bution ratio of 0.04 was an accurate indicator of the volume of the equilibrating compartment. For example, if the external medium contained 1,000 cpm/ml, cercariae contained 40 cpm/ml worm water at equilibrium. Thus, the volume (water content) of the glucose accessible compartment was only 4% of total worm water. Similarly, glucose in the remaining worm water (96%) would not readily diffuse into the bathing medium. Whatever barrier prevents the passive movement in 1 direction (influx) must equally impede movement in the other (efflux).

Embryonic tails were 2–3 times more permeable than were mature ones, and 90-min distribution ratios for early and late forms were 11% and 8%, respectively. These ratios indicate a decrease in permeability with development, but they are underestimates of compartment volumes because uptake was still increasing at 90 min.

Unlike tails, cercarial bodies are quite permeable to glucose, which approaches steady state after 60 min (Uglem, 1980). At least 40% of worm water is readily accessible to exogenous glucose. Although bodies absorb glucose by a combination of diffusion and facilitated diffusion, the ''filling rates'' for ^{14}C-glucose and ^{3}HOH are similar.

Initial uptake by cercariae indicated that the glycocalyx and external plasma membrane are permeable to glucose. Because steady state occurred so rapidly in mature forms, we reasoned that the small equilibrating compartment was the tegument. Mature cercariae are cylindrical, about 4 mm by 1 mm, with a tegument 1–2 μm thick as seen with TEM. The calculated volume of the tegumental compartment in such a cylinder is 3–5% of total volume. This structural estimate of volume agrees well with that obtained from isotope equilibration (4%).

The tegument (distal cytoplasm) of adult trematodes is separated from subtegument by a fibrous basal lamina (for review see Smyth and Halton, 1983). Distal cytoplasm and proximal cytoplasm of subtegumental cells are connected by cytoplasmic strands that pass through the basal lamina. These strands were present in embryonic cercariae, but they were absent in mature forms. Therefore, prior to emergence from the snail, the basal lamina becomes a continuous, electron-dense structure, one that appears to function as an effective barrier to passive movements of glucose. If such a barrier impedes the fluxes of other solutes and water, it would also serve to protect the cercaria from effects of osmotic stress.

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Skin Lesions Caused by *Dermophthirius penneri* (Monogenea: Microbothriidae) on Wild-Caught Blacktip Sharks (*Carcharhinus limbatus*)

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ABSTRACT: Skin lesions caused by the ectoparasite *Dermophthirius penneri* Benz, 1987 (Monogenea: Microbothriidae) on 2 wild-caught blacktip sharks (*Carcharhinus limbatus*) from the northern Gulf of Mexico were studied using light and scanning electron microscopy. Grossly, lesions appeared as multifocal, well-demarcated, ovoid or irregularly shaped, light gray patches of skin. Scanning electron microscopy of lesions revealed gaps between placoid scales apparently created by detachment and loss of placoid scales, rotated and tilted placoid scales with blunt distal tips and shallow ridges, and a frayed epithelium that covered some placoid scales and filled some spaces between placoid scales. Light microscopy of lesions revealed epithelial hyperplasia accompanied by dermal infiltrates of moderate numbers of loosely arranged lymphocytes interposed between collagen bundles in the superficial layers of the stratum compactum. This report provides the first details of microbothriid skin lesions on wild sharks. Our results indicate that *D. penneri* caused chronic skin lesions not associated with bacterial infection or severe, debilitating, skin disease in the studied sharks.

Members of Microbothriidae Price, 1936 (Monogenea) infect the skin and gills of elasmobranchs. Microbothriid infections may intensify and cause disease among sharks held in public aquaria (Cheung et al., 1982, 1988; Cheung and Nigrelli, 1983; Cheung and Ruggieri, 1983; Rand et al., 1986; Cone, 1995; Poynton et al., 1997). Heavily infected captive sharks may behave erratically, i.e., flashing and rubbing against objects in the tank, and they may also lose placoid scales, develop ulcerated skin lesions, and die from complications associated with dermatitis (Cheung et al., 1982; Poynton et al., 1997). Several workers have suggested that pathological alterations to host skin caused by microbothriids might facilitate entry and onset of lethal secondary bacterial or viral infections (Cheung et al., 1982; Grimes et al., 1985; Rand et al., 1986; Poynton et al., 1997). Grimes et al. (1985) isolated *Vibrio carchariae* Grimes, Colwell, Stemmler, Hada, Maneval, Hetrick, May, Jones, and Stoskopf, 1984 from several specimens of *Dermophthirius nigrellii* Cheung and Ruggieri, 1983 (Monogenea: Microbothri-

