Veterinary Parasitology 166 (2009) 255–261

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Evaluation of a milk ELISA for the serodiagnosis of *Dictyocaulus viviparus* in dairy cows

Christiane Fiedor [a], Christina Strube [a,*], Andrew Forbes [b], Sandra Buschbaum [a], Anne-Marie Klewer [a], Georg von Samson-Himmelstjerna [a], Thomas Schnieder [a]

[a] *Institute for Parasitology, University of Veterinary Medicine Hannover, Buenteweg 17, 30559 Hannover, Germany*
[b] *Merial, 29 Avenue Tony Garnier, Lyon 69007, France*

ARTICLE INFO

Article history:
Received 29 May 2009
Received in revised form 3 September 2009
Accepted 10 September 2009

Keywords:
Dictyocaulus viviparus
Lungworm
Major sperm protein
Milk ELISA
Dairy cows
Eprinomectin

ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against the bovine lungworm *Dictyocaulus viviparus* in milk was established. This test is based on recombinant major sperm protein (MSP) as the antigen and ELISA results are expressed as optical density ratio (ODR) values. The cut-off value of the milk ELISA was determined as the arithmetic mean of negative milk samples plus three standard deviations (SD). Specificity and sensitivity were 100% and 97.5%, respectively, using either milk or serum samples as positive control to calculate the ODR. Therefore, the presented recombinant antigen-based ELISA is suitable for routine veterinary diagnosis of exposure to bovine lungworms using milk samples instead of sera.

To assess the course of antibody titres following lungworm infection, milk and serum samples from experimentally infected dairy cows were collected over a period of 23–30 weeks in three trials. The milk and serum antibody titre curves showed strong Pearson correlation coefficients in all three trials (Trial 1 = 0.85; Trials 2 and 3 = 0.93). In milk *D. viviparus*-specific antibodies exceeded the cut-off value 30–32 days post-infection (dpi) and remained above this value until day 112–138 post-infection (pi) with an overall detection period of 79–107 days. Treatment with eprinomectin during the pre-patent period prevented larval shedding and the antibody response was eliminated; treatment during patency similarly caused a cessation of larval shedding, but had no effect on the pattern of antibody responses compared to the untreated, infected controls.

1. Introduction

The bovine lungworm *Dictyocaulus viviparus* is an important helminth parasite of cattle causing parasitic bronchopneumonia. Infections result in significant economic losses due to illness or even death of affected animals. Regardless of the broad spectrum of efficient anthelmintic drugs as well as a live vaccine against *D. viviparus* there is no sign of a decrease in the prevalence of this nematode (Eysker et al., 1994; Woolley, 1997; Holzhauer et al., 2003; Høglund et al., 2004; Jimenez et al., 2007; Wapenaar et al., 2007). Notably, clinical symptoms of the parasitic bronchitis are not pathognomonic and the sensitivity of the Baermann technique is low and inadequately reproducible (Pfeiffer and Supperer, 1980; Rode and Jørgensen, 1989; Schnieder et al., 1989; Eysker, 1997). For that reason and also to be able to perform epidemiological studies, various enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies against the bovine lungworm in serum samples have been established (Boon et al., 1982; de Leeuw and Cornelissen, 1991; Wassall, 1991; Tenter et al., 1993). These tests were based on crude or somatic antigens extracted from adult and larval stages of *D.*

* Corresponding author. Tel.: +49 511 953 8796; fax: +49 511 953 8870.
E-mail address: christina.strube@tiho-hannover.de (C. Strube).

0304-4017/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.vetpar.2009.09.002

viviparus, which require the maintenance of infected donor animals.

The application of a recombinant antigen in an ELISA, namely the major sperm protein (MSP), has increased the sensitivity for lungworm-specific antibodies in serum (Schnieder, 1992). This protein is most likely identical with the 17 kDa protein isolated from somatic antigens of adult worms which has been already used as ELISA antigen (de Leeuw and Cornelissen, 1991, 1993; Cornelissen et al., 1997). Recombinant expression of MSP has been carried out as a glutathione-S-transferase (GST) fusion protein (GST-MSP). The calculated specificity and sensitivity of the ELISA based on serum samples from experimentally infected cattle were >99% and 93%, respectively (Schnieder, 1992). Development of the recombinant antigen-based ELISA using Immobilizer™ Amino-plates (Nunc) has improved its sensitivity and thus it has been judged as an appropriate serological method for routine diagnosis of lungworm infections in cattle (von Holtum et al., 2008). The authors also have shown that there are no cross-reactions of recombinant MSP with serum antibodies from cattle infected with the gastro-intestinal nematodes *Ostertagia ostertagi* and *Cooperia oncophora*. Furthermore, in this evaluation it has been proved that GST has no impact on the detection of *D. viviparus* antibodies.

