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EFFECTS OF MODIFIED CARY AND BLAIR MEDIUM ON RECOVERY OF NONHEMOLYTIC *PASTEURELLA HAEMOLYTICA* FROM ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*) PHARYNGEAL SWABS

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ABSTRACT: Modified Cary and Blair transport medium (MCB) was evaluated for recovery of *Pasteurella* spp. from pharyngeal swabs of healthy Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). In experiment one, three pharyngeal swabs were collected from each of 25 bighorns. *Pasteurella haemolytica* was recovered from 21 of 25 swabs tested almost immediately and from 16 of 25 swabs held in MCB medium at about 22 C for 24 hr before testing ($P > 0.10$). Recovery of *P. haemolytica* decreased ($P < 0.005$) to 1 of 25 when swabs were held in MCB medium at about 22 C for 48 hr before testing. In experiment two, four pharyngeal swabs were collected from each of ten bighorns and held in MCB medium at about 5 C for ≤5, 24, 48, or 72 hr prior to testing. Recovery was unaffected by storage at 5 C; *P. haemolytica* was isolated from all 40 of these samples. All *Pasteurella* spp. isolates were nonhemolytic *P. haemolytica*. In experiment one, most isolates were serotype 4; in experiment two, serotype 3 was most common. We propose that MCB medium is effective for transporting bighorn sheep pharyngeal swabs for *P. haemolytica* screening because it imposes minimal or no effect on recovery when held ≤24 hr at 22 C or ≤72 hr at 5 C.

Key words: Rocky Mountain bighorn sheep, *Ovis canadensis canadensis*, *Pasteurella haemolytica*, transport media, modified Cary and Blair medium, bacteriology, diagnostics.

INTRODUCTION

The epizootiology of pasteurellosis in bighorn sheep (*Ovis canadensis*) has been extensively investigated. Research efforts have been directed toward understanding both host and pathogen characteristics that allow some populations of bighorns to harbor *Pasteurella haemolytica* without adverse effects (Thorne, 1982; Wild and Miller, 1991), while others experience devastating pneumonia outbreaks (Onderka and Wishart, 1984; Miller et al., 1991b). Findings from these studies have important biological and political ramifications for wildlife management practices, so data collection methods must be reliable and accurate.

Epizootiologic and diagnostic studies of pasteurellosis in bighorns rely on laboratory findings being representative of conditions *in situ*. Misleading results and inaccurate conclusions may be obtained if faulty techniques are used. Numerous factors can affect recovery of bacterial isolates from clinical specimens. These include sampling site, collection and transportation materials, transportation conditions, and elapsed time until sample testing (Ross et al., 1982; Wild and Miller, 1991). Transport media, such as modified Amies with charcoal (BBL Microbiology Systems, Becton Dickinson and Company, Cockeysville, Maryland, USA) and modified Stuart's media (Culturettes®, Marion Laboratories, Inc., Kansas City, Missouri, USA) may be used to facilitate recovery of organisms when immediate testing is not possible. Such media are designed to maintain viability of organisms without allowing marked growth for ≤72 hr. Although widely used, the reliability of these two media for isolating upper respiratory pathogens from humans (Ross et al., 1982) and bighorns (Wild and Miller, 1991) has been questioned, especially if bacteria are present in small numbers *in situ*.

The manufacturer's instructions for Port-A-Cul® transport tubes containing modified Cary and Blair (MCB) medium (BBL Microbiology Systems, Becton Dickinson

and Company) contain the suggestion that an anaerobic environment may be the preferred condition for transport of aerobic as well as anaerobic bacteria. We evaluated MCB medium for recovery of *Pasteurella* spp. from samples collected from apparently healthy bighorns. The transport medium was evaluated under two conditions, with samples held at 22 C for ≤48 hr, and at 5 C for ≤72 hr before evaluation.