idae) and suggested that *D. nigrellii* may act as a vector of *V. carchariae.*

Microscopic details of the relationship between microbothriids and their hosts in the wild have not been reported previously; however, several investigators have published incidental observations on the effects of microbothriids on host integument. Kearn (1965) studied the biology of *Leptocotyle minor* (Monticelli, 1888) and reported that the parasite eroded the epidermis of the dogfish, *Scyliorhinus canicula.* Rand et al. (1986) examined the attachment of *Dermophthirius carcharhini* MacCallum, 1926 on heavily infected captive Galapagos sharks (*Carcharhinus galapagensis*) and observed areas of skin surrounding the attachment site of *D. carcharhini* that were devoid of placoid scales and contained eroded or compressed areas of epidermis with ruptured goblet cells. Poynton et al. (1997) described hemorrhagic skin lesions characterized by placoid scale loss and excess mucous production on captive lemon sharks (*Negaprion brevirostris*) heavily infected by *Neodermophthirius harkemai* Price, 1963.

A recent elasmobranch tagging and stock assessment cruise provided us opportunity to collect and study skin samples from 2 wild-caught blacktip sharks infected with *Dermophthirius penneri* Benz, 1987 (Monogenea: Microbothriidae). This report describes the pathological alterations to host skin caused by *D. penneri.*

Two female blacktip sharks (*Carcharhinus limbatus*) infected with numerous monogeneans were caught by longline in the northern Gulf of Mexico during August 1997. One shark weighed 10 kg and was 83 cm in fork length, and the other weighed 27 kg and was 123 cm in fork length. Elliptical patches of skin (6–8 cm by 4–6 cm), each containing 2–10 monogeneans, were excised from both sharks from below the base of the first dorsal fin on the left and right sides of the body and along the dorsal ridge immediately posterior to the first dorsal fin. An additional skin lesion from the dorsal aspect of the caudal peduncle was excised from 1 shark. Skin samples included lesional areas and also wide marginal zones free of parasites and unaltered in gross appearance. Skin patches were fixed in 10% neutral buffered formalin and stored at room temperature until further processing. With the aid of a stereomicroscope, several parasites were carefully removed from excised skin patches using needle and forceps, dehydrated in ethanol, cleared in clove oil, mounted in neutral Canada balsam on glass slides, and examined by light microscopy. Skin samples processed for scanning electron microscopy (SEM) were sliced from formalin-fixed skin samples into 0.3–0.5-cm rectangular to square segments of epithelium, subjected to 3, 10-min washes in 0.1 M HEPES buffer, postfixed in 1% osmium tetroxide in 0.1 M HEPES buffer for 16 hr, subjected to 3, 10-min washes in distilled ultrafiltered water, dehydrated in ethanol, stored overnight in 100% ethanol, critical point dried for 3.5 hr in an E-3000 Polaron critical point drying apparatus (V. G. Microtech, East Sussex, U.K.), mounted on SEM stubs with silver paint, sputter-coated with gold–palladium in an E-1500 Polaron sputter coater (V. G. Microtech), and examined using a Zeiss DSM 982 field emission scanning electron microscope. Formalin-fixed samples of both lesional and normal, pathologically unaltered skin were postfixed for 24–48 hr in Bouin's fixative to partially demineralize placoid scales, processed routinely for paraffin embedding, sectioned at 4 μm, stained with hematoxylin and eosin, and examined by light microscopy.

Skin parasites collected from the blacktip sharks all conformed to the original description (Benz, 1987) of *D. penneri.* The haptor of each parasite was firmly attached to the placoid scale crown, and all parasites were oriented with the long axis of the body approximately parallel to the long axis of the placoid scale ridges, i.e., with the pharynx of the parasite facing the caudal end of the host. Parasites were present only in well-demarcated, light gray-colored areas identified as skin lesions. Lesions were irregularly shaped, 2–3 cm by 1–2 cm. When viewed at low magnification using a dissecting stereomicroscope, surface contours of lesions were slightly uneven and frayed.

