1969

Isolation of Keratinophilic Fungi from Soil and Wild Animals in South Dakota

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ISOLATION OF KERATINOPHILIC FUNGI FROM SOIL AND WILD ANIMALS IN SOUTH DAKOTA

BY

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A thesis submitted
in partial fulfillment of the requirement for the
degree Master of Science, Major in
Bacteriology, South Dakota
State University

1969

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AND WILD ANIMALS IN SOUTH DAKOTA

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Bacteriology Department Date

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ACKNOWLEDGMENT

I would like to give special thanks to Doctor Robertstad for his counseling and guidance as my major professor on this project, to Doctor Middaugh and Doctor Pengra for their suggestions and help in proofreading.

I would like to thank all the members of the South Dakota Department of Game, Fish, and Parks who helped this project by collecting specimens. The efforts of Mr. Tom Kuck, Mr. Larry Fredrickson, Mr. Warren Jackson and Mr. Barton Wittaker were appreciated.

I would especially like to thank Miss Suzanne L. Volin whose confidence and encouragement given me during this project are deeply appreciated.

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INTRODUCTION

Very little is known of the ecology of keratinophilic fungi in animal populations and their habitats in South Dakota. Recent investigation of an outbreak of ringworm in blue foxes on a fur farm in South Dakota indicated that the source of infection may have been wild animals which had been captured and raised in the kennels previous to occupancy by the foxes. In recent years, a number of investigators have demonstrated that small and large mammals may carry keratinophilic fungi in their coats (13, 29-31, 52, 56-60, 63, 70, 77) and have postulated on the role of infected animals in the transmission of ringworm infections.

Keratinophilic fungi are common in a wide range of soils from many areas of the world (3, 5, 37, 64, 78). In the United States, keratinophilic fungi have been isolated from soils in Arizona (10), Alabama, Georgia, Michigan, Tennessee and Virginia (3). South Dakota is a state of infinite variety and affords the opportunity to determine the ecology of keratinophilic fungi from semi-arid areas to areas of lush foliage. The eastern third of the state is part of the Central Plains which covers most of Central United States. The middle portion is a part of the Great Plains, which covers a large area from Canada to Mexico. The third region is the Black Hills, which is a mountainous, forested area near the western border.

The presence of buffalo (bison) herds, deer, antelope, prairie dogs, ferrets, skunks, woodchucks, coyotes, badgers, raccoons, ground

squirrels, foxes and rabbits affords an unusual and varied source of specimens for examination of keratinophilic fungi.

It was the intent of this investigation to determine the distribution of keratinophilic fungi among wild animals and their habitats in all sections of the state of South Dakota. Information gained in this study will provide data not previously available for this area of the United States. The specific objectives of this investigation were:

(1) To determine the ecology of keratinophilic fungi in wild animal populations and their habitats in South Dakota.

(2) To determine which fungi are the common causes of ringworm in wild animals in South Dakota.

LITERATURE REVIEW

Keratinophilic Fungi of Large and Small Mammals

Keratinophilic fungi are those capable of digesting keratin, which is a protein component of epidermal and skeletal tissues (39). Keratin is highly polymerized and made of polypeptide chains bound laterally by disulfide linkages and internally by secondary valency forces (12). The digestion process of keratin by keratinophilic fungi is a controversial subject. Vanbreuseghem (79) described specialized hyphae (perforating organs) which after enzymatic penetration of the hair shaft continued to grow, causing perforations in the hair.

Chesters and Mathison (21), and Mathison (55) proposed that the high alkaline environment brought on by growth of certain keratinophilic fungi and the production of proteolytic enzymes were responsible for keratinolysis. Raubitschek (73) stated that growth on keratin by fungi was due to utilization of non-keratin residues on the hair and the perforation of the hair was purely mechanical. English (27) using 9 species of keratinophilic fungi from the genera Epidermophyton, Keratinomyces, Microsporum, and Trichophyton indicated that "perforating organs" function by means of enzymes.

