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Survival of *Corynebacterium pseudotuberculosis* in axenic purulent exudate on common barnyard fomites

John L. Augustine, PhD, and Harland W. Renshaw, DVM, PhD

SUMMARY

Several inanimate surfaces (eg, plastic, wood, and steel) and particulate fomites (eg, wood shavings, hay, straw, and feces), common to the environment of confined small ruminants, were inoculated with *Corynebacterium pseudotuberculosis* in axenic purulent exudate that had been surgically removed from a naturally occurring case of caprine caseous lymphadenitis. Each inoculated fomite was incubated at 37, 22, and 4 C, and the length of time that *C pseudotuberculosis* survived was determined by isolation of bacteria from the fomite. The organism remained viable longer when caseous lymphadenitis abscess contents were mixed with particulate fomites than when spread on surfaces. Incubation at lower temperatures generally extended the survival potential of *C pseudotuberculosis*. Depending on the particulate fomite and the incubation temperature, viable *C pseudotuberculosis* organisms were isolated for mean periods ranging from 7 to 55 days, whereas recovery of bacteria from surfaces varied from 1 to 8 days.

Caseous lymphadenitis (CLA) of small ruminants is a chronic disease caused by *Corynebacterium pseudotuberculosis*.[1-8,a] The disease is characterized by caseation necrosis, mainly of superficial lymph nodes and occasionally by generalized infection, that sometimes results in emaciation and death.[3,5-8,a] Although *C pseudotuberculosis* is capable of tissue invasion,[9] available epizootiologic data indicate that contamination of superficial skin wounds is the usual mode of entrance by the microorganism into the susceptible host.[3-10] Apparently, animals occasionally acquire the infection by ingestion or inhalation.[11-13] The most common mode of transmission in sheep is contamination of superficial skin wounds caused by shearing, docking, ear cutting, tagging, and castration.[3,5,6] Transmission in Angora goats, as in sheep, is thought to occur mainly by contamination of shearing wounds with purulent exudate from ruptured lymph nodes or with common barnyard fomites containing infectious microorganisms.[10] The major methods of transmission in Spanish and dairy goats are thought to be by ingestion from a contaminated environment and by environmental contamination of head wounds resulting from head butting.[1,4,10]

The potential for survival of *C pseudotuberculosis* outside the host has important implications for those interested in developing programs for control of CLA. Results from previous studies,[14-19] using different sources of infectious microorganisms, led to conflicting conclusions about the survival potential of *C pseudotuberculosis* outside the host. Whereas one study,[18] using purulent exudate as the source of microorganisms indicated survival for over a year in sterile sheep feces, another study[19] using cultured and washed bacteria indicated that survival periods did not exceed 1 week. The purpose of the present study was to determine the time that *C pseudotuberculosis* could survive in axenic purulent exudate applied to or mixed with several common barnyard fomites.

Materials and Methods

Fomites and source of purulent exudate—Sterile inanimate surfaces (eg, plastic petri dish, white pine wood chips, and rusty nails) and sterile particulate fomites (eg, white pine wood shavings, goat feces, alfalfa hay, and wheat straw) were inoculated with purulent exudate, containing a pure culture of *C pseudotuberculosis*. Rusty nails, white pine wood chips, white pine wood shavings, goat feces, and wheat straw were sterilized in an autoclave with steam at 121 C for 20 minutes and were vent dried for 30 minutes. The purulent material that was obtained from a surgically excised abscessed lymph node from a naturally occurring case of caprine CLA contained approximately 2×10^7 colony-forming units (CFU) of *C pseudotuberculosis*/ml. To determine bacterial concentration, abscess exudate was mixed 100:1 with sterile isotonic saline solution (w/w), an aliquot of the exudate-saline mixture was sonicated for 20 s at 60% intensity[b] (to aid in disrupting clumps of bacteria), and the CFU of *C pseudotuberculosis*/ml were determined by making serial dilutions in saline solution and dispensing these dilutions into Columbia blood agar base[c] pour plates. The plates were incubated at 37 C for 48 hours, and the number of colonies on the plates were counted to determine CFU of *C pseudotuberculosis*/ml.

Recovery from contaminated barnyard fomites—Sterile plastic petri dish,[d] white pine wood chip, and rusty nail surfaces were coated with a thin layer of purulent exudate, whereas the par-

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From the Department of Pathobiology U-89, College of Agriculture and Natural Resources, University of Connecticut, Storrs, CT 06268.

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[a] Augustine JL. *Bacteriologic, ecologic, serologic, and immunogenetic studies of Corynebacterium pseudotuberculosis-induced caseous lymphadenitis in small ruminants*. PhD dissertation, Texas A&M University, College Station, 1984.

[b] Virsonic Cell Disruptor, model 16-850, Virtis Co, Gardiner, NJ.

[c] Difco Laboratories Inc, Detroit, Mich.

[d] 3025, Falcon Plastics, Division of Becton, Dickinson, & Co, Oxnard, Calif.

ticulate fomites were inoculated by mixing them with the abscess contents at a 10:1 ratio (w/w) in a tabletop blender[e] for 1 minute at high speed. Samples of the contaminated barnyard fomites were placed in sterile plastic petri dishes, with dishes containing each of the test samples prepared in triplicate and incubated at 37, 22, and 4 C. At 3-hour intervals through 7 days and at daily intervals thereafter through 67 days, 3 representative samples of the test material were removed at each period and were examined for the presence of *C pseudotuberculosis* by culture techniques. For the inoculated particulate fomites, approximately 0.1 g of a fomite-purulent exudate mixture was added to 4 ml of brain-heart infusion broth[c] (BHIB) in a test tube, and this was incubated at 37 C for 24 hours before passage onto a petri dish containing Columbia blood agar base. Plates were incubated in an aerobic system at 37 C for 48 hours, and isolates were identified as *C pseudotuberculosis*, using previously described methods.[20,21] A sterile dry cotton swab was used to scrape dried purulent exudate from the plastic petri dish surface, and the swab was placed in a test tube with BHIB. Similarly, contaminated rusty nails and white pine wood chips were inserted into test tubes with BHIB. Inoculated test samples were cultured and isolates were identified. All samples were analyzed until no bacteria were isolated from the tested sample on 2 consecutive sampling periods.