SEM showed that in normal skin, placoid scales were free of tissue debris, clearly defined, and arranged in slightly overlapping rows that covered the epidermis (Fig. 1). Light microscopy showed that the epidermis consisted of a stratified squamous to cuboidal epithelium, 3–5 cells thick, supported by a fine stratum spongiosum, contiguous with a densely collagenous stratum compactum (Fig. 2). Placoid scales were rooted in the dermis and arranged in regular repeating rows that perforated the epidermis (Fig. 2). Moderate numbers of mucous-containing goblet cells were dispersed throughout the epidermis.

SEM of lesions immediately surrounding the body and attachment site of *D. penneri* showed that placoid scales were not arranged in parallel arrays (Figs. 3, 4). There were gaps between placoid scales, apparently created by detachment, loss, or rotation of placoid scales (Figs. 3, 4). Some placoid scales were tilted out of the normal plane with their distal tips pointing toward the epidermis or away from it (Figs. 3, 4). The distal tips of some placoid scales were rounded, i.e., blunt, in comparison with those from unaffected skin (Fig. 1), and placoid scale ridges were relatively shallow (Fig. 4). Ragged and irregular mounds of epithelium corresponding to uneven and frayed areas of epidermis seen grossly were present in affected areas of skin (Figs. 3, 4). Histopathologically, epithelial hyperplasia was evident, resulting in wide columns or mounds of cells that filled spaces between placoid scales and covered some disrupted or altered placoid scales (Fig. 5). Numerous interstitial infiltrates of lymphocytes were dispersed throughout the superficial zone of the stratum compactum of the dermis (Fig. 6). Relative to normal skin, goblet cells in hyperplastic epidermis were fewer in number. There was no microscopic evidence of a concurrent bacterial or viral infection in the examined tissue sections.

These pathological alterations to host skin were observed only in the immediate vicinity of *D. penneri,* i.e., within a radius of several centimeters surrounding the body; therefore, we presumptively identified *D. penneri* as the cause of the lesions. Results of the present study indicate that wild blacktip sharks infected with *D. penneri* regenerate epithelial cells, resulting in localized hyperplasia (Figs. 3, 5). Kearn (1963, 1965) suggested that many skin-dwelling monopisthocotylineans feed on components of epidermis, probably epithelial cells, and do not harm underlying tissues such as the dermis and the associated blood vessels. It is unlikely that placoid scales provide nourishment to parasites because they are composed of dentin. Kearn (1965) stated that *L. minor* and similar microbothriids that infect shark

