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Potential for Cross-transmission of *Dictyocaulus viviparus* Between Cattle and White-tailed Deer

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ABSTRACT: Development of an in vitro culture system for infectious *Dictyocaulus viviparus* larvae made it possible to study the potential cross-transmission of *D. viviparus* between white-tailed deer (*Odocoileus virginianus*) and cattle (*Bos taurus*). Between 26 September 1995–29 February 1996, six parasite-free bull calves were individually inoculated with 15 to 50 infective third stage larvae (L_3)/kg of body weight cultured from adult *D. viviparus* collected from white-tailed deer. Three bull calves were simultaneously inoculated with 45 L_3/kg of body weight recovered from cattle either by the Baermann technique or by in vitro culture as above. All three calves inoculated with the homologous cattle strain became patently infected while all six calves inoculated with the heterologous deer strain remained negative for the presence of *D. viviparus* in the feces and in the lungs upon necropsy.

Key words: *Dictyocaulus viviparus,* cattle lungworm, white-tailed deer, cross-transmission, in vitro culture.

Skrjabin (1931) described a lungworm species in deer as morphologically dissimilar to the lungworm found in cattle and named it *Dictyocaulus eckerti.* However, Dikmans (1936) failed to reveal any reliable morphologic characteristics for distinguishing *Dictyocaulus* sp. in deer from that in cattle species and therefore synonymized the two as a single species, *D. viviparus.* He indicated that controlled experiments would be necessary to determine whether these isolates from wild ruminants could infect cattle. In Europe, wild ruminants such as red deer (*Cervus elaphus*), fallow deer (*Dama dama*) roe deer (*Capreolus capreolus*), and mouflon (*Ovis aries musimon*) were successfully infected with cattle strain *D. viviparus,* while natural infections with *D. viviparus* were described for chamois (*Rupricapra rupricapra*), camels (Camelidae), and various antelope (Enigk and Hildebrandt, 1965). In addition, bovine calves were readily infected with *D. viviparus* isolated from roe deer and red deer. These experiments prompted Enigk and Hildebrandt (1965) to conclude that *D. viviparus* infection was more adapted to deer than cattle because infections persisted longer in deer than in bovines and were less pathogenic. However, when Corrigall et al. (1988) successfully infected bovine calves with a red deer strain of *D. viviparus,* pathogenicity was greatly reduced. In a reciprocal experiment, bovine strain *D. viviparus* caused more pathology than red deer strain *D. viviparus* in red deer fawns. They concluded that cattle strain *D. viviparus* was more virulent than deer strain *D. viviparus* and that co-grazing of deer and cattle posed no serious threat to cattle. They also warned that deer brought from the wild onto pasture previously grazed by cattle could develop debilitating lungworm infection.

In North America, no mature *D. viviparus* developed in bovine calves infected with *D. viviparus* from elk (*Cervus elaphus*) (Presidente et al., 1972), moose (*Alces alces*) (Gupta and Gibbs, 1971) or black-tailed deer (*Odocoileus hemionus columbianus*) (Presidente and Knapp, 1973). However, the potential for contamination of cattle pastures with *D. viviparus* by white-tailed deer may exist. Development of an in vitro culture system for infectious *D. viviparus* larvae has facilitated a study of the potential cross-transmission of *D. viviparus* between white-tailed deer and cattle.

Adult *D. viviparus* were collected from the lungs of experimentally infected calves or from the lungs of legally harvested or road-killed white-tailed deer, and placed in warm saline. The worms were thoroughly washed in tap water to remove foreign material and placed in Petri dishes flooded with 40 mM KCl. The females were allowed to oviposit in these Petri dishes for 3 to 6 hr after which the adults were removed. If more eggs were needed, 2 to 3 large female worms were stripped of their eggs by dissection with two 18-gauge needles. Eggs were allowed to hatch at room temperature. The first stage larvae (L_1) were filtered to remove particulates by allowing them to wriggle through a submerged gauze or tissue filter. The nonfeeding larvae were returned to clean Petri dishes flooded with fresh 40 mM KCl at room temperature and allowed to develop to the infective third stage (L_3) before being refrigerated at 4 to 7 C. The number of viable L_3 was determined by counting larvae in a 1 ml aliqout from the culture.