The four main genera of keratinophilic fungi are Arthroderma, Chrysosporium, Microsporum and Trichophyton. The latter 2 are the most important because some of the species in these 2 genera are pathogenic to animals and man, causing cutaneous lesions on the skin. Originally these 2 genera were placed in the family Deuteromycetes (Fungi Imperfecti), but several of the species have been shown to have an

ascigerous state; thus, they have been placed in the family Ascomyces (6, 9, 23). The ascigerous states of Microsporum and Trichophyton have been placed in the genera Nannizzia and Arthroderma, respectively. Some keratinophilic fungi which live parasitically on the skin, hair or nails of man or other animals have been classified as dermatophytes (2). An updated classification of dermatophytes, which includes the genera Microsporum, Trichophyton and Epidermophyton has been reported by Ajello (8).

Animal ringworm is an important public health problem since infected animals are a source of indirect (62) or direct infection for humans. Direct transmission of ringworm to man from infected animals has been reported from foxes (35), hedgehogs (32, 53), mice (18), muskrats (34), porcupine (50), rats (19) and a squirrel (38).

Specimens of hair from 10,462 wild animals were cultured and keratinophilic fungi were isolated from 1,260 (12%). A summary of the results as found in the literature is presented in Tables 1, 2, and 3.

Fifty-eight isolates of T. mentagrophytes, 7 of T. rosaceum, 5 of T. simii, 8 of T. terrestre, 63 of Microsporum cookei, 29 of M. gypseum and 4 of Keratinomyces ajelloi were recovered from 2,170 mice examined (17, 18, 31, 41, 56, 57, 60, 63, 70, 77). Ajello (4) reported the recovery of 31 isolates of M. cookei from 1,159 mice examined at the National Communicable Disease Center (NCDC) in Atlanta, Georgia, during a five-year period.

Marples (52) isolated T. mentagrophytes and the hedgehog variety T. mentagrophytes var. erinacei from wild mice in Australia. Dvorak

TABLE 1 - Isolates of Keratinophilic Fungi from Wild Animals by Various Investigators

Animals	Number Examined	Number Positive	Fungi isolated																								Reference
			Trichophyton gallinae	T. gallinavum	T. indicum	T. mentagrophytes	T. persicolor	T. rosceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serratus	Chrysosporum sp.	Arthroderma curreyi	A. cuniculi	A. tuberculatum	Other		
ARTIODACTYLA																											
SUIDAE																											
Sus scrofa (European wild hog)	31	1				1																					13
CERVIDAE																											
Capreolus capreolus (Roe deer)	16	2					2																				13
Cervus elaphus (European red deer)	11	[illegible]			1	1	1																				13
Odocoileus hemionus (Mule deer)	1	1										[illegible]															43
BOVIDAE																											
(No genus given) (Buffalo)	100	10				2							7			1											17
(No genus given) (Buffalo)	2	[illegible]										[illegible]															13
CARNIVORA																											
CANIDAE																											
Canis lupus (Wolf)	[illegible]	1				1																					13
Urocyon cinereoargenteus (Gray fox)	[illegible]	6												[illegible]													44, 45, 60
Vulpes fulva (Red fox)	[illegible]	0																									44, 60
Vulpes vulpes (Red fox)	[illegible]	5			3	2																					13
FELIDAE																											
Felis domesticus (Wild house cat)	[illegible]	0																									44, 60
F. silvertris (European wild cat)	[illegible]	0																									13
Lynx rufus (Bobcat)	[illegible]	0																									44
PROCYONIDAE																											
Procyon lotor (Raccoon)	[illegible]	1													[illegible]												44, 60
MUSTELIDAE																											
Meles meles (Old-World badger)	[illegible]	[illegible]				1					[illegible]																13
Mephitis mephitis (Striped skunk)	[illegible]	[illegible]													[illegible]												44, 60
Mustela nivalis (ferret)	[illegible]	0																									77
M. vision (Mink)	[illegible]	0												[illegible]													59
CHIROPTERA																											
PHYLLOSTOMIDAE																											
Glossophaga soricina (Nectar-feeding bat)	[illegible]	[illegible]				1																					40
Pteronotus psilotus (Insectivorous bat)	[illegible]	[illegible]				[illegible]																					40
PTEROPODIDAE																											
Rousettus aegypticus (Fruit bat)	10	[illegible]																									77
VESPERTILIONIDAE																											
Lasiurus boreatis (Red bat)	[illegible]	0																									59
Minioplerus schreibers (Long-winged bat)	[illegible]	0																									70
Myotis adversus (Vespertilionid bat)	33	0																									70
Pipistrellus pipstrellus (Pipshelle bat)	1	1					1																				29
INSERTIVORA																											
ERINACEAE																											
Erinaceus europaeus (Hedgehog)	447	200				[illegible]83					17																32, 33, 44, 51-54, 75, 77