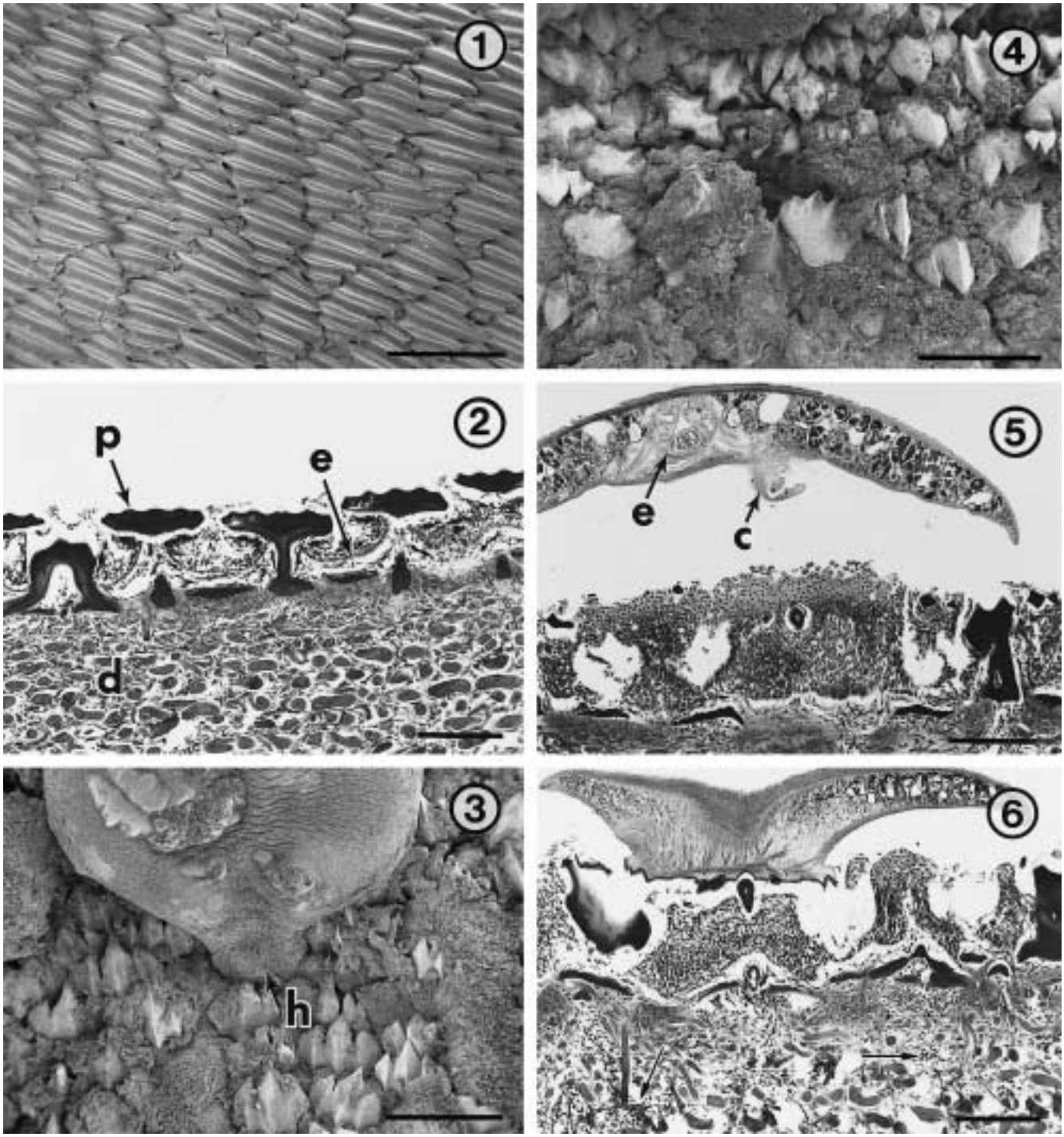

FIGURE 1. Scanning electron micrograph of normal skin from a wild-caught blacktip shark (*Carcharhinus limbatus*). Placoid scales with clearly defined raised longitudinal ridges and multiple, sharply defined points along each of their margins are arranged in an orderly array and are oriented in staggered parallel rows along an axis. Note: placoid scales cover most of the epidermis. Scale bar = 500 μm.

FIGURE 2. Histologic section of normal skin from a wild-caught blacktip shark (*C. limbatus*). The epidermis (e) consists of stratified squamous epithelium containing mucous cells and is supported by a thick dermis (d) consisting of a fine stratum spongiosum overlying a broad stratum compactum composed of densely packed collagen bundles. Placoid scales (p) perforate the epidermis, are rooted firmly in the dermis, and project above and cover the epithelial surface. Scale bar = 200 μm.

FIGURE 3. Scanning electron micrograph of *Dermophthirius penneri* and skin surrounding its attachment site on a wild-caught blacktip shark (*C. limbatus*). Haptor (h) of *D. penneri* is attached to a placoid scale crown (anterior end of the worm is out of frame). Scale bar = 500 μm.

FIGURE 4. Scanning electron micrograph of skin surrounding *D. penneri* (parasites not shown) on a wild-caught blacktip shark (*C. limbatus*). Placoid scales are apparently missing, rotated, and tilted. Free tips of some placoid scales are relatively blunt, and placoid scale ridges are relatively shallow. Scale bar = 500 μm.

skin must feed on epidermis that lies between placoid scales. In the present study, epithelial cells were the only cellular component of skin seemingly accessible to *D. penneri* (Figs. 3, 5, 6); we suspect that *D. penneri* may graze on these epithelial cells before they are sloughed. Relatively few goblet cells were present in hyperplastic epithelium; however, it is likely that these cells are ingested also. The mode of attachment used by microbothriids, i.e., the secretion of a cement (see Rand et al., 1986), seems well suited for semistationary epithelial grazing (Kearn, 1963, 1965). In addition, *D. penneri* actively probes surrounding shark skin and can occasionally detach and relocate (personal observations of S.A.B. and G.W.B.), indicating that it is able to feed in and attach to multiple sites within lesions.