METHODS

Pharyngeal swabs were collected from 25 Rocky Mountain bighorn sheep (*O. canadensis canadensis*) between 16 and 23 October 1990 for experiment one, and from ten bighorns on 28 September 1992 for experiment two. All bighorns sampled were from a healthy captive herd held at the Colorado Division of Wildlife's Foothills Wildlife Research Facility (Fort Collins, Colorado, USA; 40°355′N, 105°10′W). Animal husbandry techniques were as described by Wild and Miller (1991). To obtain samples, we immobilized bighorns with a mixture of 90 mg ketamine HCl (Bristol Laboratories, Syracuse, New York, USA) and 9 mg xylazine HCl (Mobay Corporation, Animal Health Division, Shawnee, Kansas, USA) per 80 kg body weight, administered intravenously. In experiment one, we collected three pharyngeal swabs from each bighorn by holding the mouth open and swabbing the palatine tonsil area with sterile 15 cm cotton-tipped swabs. We randomly assigned swabs from each bighorn to groups with designated holding periods (0, 24, 48 hr). Most swabs were placed into transport tubes containing MCB medium (Port-A-Cul® tubes), except that samples collected at time 0 were cultured directly onto 5% sheep blood agar plates (BBL Microbiology Systems, Becton Dickinson and Company) and incubated at 37 C within 3 hr of collection. Swabs were held in MCB medium at about 22 C for 24 or 48 hr before culturing and incubating at 37 C. We examined culture plates for bacterial growth after 24 and 48 hr of incubation. *Pasteurella* spp.-like colonies were isolated and identified using the techniques of Onderka et al. (1988) and Carter and Cole (1990).

In association with experiment one, we tested viability of pure cultures of *P. haemolytica* in MCB medium using fresh isolates collected from our bighorns during the experiment. Five replicate swabs containing an isolated nonhemolytic *P. haemolytica* colony, serotype 4, were placed in MCB medium and held at 22 C for either 24, 48, or 75 hr. Samples were cultured and identified as in experiment one.

In experiment two, we collected four pharyngeal swabs from each of ten bighorns as previously described. Swabs were held in MCB medium at about 5 C for ≤5, 24, 48, or 72 hr before evaluation. Bacteriology techniques were as described for experiment one, but were performed at the Colorado State University Diagnostic Laboratory (Fort Collins, Colorado).

Representative *P. haemolytica* colonies from each individual were serotyped using rapid plate agglutination with antisera 1–12 (Frank and Wessman, 1978) obtained from the United States Department of Agriculture, National Animal Disease Center (Ames, Iowa, USA). We tested differences in recovery of *P. haemolytica* over time in experiment one using the Cochran Q test and made *post hoc* pairwise comparisons using the chi-square analog to Scheffé's theorem (Marascuilo and McSweeney, 1977).

RESULTS

Delays of 48 to 72 hr in sample culturing and incubation decreased recovery of *P. haemolytica* from bighorn sheep pharyngeal swabs held in MCB medium at 22 C ($Q = 31$; $P < 0.005$), but not when samples were refrigerated at 5 C. In experiment one, *P. haemolytica* was recovered from 21 of 25 bighorn sheep pharyngeal swabs incubated immediately, and from 16 of 25 swabs held in MCB medium at 22 C for 24 hr before testing ($P > 0.10$). However, we isolated *P. haemolytica* from only one of 25 swabs after 48 hr at 22 C ($P < 0.005$). In viability tests of pure cultures, we isolated *P. haemolytica* from all 15 swabs held in MCB medium for ≤75 hr at 22 C. In experiment two, *P. haemolytica* was isolated from all 40 bighorn sheep pharyngeal swabs held in MCB medium at 5 C for ≤72 hr.

All *Pasteurella* sp. isolates were nonhemolytic *P. haemolytica*. Both catalase positive and catalase negative *P. haemolytica* isolates were recovered from three bighorns, one from experiment one and two from experiment two. *Pasteurella haemolytica* isolates from other bighorns all were catalase negative. Of 23 representative isolates from 22 bighorns in ex-

periment one, two were serotype 3, 17 were serotype 4, and four were serotype 3,4. Of 11 representative isolates from ten bighorns in experiment two, nine were serotype 3, one was 3,4, and one was not typable.