TABLE 2 - Isolates of Keratinophilic Fungi from Wild Animals by Various Investigators

Animals	Number Examined	Number Positive	Fungi isolated																							Reference
			Trichophyton gallinae	T. gallopavum	T. indicum	T. mentagrophytes	T. persicolor	T. rosaceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serratus	Chrysosporum sp.	Arthroderma curreyi	A. cuniculi	A. tuberculatum	Other	
LAGOMORPHA																										
LEPORDIAE																										
Lepus americanus (Snowshoe hare)	63	22				22																				1
Lepus europaeus (European hare)	75	2				2																				13, 52
Oryctolagus cuniculus (Old-World rabbit)	2	2													1	1										70
Sylvilagus floridanus (Cottontail rabbit)	219	1													1											44, 60
S. palustris (Marsh rabbit)	4	0																								44
MARSUPIALIA																										
DIDELPHIDAE																										
Didelphis marsupialis (Opossum)	379	17				6									11											44
D. virginianus (Opossum)	59	1				1																				60
Trichosurus vulpecula (Opossum)	57	3				1									1			1								51, 52
No genus given (Opossum)	64	30				25					5															13
RODENTIA																										
CAPROMYIDAE																										
Myocastor coypu (Nutria)	1	1				1																				66
CRIETIDAE																										
Arvicola terrestris (Water vole)	8	0																								63
Clethrionomyces glarcolus (Bank vole)	234	135					103				23					7		2								30, 31, 63
Meriones libycus (Sand rat)	1	0																								77
Microtus agrestis (Field vole)	114	29					29																			29, 31
M. arvalis (Field vole)	40	36									23					12		1								63
Neotoma floridana (Wood rat)	22	6													6											44, 56, 60
Pachuromys duprasi (Fat-tailed rat)	2	0																								77
Peromyscus gossypinus (Cotton mouse)	129	39				4									33	2										
P. nuttali (Golden mouse)	7	1													1											44, 57, 60
P. polionotus (Field mouse)	895	123				42		7			34				23	17										17, 44, 57, 60
Pitymys pinetorum (Pine mouse)	2	0																								57, 60
P. subterraneum (Pine mouse)	5	5									4							1								57, 60
Psammomys obesus (Diurnal sand rat)	28	0																								77
Reithrodontomys humulis (Harvest mouse)	49	6													4	2										44, 56, 60
Sigmodon hispidus (Cotton rat)	710	123				3									118	2										
HYSTRICIDAE																										
Hystrix africae-australis (Porcupine)	7	1				1																	1			50

TABLE 3 - Isolates of Keratinophilic Fungi from Wild Animals by Various Investigators