The aim of the present study was to evaluate a milk ELISA for the detection of antibodies against the bovine lungworm using recombinant MSP as the antigen. Such a test would not only offer the ability to detect exposure to infection in individual cows but also to obtain information about the infection status of dairy herds by using bulk milk samples providing an opportunity for easy herd monitoring and epidemiological studies. Another advantage is that the sampling is easier and can be done by the farmer himself in contrast to the bleeding of animals for serum samples. Furthermore, the influence of an anthelmintic treatment during prepatency and patency on the development of antibodies during induced lungworm infection has been examined to clarify its possible impact on the results of routine diagnosis and seroepidemiological studies.

2. Materials and methods

2.1. Experimental trials and sample collection

For the evaluation of the milk ELISA three trials with experimentally infected dairy cows housed at the University's research farm with no access to pasture were conducted. Twenty animals were included in Trial 1, 23 in Trial 2, and 22 in Trial 3. Each dairy cow was orally infected with 2000 third stage larvae (L3) of *D. viviparus*. Prior to experimental infection the cows were demonstrated to be lungworm-free by faecal examination and serum ELISA performed as described by von Holtum et al. (2008). Beside obtaining information on the course of antibody response in serum and milk samples, the impact of an anthelmintic treatment (eprinomectin, Eprinex®; Merial) on the antibody titre was investigated. Therefore, the cattle of Trials 2 and 3 were divided into two groups. Group I of Trial 2 (12 animals) was treated with eprinomectin during prepatency on day 18 post-infection (pi) and group II (11 animals) on day 49 pi. Group I of Trial 3 (11 animals) was treated during patency on day 49 pi whereas group II (11 animals) was left untreated.

Individual milk, serum, and faecal samples were collected at weekly intervals for 30, 24 and 23 weeks pi in Trials 1, 2 and 3, respectively. Faecal samples (2× 10 g per animal and sampling date) were examined immediately with the Baermann technique (Baermann, 1917) to verify successful experimental infections and anthelmintic treatment, respectively. The milk samples were centrifuged at 1500 × *g* for 9 min and the fluid between the sediment and the creamy top layer was recovered and stored for later use. Until performing the ELISA experiments milk and serum samples were stored at −20 °C.

2.2. ELISA

2.2.1. Determination of antigen concentration and milk sample dilution

The development of the ELISA for the detection of antibodies in milk was based on recombinantly expressed *D. viviparus*-MSP as a glutathione-S-transferase (GST) fusion protein (GST-MSP). Recombinant antigen production and purification was done as described previously (von Holtum et al., 2008). The recombinant GST-MSP concentration was determined using the Agilent 2001 bioanalyzer (Agilent Technologies). To ascertain the optimal combination of antigen and milk concentration, a chequerboard titration was performed. Immobilizer™ Amino-plates (Nunc) were coated with 0.06–4.00 μg GST-MSP/well diluted with 20 mM phosphate-buffered 150 mM saline (PBS, pH 7.4). The total volume was 100 μl/well. The plates were incubated overnight at 4 °C. After washing the plates three times for 5 min with PBS containing 0.05% Tween-20 (PBS–Tween) they were tapped dry and incubated for 1 h at 37 °C with 100 μl/well pooled positive and negative milk, respectively. These positive and negative milk samples were applied as a dilution series ranging from 1:1 (undiluted milk) to 1:1024 in PBS–Tween. Positive and negative pooled serum samples (100 μl/well) were diluted 1:40 with PBS–Tween and served as reference. The plates were washed as described above, tapped dry and incubated with 100 μl/well horseradish peroxidase-conjugated sheep anti-bovine IgG1 (Serotec) in a dilution of 1:20,000 in PBS–Tween and incubated for 1 h at 37 °C. Afterwards the plates were washed again and 50 μl/well σ-phenylenediamine dihydrochloride (0.4 mg/ml, Sigma–Aldrich) in 25 mM citrate/50 mM phosphate buffer comprising 0.04% of a 30% hydrogen peroxide solution were added followed by 10 min incubation in the dark at room temperature. The enzymatic colour reaction was stopped by adding 50 μl/well 2.5 M sulphuric acid. The ELx800 ELISA-Reader (Bio-Tek) was adjusted to a wavelength of 490 nm and the optical density (OD) of the samples was measured.

2.2.2. Selection of conjugate dilution

The secondary antibody used in the ELISA experiments was horseradish peroxidase-conjugated sheep anti-bovine IgG1 (Serotec). To select the optimal dilution for the milk ELISA, test experiments were carried out with 100 μl