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Diagnosis of ovine caseous lymphadenitis by blood and milk gamma interferon assays

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ABSTRACT

Diagnosis of *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) infection in sheep herds, caseous lymphadenitis (CLA), usually depends on individual animal testing by blood based immunoassays. This study was carried out to investigate the reliability of newly developed milk and heparinized whole blood based gamma interferon (IFN-γ) assays for the diagnosis of ovine CLA. It was conducted on 65 (16 lactating and 49 non-lactating) sheep at Semella village, Gharbia Governorate (in the central region of the Egyptian Delta). Ten (15.3%) animals, 5 lactating and 5 non-lactating, had superficial lymph node abscesses at the head region. *C. pseudotuberculosis* recombinant phospholipase D (rPLD) was used as a stimulating antigen for blood and milk IFN-γ assays. Blood IFN-γ assay had showed a sensitivity and specificity of 70% and 80% compared to 78.1% and 72.7% of milk IFN-γ assay. A total of 18 (27.6%) animals were positive by blood IFN-γ assay. In addition, 7 out of 10 (70%) of clinically affected animals were positive and 76.5% of apparently normal animals were negative by blood IFN-γ assay. On the other hand, a total of 7 (43.7%) lactating animals were positive by milk IFN-γ assay. Considering the relation between the existence of a superficial lesion and milk IFN-γ assay, 4 out of 5 (80%) affected lactating ewes were positive and 72.7% of apparently normal lactating ewes were negative. These findings declared that the existence of superficial lymph node abscesses in sheep is highly indicative of the true disease status of the tested animals. In addition, milk IFN-γ assay is a newly developed technique for the diagnosis of CLA in lactating ewes had a similar performance to blood IFN-γ assay. However, further wide scale studies are required for testing, evaluation and standardization of milk IFN-γ assay on tank milk to judge its validity to identify the disease status on the herd level, particularly in countries where there are dairy small ruminant herds.

1. Introduction

CLA is a bacterial disease of sheep which negatively affects sheep production and trading (Arsenault et al., 2003). The repulsive appearance of the affected animal, weight loss, wool loss in addition to the risk of zoonotic transmission are reasons to consider CLA as an important disease (Peel et al., 1997; Williamson, 2001). CLA is caused by *C. pseudotuberculosis* and has two forms; external and internal which are mainly characterized by abscessiation of lymph nodes (Al-Gaabary et al., 2009). Not all CLA cases show obvious clinical signs, some cases are apparently normal in spite of its ability to transmit the infection to the susceptible animals (Sunil, 2006). Therefore, it is essential to use especial diagnostic tests which are able to detect unapparent form of the disease. Despite of scientific debate about its sensitivity and specificity, serological tests are mostly used for screening against CLA (Oreiby, 2015). Humoral immunity against CLA is usually detected by antibody-targeting tests, particularly ELISA. On the other hand, gamma interferon is usually used as a marker of cellular immunity against *C. pseudotuberculosis* infection. For this purpose, IFN-γ assay is used for the diagnosis of CLA. In many previous studies, commercially available IFN-γ assay designed to diagnose bovine tuberculosis had been used successfully to detect CLA cases in both sheep and goat (Prescott et al., 2002; Menzies et al., 2004). Studies had extensively investigated the validity of blood and pus as diagnostic samples for CLA in contrast to milk on which studies are very limited. Recently, a few studies have been conducted to detect antibodies against *C. pseudotuberculosis* in milk (Nagel-Alne et al., 2015). However, studies to detect the cellular response of milk leukocytes against *C. pseudotuberculosis* had never been reported previously. So, this study aimed to investigate the validity of milk IFN-γ assay for diagnosis of CLA which is not reported previously, to compare between the performance of whole blood and milk IFN-γ assays.

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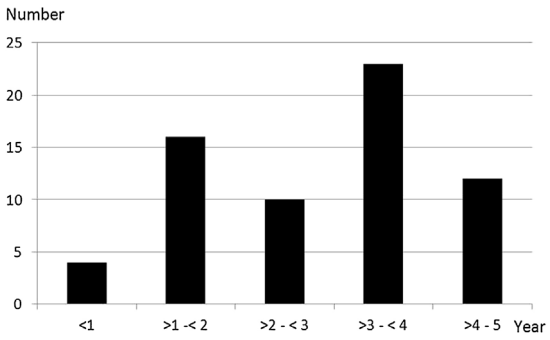

Fig. 1. Age distribution of the tested animals.