Animals	Number Examined	Number Positive	Fungi Isolates																							Reference
			Trichophyton gallinae	T. gallopavum	T. indicum	T. mentagrophytes	T. persicolor	T. rosaceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serrutus	Chrysosporum sp.	Arthroderma curreyi	A. cuniculi	A. tuberculatum	Other	
RODENTIA																										
MURIDAE																										
Acomys charinus (Egyptian spiny mouse)	326	0																								77
Apodemus flavicollis (Old-World mouse)	21	16									7					8		1								63
A. sylvaticus (Wood mouse)	32	8				3	5																			30, 63
Arvicanthis miloticus (Nile grass rat)	507	2														2										77
Hydronys chrysogaster (Water rat)	5	5																			5	1				70
Melomys cervinipes (Banana rat)	19	11													5					2	4	1			1	70
M. lutillus (Mosaic-tailed rat)	4	3														1				1	1	1				70
Micromys minutes (Old-World harvest mouse)	1	1				1																				63
Millaridia meltoda (Soft-furred rat)	15	0																								41
Mus musculus (House mouse)	964	64				52					6				3	2		1								18, 41, 44, 57, 60,63,70
M. platythrix (Brown spiny mouse)	34	5								5																41
Nesokia indica (Bandicoot rat)	35	1								1																41, 70
Ratus assimilis (Indian mole-rat)	55	38													16	2				8	27	14				70
R. bengalensis (Indian mole-rat)	13	0																								41
R. blanfordii (Blandford's rat)	1	0																								41
R. conatus (Indian mole-rat)	5	1																			1					70
R. exulans (Polynesian rat)	64	1				1																				52
R. lutreolus (Indian mole-rat)	3	2													2						1					70
R. norvegicus (Norway rat)	494	22				16									1	4		1		1					2	41, 44, 52, 60, 70, 77
R. ratus (Roof rat)	494	38				5									15	1				1	12		5		4	7, 41, 44, 52, 60, 70
Uromys caudimaculatus (Naked-tailed rat)	6	4																			3	2				70
ONDATRAE																										
Ondatra zibethica (Muskrat)	364	35*				35*																				34
O. zibethica (Muskrat)	3	0																								63
SCIURIDAE																										
Funambulus palmarum (Common Indian striped squirrel)	27	2								2																17
Ratufa indica malabarica (Malabar giant squirrel)	1	1															1									38
Sciurus carolinensis (Gray squirrel)	5	1				1																				24, 44, 60
S. niger (Fox squirrel)	57	0																								44, 60
S. vulgaris (Squirrel)	24	2													2											13

*Refers to litters which contained five or more muskrats per litter

and Otcenasek (26) have reported the following dermatophytes to be found in mice: T. gallinae, T. mentagrophytes, T. rubrum, T. schoenleinii, T. violaceum and M. gypseum.

Bennett, according to Buchanan (19) was the first to record observations on favus of mice, in 1850. Since then numerous cases of mouse favus have been reported (17). It is interesting to note that only 30 of the 2,170 mice examined (17) showed signs of an active dermatophyte infection (favus).

Menges and Georg (59) obtained 21 isolates of T. mentagrophytes, 11 of M. cookei and 2 of K. ajelloi from 471 rats representing three genera. Of this total number of rats, 16 had lesions. Culturing material from the lesions failed to yield keratinophilic fungi. Marples (52) examined 28 rats which represented 2 genera and 10 unknown species and obtained 2 isolates of T. mentagrophytes and 1 of the hedgehog variant T. mentagrophytes var. erinacei. Culturing of hair samples from 767 rats produced 20 isolates of T. mentagrophytes, 1 of T. simii, 7 of M. gypseum and 2 of M. cookei (7, 38, 76, 77). Ajello (4) reported 158 isolates of M. cookei recovered from 1,193 rats examined by the NCDC, over a five-year period. Rees (70) examined rats from Queensland, Australia, for keratinophilic fungi and isolated fungal species from several genera of which Arthroderma, Chrysosporium, Microsporum and Trichophyton predominated.

English (29) isolated T. persicolor from scaly lesions on the tail of a field vole (Microtus agrestis). Trichophyton persicolor is very common in voles; 132 isolates were obtained from 396 samples

examined (29-31, 63). English observed that 1 in 2 voles had the organism colonized on their tails and postulated that presence of the fungus on the body was usually in the nature of a contamination or minimal infection. Forty-six K. ajelloi were also isolated from voles (29-31, 63).

