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The value of a bulk-tank milk ELISA and individual serological and faecal examination for diagnosing (sub)clinical *Dictyocaulus viviparus* infection in dairy cows

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ABSTRACT

To test the value of a recently developed bulk-tank milk (BTM) ELISA for diagnosing (sub)clinical *Dictyocaulus viviparus* infection in lactating dairy herds under field conditions, bulk milk samples were collected from farms with or without clinical symptoms suspected to be caused by lungworm infection. Results of the BTM ELISA were compared against individual examinations for lungworm larvae in faeces and lungworm antibodies in serum from up to 20 heifers (parity 1) and up to 20 cows (parity ≥2) on the same farms. This also allowed, for the first time, to examine the value of individual faecal and serological examinations in the diagnosis of (sub)clinical lungworm infections.

In total, 33 farms participated. Of these, 16 reported clinical symptoms possibly related to lungworm infection (defined as a suspected positive clinical status or CS^+) and 17 reported having no such symptoms (CS^-). In total, 503 heifers and 649 cows were sampled. Of all faeces samples positive for lungworm larvae, 94 were from heifers (18.9% of all heifers) and 75 from cows (11.7% of all cows) ($P < 0.001$). Of all sera positive for lungworm antibodies, 130 were from heifers (26.1% of all heifers) and 113 from cows (17.5% of all cows) ($P < 0.001$).

Of the CS^- farms 41% had at least one heifer or cow shedding larvae and 71% had at least one seropositive heifer or cow. Of the CS^+ farms this was 81% and 94%, respectively. There were only 4 farms, all CS^-, where none of the animals were found shedding larvae and all animals tested seronegative. This implies that on 76% of the CS^- farms lungworm infection circulated unnoticed.

On all CS^+ farms the suspicion that lungworm caused the respiratory symptoms was confirmed by the individual faecal and serological examinations, whereas the BTM ELISA confirmed presence of lungworm on half of the CS^+ farms. The latter in particular occurred on farms with the more severe outbreaks. Overall, of 32 available BTM samples 10 tested positive (8 of 15 CS^+ and 2 of 17 CS^- farms). For diagnosing suspected lungworm disease it was concluded that testing a BTM sample might suffice in case of moderate to severe outbreaks. However, in case of a mild outbreak with just a few animals coughing, examining individual animals has to be preferred over testing a BTM sample. The likelihood to detect lungworm infection is higher if heifers are sampled compared to cows.

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Sensitivity of the BTM ELISA was 35.7% if the presence of at least one seropositive and/or one larvae shedding animal in the herd was used to define lungworm positive farms. On average, at least 30% of the herd had to be seropositive before the BTM ELISA was found positive for lungworm antibodies. Results indicate that the BTM ELISA in its current form does not appear to be suitable for surveys on the prevalence of lungworm presence on farms. However, this BTM ELISA might be used in large-scale surveys to detect, for instance, annual changes in percentage positive farms, as long as it is recognized that positivity is more closely related to incidence of lungworm disease than to prevalence of lungworm infection.

1. Introduction

Dictyocaulus viviparus infection was for a long time considered to be of particular clinical relevance in pastured first-year calves. However, during the last two decades lungworm outbreaks have increasingly been seen in adult dairy herds (Holzhauer et al., 2003; Van Dijk, 2004; Wapenaar et al., 2007; Muskens and Otten, 2009) with large economic consequences (Woolley, 1997; Holzhauer et al., 2011).

Current diagnosis of lungworm disease depends on either detecting larvae in the faeces or using a serological assay detecting antibodies. Detecting larvae in the faeces is time-consuming and is thought to be an insensitive technique, especially in adult cattle (Fiedor et al., 2009). The two serological assays currently available, a lungworm ELISA formerly known as the Ceditest® (Lelystad, The Netherlands) and the recombinant major sperm protein (MSP) ELISA (Hannover, Germany), have been shown to be very specific and sensitive for lungworm infections based on results from both experimental and field infections in previously lungworm-naïve animals (Cornelissen et al., 1997; Von Holtum et al., 2008). However, these tests require sampling individual animals. Recently, Fiedor et al. (2009) adapted the MSP ELISA (Von Holtum et al., 2008) for use on milk samples. Based on experimentally infected cows that were lungworm-naïve, the milk ELISA was reported to have a specificity of 97.5% and a sensitivity of 100% for individual cows. A major advantage of a milk ELISA is that it allows collecting just one bulk-milk sample which may be of great value for diagnosing lungworm outbreaks in adult herds, large-scale epidemiological studies and to monitor herd health thereby potentially preventing disease and production losses as suggested by Fiedor et al. (2009).

This recently developed lungworm milk ELISA has already been applied in large scale surveys using bulk-tank milk samples in Belgium (Bennema et al., 2009) and Sweden (Höglund et al., 2010). However, this assay has not been validated for use under natural field conditions where cows may have been exposed repeatedly to lungworm year after year, and consequently may have varying levels of immunity against reinfections. It has been observed that lungworm-diseased adult cows may not excrete lungworm larvae nor become positive in an ELISA based on adult worm derived antigens (Ploeger, 2002; Holzhauer et al., 2003; Wapenaar et al., 2007). These findings conform to observations made more than half a century ago on the epidemiology of lungworm outbreaks in adult cattle (Michel and Shand, 1955; Michel and MacKenzie, 1956). Thus, if not all cows in a lungworm-exposed herd may become serologically positive, the question is how many animals need to be serologically positive before the bulk-tank milk (BTM) ELISA returns a positive result. Fiedor et al. (2009) tried to resolve this issue by milk dilution experiments, but this cannot substitute testing validity of the milk ELISA in the field. A similar lack of validation for field conditions actually applies for the individual serum ELISAs as well.

The present study was set up to test the value of the BTM ELISA as developed by Fiedor et al. (2009) under field conditions, both with respect to diagnosing lungworm outbreaks and in detecting subclinically circulating lungworm infection *per se*. Farms with or without clinical symptoms supposed to be due to lungworm infection were sampled for bulk milk. Individual heifers and cows were sampled for faeces and serum at the same time. The study also allowed, for the first time, to assess the value of individual faecal and serological examinations in the diagnosis and detection of lungworm infections in the lactating dairy herd.

2. Materials and methods

2.1. Study design and selection of farms according to clinical status

Veterinary practices within 50 km around Utrecht were approached to report farms with and farms without signs of clinical lungworm infection for participation in this study. From early August to the end of October 2010 a total of 33 farms were visited. The dairy herds on all farms were pastured during the summer and not treated with an anthelmintic during the grazing season prior to our visit. At our visit, the herd was still pastured for at least part of the day. All visits were carried out by the same two well-instructed people.

Of the 33 farms, 16 suspected having clinical lungworm infection in their herd and 17 farms reported having no clinical problems. During our visit, it was recorded whether there were animals coughing or showed an increased respiratory rate. The number of animals showing symptoms was recorded as an indication of the severity of the clinical problems in the herd. Our observations during the visit confirmed the reported clinical status on 31 farms. Of the remaining two farms one had reported having no problems, but during our visit cattle showed obvious clinical respiratory signs (coughing and increased respiratory rate). The other farm reported having lungworm problems, but during the visit no coughing or other signs of respiratory