In addition, L_1 were recovered from feces of experimentally infected calves using the Baermann technique (Baermann, 1917) and cultured to L_3 at room temperature in tap water before being refrigerated at 4 to 7 C. The number of viable L_3 was determined by counting larvae in a 1 ml aliquot from the culture.

Nine, 5- to 8-wk-old parasite-free Holstein or mixed breed bull calves with no history of exposure to *D. viviparus* were individually stalled in isolation barns at Merck and Co., Fulton Missouri between 26 September 1995 to 29 February 1996. Six calves were inoculated per os with 15–50 L_3/kg of body weight deer strain *D. viviparus* (Table 1). Fecal samples were collected regularly and the Baermann technique (Baermann, 1917) used to demonstrate lungworm larvae in these samples. Larvae did not appear in the feces throughout the study (Table 1). Study calves were euthanized between day 49 to 135 post-inoculation. Lungs were removed from the six study calves, cut open along

TABLE 1. Results of transmission experiment in calves inoculated with *Dictyocaulus* recovered from either white-tailed deer or cattle.

Calf number	Worm strain and (number of L_3/kg)	Culture method	Baermann results	Gross necropsy and day post-inoculation	Arrested larvae results
L80	Bovine (45)	Fecal	Positive	—	—
L81	Bovine (45)	in vitro	Positive	—	—
L79	Bovine (45)	in vitro	Positive	—	—
L82	Deer (45)	in vitro	Negative	Negative Day 58	Positive (?)
L90	Deer (45)	in vitro	Negative	Negative Day 48	Negative
L3	Deer (45)	in vitro	Negative	Negative Day 137	Negative
L2	Deer (15)	in vitro	Negative	Negative Day 116	Negative
L6	Deer (50)	in vitro	Negative	Negative Day 113	Negative
L116	Deer (30)	in vitro	Negative	Negative Day 55	Negative

the bronchi and bronchioles, and systematically searched for adult *D. viviparus*. At necropsy, none of the calves had adult worms in their lungs (Table 1). Each lung was then minced in a meat grinder, and subjected to the Baerman technique (Baermann, 1917) overnight to recover arrested or immature larvae from the lung parenchyma. Forty-five unidentified larvae were found in the minced lungs of Calf L82 (Table 1). These larvae measured between 340 and 360 μm and were not ensheathed. The larvae were dead and in some cases degenerated. The funnel used for the minced lungs had been used previously to isolate *D. viviparus* L_1 from cattle feces and may have been contaminated with L_1. The remaining 5 calves were held until days 55–137 post-inoculation to allow maturation of arrested larvae if present. All five of the remaining calves were negative for arrested or immature larvae in their lungs (Table 1).

In addition, two control calves were inoculated with the Merck cattle strain *D. viviparus* cultured in vitro while one control calf was inoculated with the Merck bovine strain *D. viviparus* collected from Baermann funnels. The three control calves inoculated with cattle strain *D. viviparus* were monitored using the Baermann technique (Baermann, 1917) only. All three control calves were positive for *D. viviparus* by day 35 (Table 1).

No first stage larvae were found in calves inoculated with deer strain *D. viviparus* whereas larvae were detected in calves inoculated with cattle strain *D. viviparus* (Table 1). Culture methods did not affect the infectivity of this parasite as was demonstrated by patency of all three control calves inoculated with homologous cattle strain L_3 (Table 1). This was important because access to infected white-tailed deer feces from which to collect adequate numbers of larvae were unavailable during the study period. The in vitro method produced enough larvae to inoculate 1 to 2 calves from as few as one or two viable female worms collected from the lungs of dead white-tailed deer.