English (29) isolated T. persicolor from a pipishelle bat (Pipistrellus pipistrellus). Grose (40) isolated M. canis from the hair of the insectivorous bat (Pteronotus psilotus) collected from a cave and T. mentagrophytes from the hair of the nectar-feeding bat (Glassophaga sericina) caught in a mine.

Errington (34) observed a skin disease in young muskrats (Ondatra zibethica) in Iowa during the breeding season of 1935, 1936, and 1938. Thirty-five (9.6%) of 364 litters examined had a mycotic infection of the skin (20, 34). In 1936, an animal caretaker contracted ringworm of the arm from a diseased muskrat. Cultures isolated from arm lesions were identified as T. mentagrophytes (20). Dozier (25) reported on a possible Trichophyton infection in muskrats in Maryland. Otcenasek and Dvorak (63) failed to isolate keratinophilic fungi from three muskrats examined during a survey of small animals. Trichophyton mentagrophytes has been isolated from nutria (66). Nutria (Mycocastor coypu) are water rodents commonly found in South America, where they are valued as a fur bearing animal.

DeLamater (24) isolated T. mentagrophytes from a common gray squirrel (Sciurus carolinensis) which was severely infected with the fungus. This was the first time squirrels were reported as hosts for

T. mentagrophytes. Microsporum vanbreuseghemii was isolated from lesions typical of ringworm in a Malabar giant squirrel (Ratufa indica malabarica). This was the first recorded isolation of this dermatophyte (38). Fifty-three squirrels were examined for keratinophilic fungi and 2 isolates of K. ajelloi and 2 of T. simii were recovered (13, 41).

Opossums have been found to carry keratinophilic fungi on their coats (29). Thirty-three isolates of T. mentagrophytes, 5 of T. terrestre, 12 of M. cookei and 1 of K. ajelloi were recovered from 559 opossums (13, 44, 51, 60). During a five-year period of study, the NCDC examined 874 opossums and recovered 22 isolates of M. cookei (4).

Menges et al. (60) failed to demonstrate the presence of dermatophytes in 42 raccoons (Procyon lotor), but Ajello (4) reported the recovery of 2 isolates of M. cookei from 1,177 raccoons examined by the NCDC. Alters et al. (13) examined 20 badgers and recovered 1 isolate each of T. mentagrophytes and T. terrestre.

Microsporum cookei was isolated from 2 of 248 hair samples collected from striped skunks (44, 60). Ajello (4) recovered 4 isolates of M. cookei recovered from 487 skunks examined over a five-year period.

Marples (51), Marples and Smith (53, 54) and LeTouche and Forster (47) have shown that the hedgehog (Erinaceus europaeus) is heavily infected (62 of 187 samples positive) with T. mentagrophytes. Smith and Marples (74) isolated a variant of T. mentagrophytes from 51 of 114 wild hedgehogs. Physiological tests indicated that the hedgehog isolates formed a distinct group, which differed from the granular

and interdigital varieties by producing a bright yellow pigment and having larger microconidia than the microconidia of T. mentagrophytes var. interdigitale or T. mentagrophytes var. granulare. These investigators placed the variant in a separate variety of T. mentagrophytes and proposed the name of T. mentagrophytes var. erinacei. English et al. (32) isolated T. mentagrophytes from ringworm lesions of human patients who had been in contact with hedgehogs. The isolates produced the yellow pigment and large microconidia similar to T. mentagrophytes var. erinacei. A survey by English et al. (33) resulted in the recovery of 56 isolates of T. mentagrophytes var. erinacei from 117 hedgehogs. The failure of those variants to survive in unsterile soil seemed to indicate soil as an unlikely source of infection for hedgehogs and further, the variant was a true inhabitant of hedgehog skin.

