (Charlier et al., 2009a,b). Apart from the economic losses caused by the direct effects on production, much attention has lately been focused on the immune-modulating effects of helminth-infections in humans and livestock. Chronic helminth infections are characterized by polarizing the immune response towards a T helper 2 type response as well as regulatory responses. The regulatory network is thought to prevent strong immune responses to parasitic worms, but also to temper responses to non-helminth antigens, including those present in anti-viral and anti-bacterial vaccines (Mulcahy et al., 2004; van Riet et al., 2007). In livestock, this may result in important indirect economic losses caused by reduced efficacy of vaccination and increased susceptibility to concurrent disease.

The effects of nematode infections on vaccination have been studied in rodent and swine models (Urban et al., 2007), but few recent data are available for cattle. In this livestock species, *O. ostertagi* infections are considered to cause transient non-specific suppression of cell-mediated immune responses after infection (Cross et al., 1986; Snider et al., 1986; Yang et al., 1993a,b), but the effect on humoral responses against heterologous vaccines is not well

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understood. Yang et al. (1993a) reported that the antibody response to vaccination with *Brucella abortus* arose more slowly in GI nematode infected and infected-treated calf groups when compared to uninfected controls. However, in a second study these results could not be confirmed (Yang et al., 1993b).

Recently, an *O. ostertagi* ELISA has been developed and commercialized for evaluating levels of exposure to GI nematodes at the dairy herd or animal level (Sanchez et al., 2002; Charlier et al., 2009b,d). This offers a new possibility to study the relationship between GI nematode infections and heterologous vaccination responses. Moreover, if a relationship is present, this could enable a means to identify poor responding animals and consequently enhance vaccination responses, for example by advocating anthelmintic treatment before vaccination. The objectives of this study were to investigate the relationship of *O. ostertagi* ELISA results with (1) the magnitude and (2) the kinetics of the antibody response to rabies vaccination.

2. Materials and methods

2.1. Study design

The study was conducted on a commercial dairy herd of 46 adult cows. The mean cow age at the onset of the study was 4 years and 4 months. The distribution of the animals over first, second and third or higher lactation was of 33.3%, 28.9% and 37.8%, respectively. The rolling year average milk production per cow was 11,397 kg. The herd was given access to pasture day and night from April to November 2008. The diet during the grazing period consisted of 50% pastured grass combined with 50% total mixed ration. The study was conducted between end of January (day 0) and mid June (day 133) 2009. On day 0, all animals were given a single intramuscular administration with an inactivated rabies vaccine (Rabisin®, Merial) according to the manufacturer's instructions. Rabisin® comprises rabies virus glycoproteins in an aluminium hydroxide adjuvant. The recommended dose of 1 ml contains $\geqslant$1 IU of rabies glycoprotein and the vaccine can be administered to cattle by either the intramuscular or subcutaneous routes. Following initial vaccination of cattle during the first year of life, annual revaccination is recommended, though challenge studies (unpublished) have shown protection for up to three years. Blood samples were collected on day 0, day 7, day 14, day 21 and further at the end of the stabling period (day 70) and 2 months after turnout on pasture (day 133). All 46 animals were sampled up to day 21, but on day 70 and 133 only 40 remained available for sampling.

The herd was judged to be free of *Fasciola hepatica* and Bovine Viral Diarrhoea (BVD) infection based on serological examination for antibodies against *F. hepatica* of all studied animals and the results of the BVD monitoring programme in which the herd participated. The study protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Ghent University, Belgium).

2.2. Laboratory methods

Sera obtained from the blood samples collected on day 0, day 70 and day 133 were diluted 1/140 and analyzed by an *O. ostertagi* ELISA (Svanova Biotech AB, Uppsala) according to the manufacturer's instruction to assess the IgG levels against crude adult worm antigens (Charlier et al., 2009a,c). The results were expressed as optical density ratios (ODR).

Sera obtained from the blood samples collected at all sampling occasions were subjected to a commercial Rabies ELISA (Platelia Rabies II, Bio-rad), which was adapted in order to measure isotype-specific antibodies of cattle. The serum was diluted 1/500 in the dilution buffer delivered with the Rabies ELISA kit. Instead of using the conjugate supplied, secondary sheep antibodies directed against bovine IgM, IgA, IgG1 and IgG2, were used in a dilution of 1/1000 (AbD Serotec, AAI19P–AAI22P). After the incubation with the secondary antibody, the plates were developed for 10 min instead of the 30 min as recommended by the manufacturer. A conjugate control was integrated on each plate.

Virus-neutralizing (VN) antibodies were measured with the Rapid Fluorescent Focus Inhibition Test (RFFIT), according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Anonymous, 2009). Virus-neutralizing antibody titers are expressed in International Units (IU)/ml in reference to "The Second International standard for Anti-rabies Immunoglobulin", purchased from the United Kingdom National Institute for Biological Standards and Control. A serum titer of 0.5 IU/ml is considered protective *in vivo*.

2.3. Data analysis

The data analysis was conducted in 2 parts. First, the magnitude and kinetics of the rabies antibody response during the housing period were described by the mean OD between day 0 and day 21 (= magnitude of response), the difference in OD between day 14 and day 0 (= rate of increase) and the difference in OD between day 70 and day 14 (rate of decrease) for the different isotypes. These parameters were linearly regressed on the *O. ostertagi* ODR at day 0.

Second, because new infections during the pasture season could also influence the kinetics of the rabies antibody response, the difference in rabies antibody responses at 133 and 70 days was regressed on the difference in *O. ostertagi* ODR at 133 and 70 days. The difference in *O. ostertagi* ODR was regarded as a measure of exposure to new infections during the first months at pasture.

3. Results

3.1. Antibody levels against O. ostertagi and rabies vaccination

The mean ± standard deviation *O. ostertagi* ODR at day 0, 70 and 133 was 0.58 ± 0.25, 0.54 ± 0.21 and 0.58 ± 0.18, respectively. The results of the RFFIT assay (Fig 1) showed that VN antibodies were already detectable at day 7 and reached maximum levels at day 14. From day 14 onwards antibody levels decreased to reach lowest levels at day 133, when the study was terminated. The same general pattern was observed in the results of isotype-specific ELISA's. The antibody response consisted mainly of a IgG1-response,

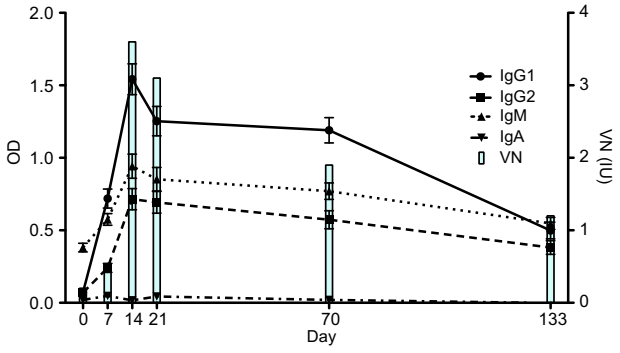

Fig. 1. Antibody responses to a single rabies vaccination on day 0 in a herd of 46 dairy cows. The mean optical density (OD) of isotype-specific ELISAs are plotted on the left *Y*-axis. Virus-neutralizing (VN) antibody results are plotted on the right *Y*-axis. The cows were turned out on pasture on day 70. Error bars represent the standard error of the mean.

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