Investigation of a listeriosis epizootic in sheep in New York state

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Objective—To investigate potential sources of an epizootic of listerial encephalitis, using molecular diagnostic and typing methods.

Sample Population—A flock of about 655 sheep.

Procedure—An epizootiologic investigation was performed. Clinical, feed, and environmental samples were tested for *Listeria monocytogenes*, using polymerase chain reaction and culture methods; recovered isolates were "fingerprinted," using an automated ribotyping system.

Results—*Listeria monocytogenes* was recovered from brain specimens of 7 sheep with clinical signs of listerial encephalitis. All clinical isolates had fingerprints identical to those of isolates from farm equipment used to transport silage. Corn silage, which was not fed to the sheep, also contained *L monocytogenes* of the same pattern type as defined by ribotyping. *Listeria monocytogenes* was not isolated from the stored haylage designated for feeding the sheep (the cut-off point for isolation being $< 10^2$ colony-forming units/g).

Conclusions—Corn silage was implicated as the source of a listeriosis epizootic. It appears to have cross-contaminated the haylage destined for the sheep during handling with a front-end loader. Suspension of silage feeding coincided with cessation of listeriosis cases.

Clinical Relevance—Use of advanced molecular techniques can help to identify the sources and restrict the scope of an epizootic. In epizootics, a single *L monocytogenes* strain can lead to infection of multiple animals, with rapid progression of the disease. (*Am J Vet Res* 1997;58:733–737)

Listeria monocytogenes is an important pathogen of human beings and animals.[1] The incidence of listeriosis in ruminants has markedly increased over the past few decades,[2,3] and feeding of poor-quality silage seems to have an important role in transmission of this disease.[2,4,5] The identification of the source in cases of animal listeriosis is often complicated because *L monocytogenes* is ubiquitous. This can lead to a situation where its isolation from feed or environmental samples may be coincidental rather than evidence for a causal relation. Application of an accurate, discriminatory strain typing method in conjunction with an epizootiologic investigation is, therefore, necessary to allow reliable and rapid identification of the source.

Phage typing,[6,7] pyrolysis mass spectrometry,[4] multilocus enzyme electrophoresis,[8] and randomly amplified polymorphic DNA[9-12] have been used to type *L monocytogenes* strains. A recently described automated ribotyping system for differentiating *L monocytogenes* strains[13] offers substantial advantages over other typing methods. This system allows species identification as well as discrimination beyond the species level. For example, a total of more than 50 pattern types have been described for *L monocytogenes*.[14] Furthermore, an appreciable database cataloging these pattern types in general,[14] as well as among ruminant isolates,[15] has been established. These baseline data help to assess the relevance of finding a particular pattern type in epidemiologic investigations. Results obtained through this automated ribotyping system are standardized and comparable between laboratories[13] and will, therefore, allow maintenance of a database of human and animal outbreak strains, potentially permitting global assessment of the epidemiology of *L monocytogenes*. We describe the use of ribotyping and **polymerase chain reaction** (PCR)-based diagnostic tools to aid in the investigation and rapid resolution of an epizootic of listerial encephalitis in sheep.

Materials and Methods

Outbreak investigation—On Dec 31, 1995, we were contacted because 4 animals in a 655-head sheep flock had clinical signs typical of listerial encephalitis. A case of listeriosis was defined as an animal with typical clinical signs of listeriosis. Clinical signs of disease were evaluated by a veterinarian. All sheep on the farm were inspected twice daily by the farm manager to detect animals with signs of disease. A questionnaire was constructed and data were collected by personal interview with the farm manager.

Silage pH was determined as described.[5] Silage samples were collected from the open end of the silo bags; if visual inspection revealed areas with apparently improper fermentation, samples were collected from these locations.

Statistical analysis—Significance of association between each hypothesized categorical factor and the risk of developing listeriosis was evaluated, using χ^2 analysis. Factors examined included sex of the animal, pregnancy status, and location at the time of the epizootic. The magnitudinal effect of each factor on the risk was quantified, using the odds ratio.

Pathologic examination—Brain specimens of affected animals were fixed in buffered 10% formalin. After progressive dehydration in alcohols, the tissues were embedded in paraffin and 5-µm sections were stained with H&E. Immu-

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