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Isolation of *Pilobolus* spp. from the Northern Elk Herd in Yellowstone National Park

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ABSTRACT: *Pilobolus* spp. were recovered from all fecal samples collected from an elk (*Cervus elaphus nelsoni*) herd in Yellowstone National Park (USA) with a high prevalence of *Dictyocaulus viviparus* infection. *Pilobolus* spp. have been shown to be important in the epizootiology of *D. viviparus* infections in cattle because these fungi aid in dissemination of larvae away from feces to areas where animals are more likely to ingest them, and protect larvae against dehydration and thus prolong survival. The same mechanism of dissemination of *D. viviparus* larvae may play a role in the epizootiology of these infections in elk.

Key words: *Pilobolus* spp., *Dictyocaulus viviparus*, elk, lungworm, Yellowstone National Park.

Species of the coprophilous fungi, *Pilobolus* spp., have an unusual method of spore dispersal. When mature, their sporangia are forcibly discharged and propelled to a distance of nearly 3 m (Buller, 1934). Robinson (1962) saw third-stage larvae of *Dictyocaulus viviparus* on the sporangia of *Pilobolus* sp. and demonstrated that these larvae could be transported to >3 m from the fecal mass when the sporangia were discharged; this provided an effective means of transferring larvae from feces to herbage without gross fecal contamination.

Doncaster (1981) filmed infective third-stage larvae of *D. viviparus* climbing sporangiophores of *Pilobolus* sp. and found that discharged sporangia could aid in the survival of the larvae by retarding desiccation. Jorgensen et al. (1982) discovered that *Pilobolus* sp. harbored the majority of infective larvae of *D. viviparus* in pastures and that the numbers of infective larvae of *D. viviparus* were greatly reduced in the absence of *Pilobolus* sp. Boon et al. (1983) found *Pilobolus kleinii* associated with infective *D. viviparus* larvae in cattle in the Netherlands and reported that *Pilobolus* sp. sporangia were propelled while harboring the larvae. Rodriguez Diego et al. (1983) examined the dissemination of invasive *D. viviparus* larvae by *Pilobolus* sp. in Cuba and located most larvae 0.5 to 1 m from animal feces and associated with the fungus. Roque et al. (1983) noted that *Pilobolus* sp. sporangia developed at the same rate as the infective larvae of *D. viviparus* and this parallel development provided the appropriate time sequence for *Pilobolus* sp. dissemination of the infective nematode larvae.

Worley and Barrett (1964) reported the northern elk (*Cervus elaphus nelsoni*) herd in Yellowstone National Park, Wyoming (USA) to have a high prevalence of lungworm infection caused by *D. viviparus*. Feces were collected from this elk herd and examined for *Pilobolus* sp. to determine whether this fungus could be involved in dissemination of *D. viviparus* larvae.

During June and July 1986, feces were collected from 10 elk in Gibbon Meadows (44°42′N, 111°46′W), Elk Park (44°43′N, 111°44′W) and Virginia Meadows (44°44′N, 111°46′W) areas of Yellowstone National Park, Wyoming (USA) for *Pilobolus* sp. Collections of fresh feces were made aseptically in plastic bags and were transferred within hours to aseptic plastic cups (Foos and Royer, 1986). During the 3-wk period in the field, light and temperature varied considerably. Cultures were maintained under shaded outdoor conditions. However, upon return to the laboratory all cultures were maintained at room temperature under cool white fluorescent lights with an intensity of 2,000 lx and alternating 12 hr light and dark periods.

Isolates were obtained by removing single sporangia from the sides or tops of the plastic cups with sterile inoculating needles and transferring these sporangia to petri

dishes containing dung agar (Buller, 1931). Subsequent cultures were maintained through hyphal tip transfers.

Sporangia were collected from the lids of petri dishes with sterile inoculating needles and mounted in lactophenol. One hundred spores per sporangium were examined and measured at 1,000× using brightfield microscopy. Columellae were observed by removing sporangia with microforceps. Measurements of spores, sporangiophores and sporangia were made from the original isolates on feces and from growth on dung agar in the laboratory.

Eleven isolates of *Pilobolus* spp. were made from 10 fecal samples. *Pilobolus crystallinus*, *P. kleinii* and *P. roridus* were isolated. Nine samples contained a single species; one contained two species.

Four isolates of *P. crystallinus* were identified based on the redescription by Fries (1823). *Pilobolus crystallinus* sporangiophores developed in 3 to 4 days, were 1 to 6 mm long, and were clear to pale yellow in color. Trophocysts developed submerged in the substratum and were usually 300 to 400 μm long. Sporangia were covered with a dark, cutanized wall and ranged from 80 to 250 μm in diameter. Columellae were papillate and penetrated deeply into the sporangia. Sporangiospores were pale yellow ellipsoids 10.0 ± 0.7 (range 7.5 to 12) μm in length by 6.3 ± 0.9 (range 5 to 8) μm in width producing a length to width ratio of 1.6.

Five isolates of *P. kleinii* were identified based on the original description of van Tieghem (1876). *Pilobolus kleinii* sporangiophores measured 1 to 6 mm in length and arose from dark yellow turnip shaped trophocysts measuring 300 to 500 μm in diameter. The trophocysts were often partially submerged within the substratum. Sporangia were dark, smooth, and cutanized. They measured 80 to 300 μm in diameter. Columellae were papillate and extend deeply into the sporangia. Sporangiospores were yellow to orange ellipsoids 12.2 ± 1.4 (range 9.5 to 16) μm in length by 7.1 ± 0.9 (range 4.5 to 10.5) μm in width with a length to width ratio of 1.7.

Two isolates of *P. roridus* were identified based on the description of Persoon (1801). *Pilobolus roridus* sporangiophores were 2 to 3 mm long. Sporangia were 80 to 250 μm in diameter, smooth and hemispherical. Columellae were rounded and did not penetrate into the sporangia appreciably. Sporangiospores were pale yellow to colorless, oval 5.3 ± 0.5 (range 4 to 7) μm in length and 3.7 ± 0.5 (range 3 to 5.5) μm in width with a length to width ratio of 1.4.

The role of *Pilobolus* spp. in the dissemination of infective larvae of *D. viviparus* in cattle has been well documented. The isolation of *Pilobolus* spp. from elk feces in Yellowstone National Park suggests a similar mechanism may occur in elk. This well studied herd of elk, shown to have a high prevalence of lungworm infection, also had an unusually high prevalence of *Pilobolus* spp. While one might expect to isolate *Pilobolus* spp. in 75% of samples collected (Foos and Royer, 1989), every fecal sample contained this fungus. Isolation of *Pilobolus* spp. from samples from three different locations within the range of this elk herd demonstrates wide distribution of the fungus in the area. A general survey for *Pilobolus* spp. in Yellowstone National Park (Foos and Royer, 1989) found that the three species isolated from elk were the same as those isolated from the general population of herbivores found in the Park.

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