Marples and Smith (54) isolated the geophilic fungus T. terrestre from 12 of 85 hedgehogs examined. Four of the isolates were unpigmented and were identical macroscopically and microscopically with soil isolates. The remaining 8 isolates were slow growing and produced large amounts of wine-colored pigment in the medium. Physiological tests indicated that these variants form a homogeneous group with several characteristics not present in the soil isolates. The failure of these organisms to grow in sterile soil suggests that they are true inhabitants of the skin of hedgehogs rather than soil organisms.

Marais and Oliver (50) investigated another spine-bearing animal, the porcupine (Hystrix africa-australis). Trichophyton mentagrophytes was isolated from a porcupine which had an extensive white,

scaly eruption covering its snout. The authors indicated this was the first record of dermatophytosis in a member of the Hystricidae.

Honess and Winter (43) reported the isolation of T. faviforme (T. verrucosum) from a mule deer (Odocoileus hemionus) in Wyoming. The isolation was made from lesions of the face, neck, legs and around the eyes. Alters et al. (13) recovered 2 isolates of T. terrestre from 16 deer (Roe Buck) and 1 isolate each of T. mentagrophytes, T. terrestre, and T. indicum from 11 stag. Trichophyton mentagrophytes, T. verrucosum, T. violaceum and M. gypseum have been isolated from buffaloes (17).

Menges et al. (60) failed to isolate dermatophytes from 5 gray foxes (Urocyon cinereoagenteus) and 2 red foxes (Vulpes vulva). Alters et al. (13) obtained 2 isolates of T. mentagrophytes and 3 of T. indicum from 16 foxes (Vulpes vulpes) and T. mentagrophytes from 4 wolves (Canis lupus). Levenberg (48) reported the isolation of Microsporum lanosum (M. canis) from 6 silver-gray foxes.

Rabbits, hares and cottontails have a low incidence of dermatophyte infection (44, 52, 59, 70, 72). Only one case of enzootic dermatophytosis has been reported (1). This occurred on an isolated island within a lake in Montana. Many members of a wild snowshoe hare (Lepus americanus) population were observed to have a disease condition of the skin. Material from 63 hares was cultured and 22 isolates of T. mentagrophytes were recovered. It was noted that throughout the duration of the study (9 months) the number of infected animals neither increased nor decreased, which indicated the disease was endemic.

Thirty-one European wild hogs (Sus scrofa) were examined for keratinophilic fungi and 1 isolate of T. mentagrophytes was recovered (13).

No isolations of keratinophilic fungi have been reported from wild house cats, European wild cats, bobcats, ferrets or mink (13, 44, 60, 77).

Keratinophilic Fungi of Birds

Keratinophilic fungi are commonly found on feathers of fowl (Table 4). Rees (72) isolated 17 genera of keratinophilic fungi from 546 (97.8%) of 558 feather samples from domestic fowls in Southern Queensland, Australia. Chrysosporium spp. (84.8%) and Ctenomyces serrutus (76.5%) predominated.

A mycotic disease of poultry known as favus or white comb disease is caused by the keratinophilic fungus, Trichophyton gallinae. Favus infection is manifested by the formation of dry, white scaly deposits which appear on the comb and wattles and sometimes on the neck (16). King (45) indicated that lesions also appear in the mouth and upper respiratory system and may extend down to the crop.

Londero et al. (49) isolated T. gallinae from a domestic chicken in which heavy incrustation of the comb, wattles, and distal portion of the neck was observed. Pepin and Austwick (66) recovered 3 isolates of T. gallinae from 41 favic chickens.

Isolation of T. gallinae from turkeys has been reported by Hinshaw and Rosenwald (42) and Menges and Georg (58). A recently reported dermatophyte described under the name Trichophyton gallopavum was isolated from favic turkeys in France (61). The author described the dermatophyte as being intermediate between T. gallinae and T. quinckaneum (T. mentagrophytes).

Micropsorum gypseum, a keratinophilic fungus commonly found in soil has been reported as a cause of favus in fowl (49).

