Validation of indirect milk-ELISA based on LPS antigen for screening dairy brucellosis

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Introduction

In dairy cattle the milk ring test(MRT) has been widely used for screening of brucellosis but its specificity is not good when prevalence is low. An indirect ELISA has developed to detect antibodies against *Brucella* in milk samples as improving non-specific reactions in MRT. But practically little has been screened ELISA for diagnosis of dairy brucellosis in Korea.

Aim

Thus, we evaluate indirect milk-ELISA coated by *Brucella abortus* smooth LPS for detecting *Brucella* antibodies in milk of cows including one suspected of being infected.

Methods

The assays used *B. abortus* smooth LPS antigen that was produced by hot-phenol extraction. Following by coating LPS in 0.01M phosphate buffered saline on a polystyrene matrix, all plates were incubated at 4°C and then evaluated. A total of 3890 bulk milk samples were collected from dairy herds whose results of MRT were positive or negative together with comparing to commercial milk-ELISA and serological tests.

Results

The cut-off value determined using receiver operating characteristic (ROC) analysis was fixed at 450nm OD of 0.22 [21.9 likelihood ratio (LR)] giving the sensitivity of 93.8 % with a 95% confidence interval (CI) of 73.97–99.02% and the specificity of 93.62% (CI, 94.64–99.42). LPS-milk ELISA moderately correlated with MRT (agreement, 99.3%; kappa, 0.431) and commercial milk-ELISA test (agreement, 99.8%; kappa, 0.799) with an acceptable specificity (99.82% and 99.92%, respectively). The result exhibited moderate or good correlation with serological tests (agreement, 78.4%; kappa, 0.460).

Conclusion

In conclusion, this study validated that LPS-coated indirect ELISA designed for diagnosis of dairy brucellosis with milk samples had reliable efficiency. We suggest newly designed in-house LPS-milk ELISA could be a procedure which can readily apply, furthermore, it could help to establish as a screening test for dairy brucellosis in Korea.