Results

The time that *C pseudotuberculosis* remained viable in axenic purulent exudate was greater when CLA abscess contents were mixed with particulate fomites than when spread on surfaces (Table 1). The *C pseudotuberculosis* cells in purulent exudate survived on average for 27, 72, and 99 hours on plastic petri dish surfaces at 37, 22, and 4 C, respectively. Viable bacteria were recovered from the inoculated surfaces of wood chips from 51 hours at 37 C to 129 hours at 4 C. Living *C pseudotuberculosis* cells were recovered from inoculated rusty nail surfaces for 39 to 192 hours depending on the incubation temperature. The etiologic agent of CLA in small ruminants survived for up to 55 days at 4 C in wood shavings and alfalfa hay. The incubation temperature did not markedly influence the length of survival in wheat straw with organisms recovered up to 19, 23, and 24 days when incubated at 37, 22, and 4 C, respectively. Unlike the situation with any of the other fomites, *C pseudotuberculosis* survived longer in goat feces at 22 C than at 4 C. In all other instances, incubation temperature and length of survival time for the organism were inversely related with *C pseudotuberculosis* surviving longest at 4 C and shortest at 37 C.

[e] Model 7010S, Waring Products Division, Dynamics Corp of America, Greenwich, Conn.

TABLE 1—Survival of *Corynebacterium pseudotuberculosis* in axenic purulent exudate when applied to or mixed with sterile barnyard fomites

Fomite	Survival time (days)		
	37 C	22 C	4 C
Surface coated			
Plastic dish	1.125 ± 0.18	3.000 ± 0.71	4.125 ± 0.71
White pine wood chip	2.125 ± 0.18	4.125 ± 0.62	5.375 ± 0.88
Rusty nail	1.625 ± 0.53	4.125 ± 0.35	8.000 ± 1.00
Mixed with homogenizer			
Wheat straw	19.00 ± 4.00	23.00 ± 2.65	24.00 ± 2.00
Caprine feces	10.00 ± 2.65	53.33 ± 7.64	38.00 ± 2.65
Alfalfa hay	7.00 ± 3.61	24.00 ± 2.00	55.00 ± 1.00
White pine wood shavings	11.00 ± 3.60	25.33 ± 7.09	55.00 ± 10.00

Data are expressed as mean ± SD.

Discussion

Seemingly, *C pseudotuberculosis* can survive for extended periods when inoculated onto sterile fomites common to the environment of small ruminants. Previously, speculations about the spread of *C pseudotuberculosis* in small ruminant populations has been based on the premise that the organism has a relatively short life span outside the host.[5,6,22] The organism was reportedly only able to survive for 1 to 8 days when cultured and washed cells were inoculated onto sterile feed, bedding, soil, and water.[19] In contrast to those results, the organism was able to survive for over a year when sterile sheep feces were inoculated with axenic purulent exudate.[18] Because purulent exudate is the presumed vehicle that is responsible for environmental contamination,[1-10] studies[14-16,18] using this as the inoculum, rather than washed cultured cells, should provide more useful insights about the role specific fomites may have in the transmission of the agent.

The pathogen was able to survive longer when axenic purulent exudate was mixed with finely chopped particulates such as wood shavings, straw, hay, and feces than when exposed on surfaces such as plastic, wood chips, or nails. During the mixing procedure used in this study, the purulent exudate was coated with the particulate matter to form small particles or micelles that may be a major factor in bacterial survival. The formation of micelles may favor retention of nutrients and moisture. The rapid loss of moisture may account for the shorter periods of survival on sterile inanimate surfaces. The consistency of purulent exudate that escapes from ruptured abscesses favors micelle formation when it is mixed with particulate materials. The potentially extended period of survival that occurs because of micelle formation may provide extended opportunities for infection of healthy animals.

In the present study, *C pseudotuberculosis* was inoculated onto sterile fomites, a situation markedly different from what would be expected to occur under natural conditions. Although the results from this study may not be directly applicable to circumstances occurring in the environment of small ruminants, they provide important insights about the potential that *C pseudotuberculosis* has for extended survival outside the host at different environmental temperatures. Considered collectively, data from this and other studies[4,11,12,14-19] indicate that management techniques (eg, shearing, docking, castration, and dipping) may not be the only means of transmission and that purulent material containing the organism may not be the only fomite. Previous reports have recommended measures that can be adapted by producers to reduce the hazards of infection.[3-7,10,17] Some measures that may prove beneficial include separation of infected animals from animals without abscesses; frequent removal and proper disposal of bedding, feed, and water which are contaminated with purulent exudate; cleaning and disinfection of inanimate surfaces contaminated with purulent exudate; segregation and observation of animals added to the production unit or returning to the unit from shows or exhibitions; use of sterile instruments for surgical procedures; shearing animals according to age and proper disinfection of shears between animals; and frequent changes in pasture locations when possible. Efforts directed at controlling the opportunities for transmission by direct and indirect contact can be helpful in limiting

the occurrence of CLA in a production unit where the disease is a problem.

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