This is the first report of lymphocytic infiltrates associated with a microbothriid infection. Roubal and Whittington (1990) reported a similar host response induced by *Anoplodiscus australis* Johnston, 1930 (Monogenea: Anoplodiscidae) on the fin of the yellowfin bream *Acanthopagrus australis*. They concluded that *A. australis* elicited a host response involving cellular elements indicative of long-term, i.e., chronic, inflammation. In the present study, lymphocytic infiltrates in dermis beneath specimens of *D. penneri* suggested that these lesions were also chronic. A comparable, chronic host response was expected given that members of Anoplodiscidae and Microbothriidae cement themselves to the host and may not often detach and relocate.

There was neither deep, extensive, integumentary damage nor concurrent infection (of any type) in the examined tissue samples. However, in captive environments such as public aquaria, microbothriid infections may intensify and cause severe dermatitis and disease (Cheung et al., 1982; Poynton et al., 1997). If microbothriids feed on epithelial cells, it is conceivable that dense parasite populations may consume more epithelial cells than the host is capable of regenerating, resulting in epidermal erosion and hemorrhagic or ulcerated lesions. Once the epidermis is breached and the dermis is exposed, secondary bacterial and viral infections would be facilitated. Cheung et al. (1982) reported that open wounds preceded development of ulcerated skin lesions that were soon afterward infected with bacteria. If not treated, heavily infected captive sharks become moribund or die.

There is no report that describes a disease caused by a microbothriid in a wild shark, and we (casual observations of S.A.B. and G.W.B.) have not seen any hemorrhagic or ulcerated microbothriid skin lesions on wild sharks. The literature, our personal observations, and the results of the present study suggest that microbothriids are not typically pathogenic in wild hosts and probably become so only when infected sharks are kept in relatively confined environments that facilitate development of large populations of parasites (Cheung et al., 1982; Cheung and Nigrelli, 1983; Poynton et al., 1997). Among infected wild sharks, microbothriid cement and shark placoid scale may serve as an acellular barrier that shields *D. penneri* from a host immune response.

We do not know the mechanism through which placoid scales near *D. penneri* are altered or lost. However, it should be noted that placoid scale loss may eliminate some parasites, as members of *Dermophthirius* MacCallum, 1926 are only known to attach to placoid scales. Rand et al. (1986) suggested that compressed and eroded areas of epidermis and ruptured goblet cells near *D. carcharhini* altered the physiologic activity of tissue surrounding the basal plate of the placoid scale, thus facilitating placoid scale loss. In addition, they suggested that *D. carcharhini* may act as a lever to loosen or dislodge placoid scales when extending or rotating its body during feeding. In the present study, we did not observe ruptured goblet cells or evidence of leveraging. Some monogeneans secrete proteolytic enzymes that aid in chemical erosion of host epidermis prior to its ingestion (Kearn, 1965). The presence of such an enzyme in *D. penneri* perhaps could explain placoid scale loss, but we saw no evidence to support this. Although we did not observe any significant epidermal erosion in our samples, we did observe small, isolated areas of skin that lacked placoid scales and contained mounds of hyperplastic epithelium. These observations suggest that epithelial proliferation may be the chronic host skin response to placoid scale loss.

We thank Jim Romanow (Electron Microscopy Laboratory, University of Connecticut) for assistance with SEM; Robin Overstreet (Gulf Coast Research Laboratory [GCRL], Department of Coastal Sciences, The University of Southern Mississippi) for financial support and laboratory supplies; Reg Blaylock (GCRL) for helpful comments on the manuscript; Brian Smith (Southeast Aquatic Research Institute) for collecting parasites; the captain, officers, and crew of the research vessel *Oregon II* (National Oceanic and Atmospheric Administration [NOAA]) and Mark Grace and Lisa Jones (both Southeast Fisheries Science Center, National Marine Fisheries Service [NMFS]) for facilitating some of the field work; and Joyce Shaw (Gunter Library, GCRL) for gathering pertinent literature on monogeneans. This study is contribution no. 1894 from the Storrs Agricultural Experiment Station and was supported by NOAA, NMFS award NA86FL0476 and a National Science Foundation Research Experiences for Undergraduates (REU) award to S.A.B. that was kindly facilitated by Janine Caira (University of Connecticut).