TABLE 4 - Isolates of Keratinophilic Fungi from Domestic and Wild Birds by Various Investigators

Orders of Birds	Number Examined	Number Positive	Fungi Isolated																							Reference
			Trichophyton gallinae	T. gallopavum	T. indicum	T. mentagrophytes	T. persicolor	T. rosaceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serrutus	Chrysosporum sp.	Arthroderma curreyi	A. cuniculi	A. tuberculatum	Other		
Caprimulgiformes	4	0																								70
Casuariiformes	1	1																		1	1					70
Charadriiformes	12	4																		3	1					70
Ciconiiformes	10	5																		4	1					70
Columbiformes	21	8													1					4	2		2			70
Coraciiformes	26	7																		5	2		1	1		70
Cuculiformes	8	3													1					1	1					70
Falconiformes	11	4																		4	1			1		70
Galliformes																										
Domestic fowl	602	552	5	1												2			427	473	14		98	266		45, 46, 60, 65, 71
Wild fowl	6	6													3	1			1		4		1			64, 70
Gruiformes	2	1																			1					70
Passeriformes	209	94									1				6				3	32	56	1	13	4		53, 66, 70
Pelecaniformes	3	2																		2						70
Podicipiformes	2	1																		1						70
Psittaciformes	20	10																		3			7	1		70
Stigiformes	4	2																		2						70
Turniciformes	1	0																								70

Trichophyton schoenleinii (37) and T. gallinae (58) have been isolated from favic canaries.

Rees (71) isolated 5 genera of keratinophilic fungi from 116 (40.7%) of 285 feather samples collected from wild birds representing 113 species. Chrysosporium spp. (21.8%), Arthroderma curreyi (14.0%), Arthroderma tuberculatum (8.5%) and Microsporum cookei (3.9%) predominated. Pugh (67) isolated the keratinophilic fungi A. curreyi and T. terrestre from the feathers of blackbirds (Turdus merula). Marples (52) failed to isolate keratinophilic fungi from the wild thrush (Turdus ereceterum) and wild Whiteeyes (Zosterops lateralis).

Patiala (65) reported the presence of mycotic infection in the black grouse (Tetra tetrix) population in Finland. Trichophyton gallinae was isolated from featherless lesions of the neck. This condition in Finnish grouse caused concern because of the popularity of the grouse as a game bird.

Pugh (68, 69) baited birds' nests with sterile wool and isolated keratinophilic fungi. The pH of the nest substrates upon which individual fungi occurred was determined. The author postulated that pH of the substrate might explain the differences in distribution of fungi in nests and the variations between fungi found on feathers taken from birds and the nest flora. Rees (71) suggested that the pH and the amount of keratin available are important in determining the numbers and types of keratinophilic fungi isolated from a particular source.

Material and Methods

Collections of hair samples from wild animals and soil samples were made in numerous counties of South Dakota from June, 1966 to June, 1968. The counties from which hair samples and soil samples were collected are shown in Figures 1 and 2, respectively. The procedure for collecting, examining and culturing the samples are as follows:

1. Collection of specimens

a. Hair samples. Using sterile disposable gloves, hairs were plucked from the face, back and abdomen of the animals. If the animal showed any evidence of skin lesions, the hairs were removed from this area. The following information was recorded on a card in the sterile plastic bag with each sample: Date, Biologist, Location in State (section, range, township), Name of animal and any pertinent comments.

b. Soil samples. Soil samples were collected from the surface soil in the immediate area of the animal habitat. The samples were collected with a stainless steel spatula and placed in a plastic bag for storage. Whenever possible, soil samples were collected from habitats from which an animal had been trapped and hair specimens had been obtained. Such associations were noted and proper identification made.