Dictyocaulus sp. has been isolated from a wide range of wild ruminants and many are closely associated with grazing cattle. Concern about wild ruminants contaminating pastures has led to a variety of cross-transmission studies. Corrigall et al. (1988), felt that deer strain *D. viviparus* was less pathogenic than cattle strain *Dictyocaulus,* and posed no serious threat to cattle grazing pastures occupied previously or simultaneously with red deer. In North America, studies involving cross-transmission with *D. viviparus* isolated from elk (Presidente et al., 1972), moose (Gupta and Gibbs, 1971), and black-tailed deer (Presidente and Knapp, 1973) indicated that these strains do not mature in cattle. However, when 25,000 L_3 from black-tailed deer were inoculated into three calves, all displayed signs of coughing, dypsnea and nasal discharge. One of the three calves died before patency of streptococcal pneumonia, and a few immature *D. viviparus* were recovered from the air passages of the lung, although no other larvae were recovered from the lung tissue. The other two calves showed similar clinical signs but none became patent throughout the study. In this same study, a fourth calf and a black-tailed deer fawn grazed on pasture heavily contaminated with *D. viviparus* larvae from a naturally infected black-tailed deer. The fawn responded clinically to the exposure and developed a patent infection while the calf responded with only mild coughing and no patent infection (Presidente and Knapp, 1973). Presidente et al. (1972), reported that four Holstein calves did not develop patent infections after being inoculated with 6,000 to 10,000 elk strain *D. viviparus* L_3. They also noted partial protection from *D. viviparus* reinfection with cattle strain *Dictyocaulus* sp. after being inoculated with elk strain *D. viviparus,* as characterized by longer prepatent period, short patent period and low larval production compared to the controls. Yet in prelimi-

nary studies, these authors were able to infect 2 out of 4 yearling Hereford cattle after 4,000 and 43,000 elk strain *D. viviparus* L_3 were given orally by stomach tube, and also were able to infect one Jersey steer that was injected intraruminally with 6,300 L_3. They speculated that the method of inoculating the calves and genetic differences in the breed of calves used were responsible for the success of infecting these animals.

Lastly, a genomic analysis of *Dictyocaulus* spp. using RAPD-PCR, indicated that *Dictyocaulus* sp. isolated from fallow deer (*D. eckerti*) had a different genomic profile from *Dictyocaulus* sp. isolated from cattle (*D. viviparus*), horses (*D. arnfieldi*), and sheep (*D. filaria*) (Epe et al., 1995). This technique used random primers to amplify total DNA isolated from each strain of worms. The authors showed that the four *Dictyocaulus* spp. had different characteristic banding patterns and were different species. While the similarity coefficients were the highest for *D. viviparus* and *D. eckerti* (32%) it was far below the values calculated for two separate isolates of the same *Dictyocaulus* spp. (93%). They concluded that lungworms collected from fallow deer were a separate species and not a *D. viviparus* isolate of wild ruminants. Subsequently, studies using PCR and restriction-fragment-length polymorphisms on the second internal transcribed spacer (ITS2) of the ribosmomal DNA (Schneider et al., 1996), ITS2 sequence analysis (Epe et al., 1997) and a recent development of a specifies specific PCR for differentiation of *D. viviparus* and *D. eckerti* larvae (von Samson-Himmelstjerna et al., 1997) have provided further evidence of species differences between *D. viviparus* and *D. eckerti.*

Therefore, we can not recommend a taxonomic reclassification for *Dictyocaulus* sp. isolated from white-tailed deer until more definitive DNA work is performed on North American deer and cattle strains and further cross-transmission studies are conducted. From our results and experience as well as previous published literature, it appears that *D. viviparus* from a given host species may be minimally capable of infecting other ruminant species. Yet, it may not be a good source of infection or serve as a reservoir for the infection. A useful next step would be to challenge cattle after inoculating them with white-tailed deer strain *D. viviparus,* with a homologous strain and note whether deer strain *D. viviparus* confers protection. If this was true, then there may be a potentially positive impact of deer grazing simultaneously with cattle.

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