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Detection of antibodies to *Salmonella* lipopolysaccharide in muscle fluid from cattle

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Objective—To compare muscle fluid with serum samples for detection of antibodies to *Salmonella* lipopolysaccharide.

Sample Population—Muscle fluid and serum samples from 2 cattle populations: 1 from the island of Bornholm with no history of salmonellosis (n = 39), and the other from the *S dublin*-enzootic areas of Jutland (n = 144).

Procedure—*Salmonella dublin* (O:1,9,12), *S typhimurium* (O:1,4,5,12), and *Salmonella* O:9-blocking ELISA were used for testing the samples.

Results—In the *S dublin* ELISA, all serum and muscle fluid samples from cattle on the island of Bornholm had OD_{450} values well below the cutoff value (0.5). For samples obtained from cattle in the enzootic areas of Jutland, high correlation was found between serum and muscle fluid samples ($r_s = 0.89$, $P < 0.001$). In addition, 19% (28/144) of the cattle had ELISA-positive muscle fluid and serum samples; 2% (3/144) had positive results for muscle fluid only, whereas 1 animal had positive results for serum only ($\kappa = 0.91$, $P < 0.0001$; sensitivity and specificity of 97%). The same samples had similar significant correlation in the *S typhimurium* ELISA ($r_s = 0.88$, $P < 0.001$, $\kappa = 0.7$, $P < 0.001$; sensitivity of 73% and specificity of 98%) and the O:9-blocking ELISA ($r_s = 0.49$, $P < 0.001$).

Conclusion and Clinical Relevance—Muscle fluid samples taken at slaughter can be used as a practical alternative to serum samples for surveillance of *Salmonella* infections in cattle. (*Am J Vet Res* 1997;58:334-337)

Salmonellosis is an important disease in cattle, and is mainly caused by the *Salmonella* serotypes *dublin* and *typhimurium*.[1] Most infected animals develop humoral immune response against the O antigens.[2] In previous studies,[3-5] we tested, in a lipopolysaccharide (LPS)-based ELISA, serum and milk samples from herds in enzootic areas with clinical history of *S dublin* infection, and herds from an island with no reports of bovine salmonellosis. Results indicated sensitivity and specificity of 100% each for serum samples,[3] and 100 and 95%, respectively, for milk samples.[5] Testing of bulk milk samples in an LPS-ELISA was found to be a useful alternative to testing serum samples for screening dairy herds.[5] However, by use of retrospective identification of infected herds, muscle fluid samples from slaughtered animals at abattoirs may be another useful alternative to serum samples, particularly in beef cattle herds. Muscle fluid samples are easy to obtain and are economically useful for large-scale screening programs. This aspect has been investigated in swine, but not in cattle.[6] Thus, the purpose of the study reported here was to evaluate usefulness of muscle fluid from cattle for detection of antibodies to *Salmonella* LPS, using various ELISA.

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Materials and Methods

Study design—Blood and muscle samples from 2 populations of cattle were included: 1 from the island of Bornholm with no history of bovine salmonellosis (n = 39), and another from the *Salmonella dublin*-enzootic areas of Jutland (n = 144). The 2 cattle populations from different herds were slaughtered at different abattoirs.

At the abattoirs, 10 ml of blood was collected from each animal at the beginning of the slaughter line. The serum was collected after overnight coagulation of blood samples.[a] At the end of the slaughter line, approximately 10 g of the sternomastoideus muscle of each animal was collected and transferred to a specially designed muscle fluid collection system, consisting of a polyethylene funnel connected to a 10-ml polyethylene tube.[b] The funnel was covered and placed in a –20 C freezer overnight. In the morning, the collection system was incubated at 20 to 22 C (room temperature) for approximately 5 hours to allow muscle to thaw. On average, 0.5 to 2 ml of fluid was collected in the underlying tube from the muscle samples. The muscle fluid and serum samples were kept at –20 C until analysis.

Enzyme-linked immunosorbent assay procedures—Indirect and blocking ELISA for detection of antibodies to *S dublin* and an indirect ELISA for detection of antibodies to *S typhimurium* were performed as described.[4,6] The LPS extracts were essentially free of proteins, as tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis,[3] and 3 different preparations had similar ELISA reactions (data not shown). All serum and muscle fluid samples were tested in triplicate on the same occasion, and the results were recorded as the geometric mean of 3 measurements.

In a preliminary study, we found that a tenfold-less dilution of muscle fluid, compared with serum, resulted in comparable OD or blocking values for paired samples. The serum ELISA was reported to have sensitivity and specificity of 100% for *S dublin*-infected herds,[3] and sensitivity of 92% and specificity of 100% for *S typhimurium*-infected herds.[4]

In the indirect ELISA (O:1,9,12 and O:1,4,5,12, both with cutoff OD_{450} of 0.5), the test sera were diluted 1:100, and the muscle fluid samples were diluted 1:10 in phosphate-buffered saline solution containing 0.5*M* NaCl, 0.1% Tween-20, and 1% polyvinylpyrrolidone-40T.[c] The dilutions were added to the LPS-coated micro-well plates Horse radish peroxidase-labeled goat antiserum to bovine IgG diluted 1:2,000, indicator (tetramethylbenzidine),[d] and hydrogen peroxide substrate were used. A test set-up was considered valid with negative-control OD_{450} of 0.02 to 0.09 in both tests and positive-control OD_{450} of 3.0 to 3.2 in the *S dublin* ELISA and 2.0 to 2.2 in the *S typhimurium* ELISA