DISCUSSION

Wildlife biologists and veterinarians frequently collect diagnostic samples from animals in remote locations. Transporting samples to a laboratory is a formidable obstacle to gaining reliable data on wildlife diseases. Pharyngeal samples from bighorns swabbed directly onto blood agar plates have yielded reliable results, but require almost immediate culture and incubation (Wild and Miller, 1991). The Baltimore Biological Laboratories (BBL) 1978 package insert for Prepared Transport Media and the BBL 1984 package insert for Port-A-Cul® tubes both include information suggesting that samples should be transported to a laboratory promptly, preferably in <24 hr and no longer than 72 hr, to avoid overgrowth by rapidly growing organisms. Understanding the effects that transportation time and condition have on diagnostic samples is necessary so that biases and technique limitations are recognized. Otherwise, erroneous conclusions can be reached.

Recovery of nonhemolytic *P. haemolytica* from pharyngeal swabs of healthy bighorns was markedly greater in this study when MCB medium was used than in a previous study when modified Stuart's media and modified Amies with charcoal were used under similar conditions (Wild and Miller, 1991). This success using MCB medium may be attributable to maintenance of an anaerobic environment during transport, thus inhibiting overgrowth by other organisms. Additionally, because the medium is solid, organisms are kept moist but are not diluted as they can be in liquid media. This may be most important when *P. haemolytica* is present in small numbers.

Refrigeration of swabs in MCB medium further extended the recoverability of *P. haemolytica*. At 5 C, overgrowth by rapidly growing species of bacteria may have been inhibited due to unfavorable conditions for reproduction, thus allowing *P. haemolytica* to remain detectable. We acknowledge that, because 2 yr separated these two experiments, *P. haemolytica* strains could have changed in some way making them less fragile during transport in experiment two. No change in clinical health of the bighorns occurred during the period between experiments, but based on our serotype data, there was an apparent shift from predominantly serotype 4 in 1990 to serotype 3 in 1992. Alternatively, both serotypes may have been present during both experiments but varied transport conditions selected recovery of one serotype over the other. Ribotypes of *P. haemolytica* isolates in this study were not determined, but *Eco*RI ribotypes 1 and 11 have previously been identified in similar isolates from this herd (Snipes et al., 1992).

Data from field collections appear to support our experimental findings for the superiority of MCB medium for recovery of nonhemolytic *P. haemolytica* from healthy bighorns. In some bighorn herds in Colorado, detection of *P. haemolytica* in healthy bighorns has increased dramatically recently. Increased detection occurred when researchers began collecting pharyngeal swabs instead of, or in addition to, nasal swabs. Another marked increase in detection occurred when MCB medium was used for transport rather than modified Amies or modified Stuart's media (Miller et al., 1991a). We attribute increased detection to improved sampling techniques based on increased understanding of the effects of sampling site and transportation rather than to widespread increased prevalence of *P. haemolytica* among numerous isolated herds. Such results also have encouraged further investigation into the effects of transportation on *P. haemolytica* recovery.

The sampling techniques described here have been developed for the specific pur-

pose of detecting nonhemolytic *P. haemolytica* in apparently healthy bighorns. Epizootiological studies of pasteurellosis in bighorns rely on these data, but other potential pathogens should not be ignored in investigations of pneumonia epizootics. We do not know the suitability of MCB medium for transport of other pathogens, or even other types of *Pasteurella* spp. Recognizing these limitations, we suggest that when pharyngeal swabs from bighorns cannot be tested immediately, they should be transported under refrigeration in MCB medium. This technique appears to provide for reliable detection of nonhemolytic *P. haemolytica* even when samples are held for 72 hr before testing.

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