2. Material and methods

2.1. Study area and sample collection

This study was conducted at Semella village, Gharbia Governorate, which is located in the central region of the Egyptian Delta. Due to the abundance of cultivated lands at this locality, shepherds are allowing their animals to graze at day light then animals are gathered in a fixed pen at night. A flock consisting of 65 sheep, some of which had a previous history of CLA cases confirmed by PCR, were used in this study. The animals were of different ages, which is illustrated in Fig. 1. Clinical signs, pregnancy and lactation status were recorded. Whole heparinized blood samples were collected from all animals in addition to milk of lactating ewes. Samples were collected aseptically and transported to the laboratory at ambient temperature with minimal of delay.

2.2. IFN-γ assay

For the detection of cellular immunity against *C. pseudotuberculosis*, a commercially available bovine IFN-γ BOVIGAM® kit (Prionics AG, Switzerland) was used. Leukocytes in blood and milk were sensitized by a commercially available *C. pseudotuberculosis* rPLD antigen (Hyphen Biomed, France). Different amounts of rPLD were tested to determine a suitable concentration for blood and milk IFN-γ assays. In 6 wells tissue culture plates (CELLSTAR® Tissue Culture Plates, Greiner Bio-One), blood and milk samples (1.5 and 3 ml, respectively) were added after a gentle mixing. Leukocytes stimulating antigen was added to each sample followed by gentle repeated pipetting to ensure homogenization. After 12 h incubation at 37 °C in a humidified atmosphere, plasma and milk serum samples were harvested by centrifugation at 500*g* for 10 min at 22 °C. The IFN-γ assay was conducted according to the producer. A minor modification for milk serum samples was performed; volume of milk serum was the double of plasma to overcome the dilution effect of the initial milk sample volume. Reading of results was performed at 450 nm with 630 nm as a reference wavelength.

2.3. Statistical analysis

Nonparametric ROC analysis based on atypical gold standard (clinical status of the tested animal) was performed according to Zhou et al. (2005). ROC curves, area under curve (AUC), optimum cutoff, sensitivity and specificity were estimated for both whole blood and milk IFN-γ assays using STATA 13.1® software.

Table 1
IFN-γ production in response to different antigen concentrations.

Animal	Clinical status	Sample type	Antigen concentrations[a]		
			2 μg	4 μg	8 μg
A	Affected	B	0.089	0.274	0.314
		M	0.057	0.074	–
B	Normal	B	0.066	0.076	0.075
		M	0.081	0.103	–
C	Normal	M	0.075	0.076	0.088
D	Normal	M	0.103	0.095	0.098

B: blood, M: milk, –: insufficient sample volume.
[a] Sample volume used (1.5 ml blood and 3 ml milk).

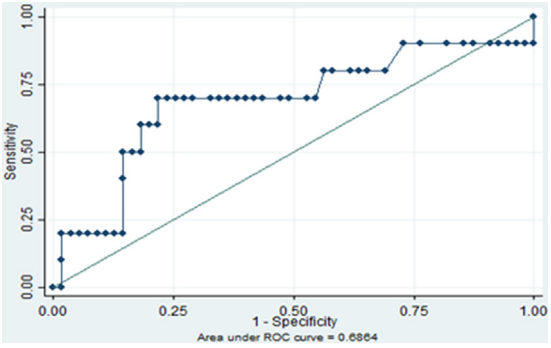

Fig. 2. ROC curve of whole blood IFN-γ assay.

3. Results

3.1. Clinical and descriptive aspects

Superficial lymph node abscesses were detected in 10 (15.3%) out of 65 animals. All lesions were detected in parotid lymph nodes, except for one animal which had a mandibular lymph node abscess. Sixteen animals were lactating (24.6%) of which 5 were suffering from lymph node abscesses.

3.2. Determination of the antigen concentration

Serial concentrations of the stimulating antigen were added to the blood and milk samples. Accordingly, 4 μg per sample was selected as a suitable concentration. IFN-γ response to different antigen concentrations in blood and milk samples is shown in Table 1.

3.3. Whole blood IFN-γ assay

Based on ROC curve analysis, AUC was 0.686 and the optimal cutoff point of the highest sensitivity (70%) and specificity (78.1%) was ≥0.110. ROC curve is shown in Fig. 2.

A total of 18 (27.6%) animals were positive by whole blood IFN-γ assay. Out of the 47 apparently normal cases, 11 (23.4%) were blood IFN-γ assay positive and 36 (76.5%) were negative. On the other hand, 7 (70%) out of 10 cases with suspected lymph node lesions were positive and 3 (30%) were negative.

3.4. Milk IFN-γ assay

The optimum sensitivity (80%) and specificity (72.7%) of milk IFN-γ assay was observed at a cutoff value of ≥0.111. The AUC was 0.846 which indicates the high reliability of the test as a predictor of clinical status of the animal. The ROC curve of milk IFN-γ assay is shown in Fig. 3.