Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Preventive Veterinary Medicine 68 (2005) 277–288
PREVENTIVE VETERINARY MEDICINE
www.elsevier.com/locate/prevetmed

Assessment of the repeatability of a milk *Ostertagia ostertagi* ELISA and effects of sample preparation

Johannes Charlier [a,*], Luc Duchateau [b], Edwin Claerebout [a], Jozef Vercruysse [a]

[a] *Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[b] *Department of Physiology, Biochemistry and Biometrics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*

Received 16 August 2004; received in revised form 14 January 2005; accepted 19 January 2005

Abstract

An indirect *Ostertagia ostertagi* ELISA on milk is a promising diagnostic tool in bovine parasitology. Interpretation of the test results requires a good knowledge of the test characteristics. In this study, border effects, the repeatability of the ELISA and the effect of different factors such as storage, skimming and freeze–thaw cycles of the milk samples were investigated. The border effects trial showed that significant border effects can occur. The repeatability trial was conducted over 3 days. An alternative graphical technique to assess the repeatability over a large number of ELISA plates measured over different days was developed. From these graphs, it was obvious that the ODR values obtained on the third day were deviating from the values on the first and second day. On the third day, also abnormal control values were observed. When the control values were normal, 94% of the variability was explained by the milk sample and 6% by assay variability. The expected 95% range of the difference of 2 ODR readings of the same sample on the same plate and the same sample on different plates was −0.14 to 0.14 and −0.16 to 0.16. No extra variability was observed when samples were tested on a different day, however these results are based on the measurement of 2 days. Storage for 2–4 days at 4 °C, using whole milk instead of skimmed milk and up to 2 extra freeze–thaw cycles of the milk samples did not significantly affect the test results.

Keywords: *Ostertagia ostertagi*; ELISA; Validation; Repeatability; Sample preparation

* Corresponding author. Tel.: +32 9 264 7400; fax: +32 9 264 7496.
E-mail address: johannes.charlier@ugent.be (J. Charlier).

0167-5877/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2005.01.011

1. Introduction

Although gastrointestinal (GI) nematode infections in adult cows are usually subclinical, they have been associated with decreased levels of milk production (Gross et al., 1999; Sanchez et al., 2004). The major problem however remains to identify the herds where the infection level is high enough to justify an anthelmintic treatment (Vercruysse and Claerebout, 2001). Diagnostic techniques such as fecal egg counts and serum pepsinogen assays have been shown to be of limited use in adult dairy cattle (Ploeger et al., 1989, 1990; Berghen et al., 1993). Currently, detection of anti-*Ostertagia ostertagi* antibody levels is considered as a very promising parameter (Eysker and Ploeger, 2000; Sanchez et al., 2002a) and especially determination of antibody levels in bulk tank milk would be suitable for a regular monitoring of the parasitic infection level of a herd. In previous studies, significant associations were found between bulk tank milk antibody levels and certain management practices known to be associated with infection levels (Guitián et al., 2000; Caldwell et al., 2002; Sanchez and Dohoo, 2002). In recent surveys in Canada and Belgium, significant negative relationships were found between bulk tank milk antibody levels and milk production (Sanchez and Dohoo, 2002; Charlier et al., 2005). These results demonstrate the potential of this technique as a standard monitoring tool of the GI nematode herd infection level.

However, a good interpretation of test results is impossible without knowledge about the test characteristics. Previously, the repeatability of the test was evaluated without discriminating between serum and milk samples. Two different graphical tools, the concordance correlation coefficient (CCC) plot (Lin, 1989) and the Bland–Altman plot (Bland and Altman, 1986) were used for this purpose (Sanchez et al., 2002b). Both plots indicate to what extent 2 replicates of a set of samples have similar values. In both plots one replicate is compared with another. It is obvious that the large amount of replication used in this validation study leads to an even larger number of plots as relationships can only be represented pairwise. Therefore, in this paper a new graphical method is proposed to assess the variability of several measurements of a milk *O. ostertagi* ELISA on the same sample over time. Furthermore, the effect of different sample preparations on the test results is investigated.

2. Materials and methods

2.1. Milk samples

A total of 82 milk samples were collected at 42 dairy herds in Flanders by convenience sampling. The samples consisted out of 42 bulk tank milk samples from an equal number of herds and 40 individual milk samples from two herds.

After collection, the samples were handled following a standard procedure. The samples were centrifuged ($16{,}000 \times g$ for 5 min), the fat was removed and the underlying supernatant was collected and frozen ($-20\,^{\circ}C$). All this was done on the day of sample collection. To remove all fat, the samples were thawed and recentrifuged ($16{,}000 \times g$ for