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FIGURE 5. Histologic section of the skin of a wild-caught blacktip shark (*C. limbatus*) beneath a transverse section of *D. penneri*. The parasite's muscular esophagus (e) and ventrally everted cirrus (c) are visible. The regular repeating array of placoid scales is disrupted, and some placoid scales are apparently missing. Epithelial hyperplasia creates wide columns or mounds of cells that fill spaces without placoid scales and/or that reach above the surfaces of placoid scales. Scale bar = 200 μm.

FIGURE 6. Histologic section showing skin of a wild-caught blacktip shark (*C. limbatus*) and tangential section at the haptor of *D. penneri*. Moderate numbers of lymphocytes (arrows) are loosely interposed between collagen bundles in the superficial zone of the stratum compactum beneath the attachment site of *D. penneri*. Scale bar = 200 μm.

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J. Parasitol., 86(3), 2000, p. 622–623

Seroprevalence of *Toxoplasma gondii* in Rocky Mountain Bighorn Sheep (*Ovis canadensis*)

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ABSTRACT: Serum samples from 697 Rocky Mountain bighorn sheep (*Ovis canadensis*) from North America were examined for antibodies to *Toxoplasma gondii* by the modified agglutination test incorporating mercaptoethanol and formalin-fixed tachyzoites. Antibodies to *T. gondii* were found in 25 of 697 (3.6%) sheep in titers of 1:25 (8 sheep), 1:50 (4 sheep), 1:100 (7 sheep), 1:200 (1 sheep), 1:400 (1 sheep), 1:800 (1 sheep), and 1:1,600 (3 sheep). This is the first record of *T. gondii* exposure in bighorn sheep.

Toxoplasma gondii infections have been reported from numerous domestic and wild species of animals (Dubey and Beattie, 1988). It is a major cause of abortion in goats and sheep. We are not aware of any report of *T. gondii* infection in Rocky Mountain bighorn sheep (*Ovis canadensis*), therefore the present survey was conducted to determine the level of exposure rates.

Blood samples were collected routinely when bighorn sheep were captured for herd health evaluations or translocation. Blood was collected between 1982 through 1999 in 6 western states and Canada, and sera were stored at −20 C.

Sera were initially screened at dilutions of 1:25, 1:50, and 1:500 using the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). Seropositive sera were end-titrated using 2-fold dilutions.

Antibodies to *T. gondii* were found in 25 of 697 sheep (3.6%); 20 of 411 from Washington, 1 of 24 from Idaho, 1 of 70 from Oregon, 1 of 107 from Nevada, 1 of 8 from Wyoming, 0 of 22 from Montana, 0 of 5 from Alberta, Canada, and 1 of 50 from unknown sources. The antibody titers were 1:25 (8 sheep), 1:50 (4 sheep), 1:100 (7 sheep), 1:200 (1 sheep), 1:400 (1 sheep), 1:800 (1 sheep), and 1:1,600 (3 sheep). Most positive animals (20 of 411) were from the state of Washington (Table I).

The only cluster of positive titers in the bighorn sheep samples was from a population of bighorn sheep in northeastern Washington on Hall Mountain (48°50′N, 117°15′W). Four of 14 bighorn sheep including 2 adult ewes, 1 adult ram, and 1 female lamb in this population were positive on 15 December 1998. Over 200 bighorn sheep have been captured and sampled from this population since 1982 (Foreyt et al., 1996), but other than the 4 positive samples in 1998, only 2 other sheep, including 1 adult male in 1993 and 1 adult female in 1999 were positive.

TABLE I. Prevalence of *Toxoplasma gondii* antibodies in sera of 697 bighorn sheep.

Source of bighorn sheep	No. of samples	No. of sera positive (≥1:25)
Washington	411	20
Idaho	24	1
Oregon	70	1
Montana	22	0
Nevada	107	1
Wyoming	8	1
Alberta, Canada	5	0
Unknown source	50	1