2. Examination of specimens for fungi

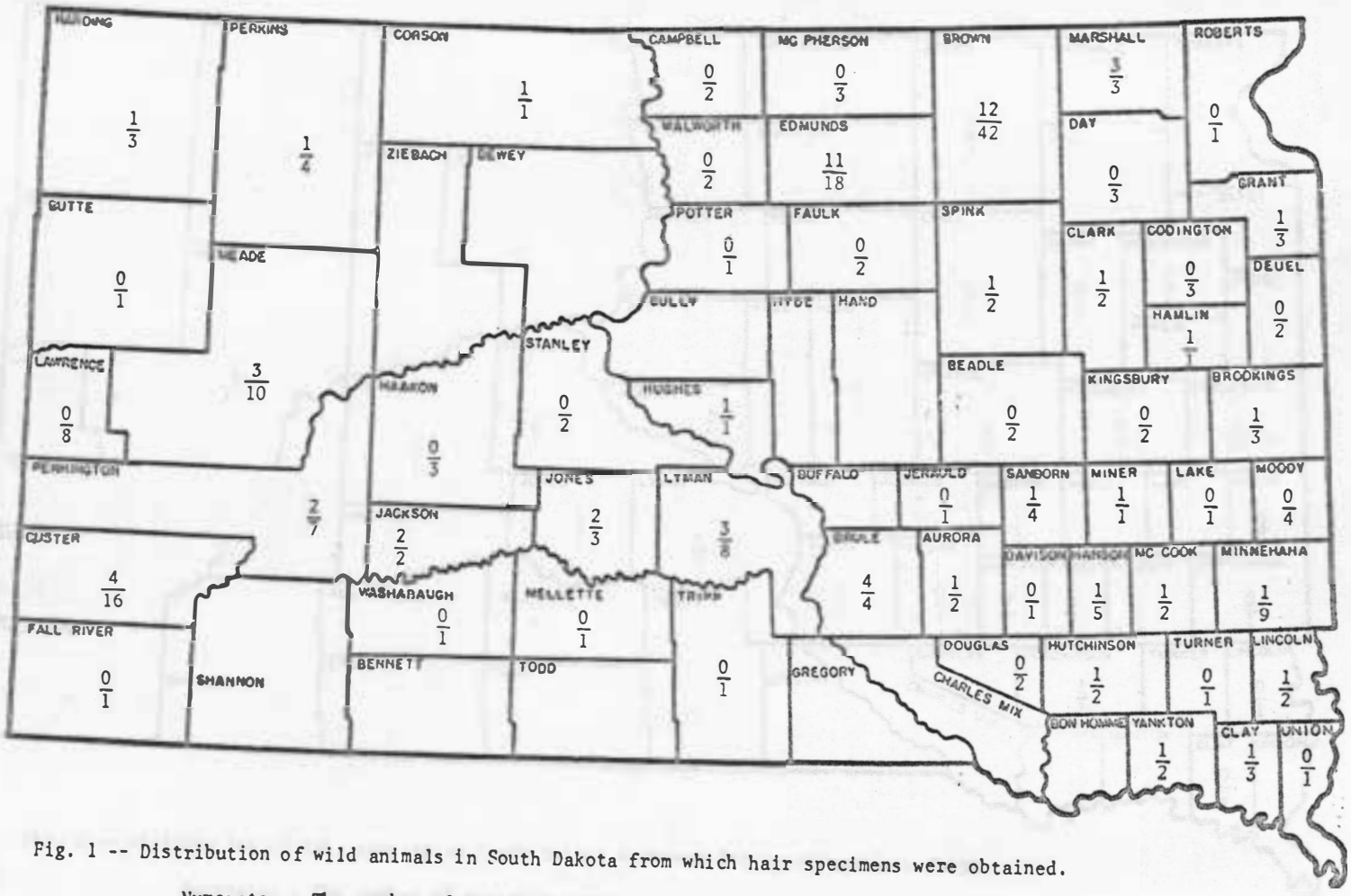

Fig. 1 -- Distribution of wild animals in South Dakota from which hair specimens were obtained.

Numerator = The number of positive samples.
Denominator = The number of wild animals tested.

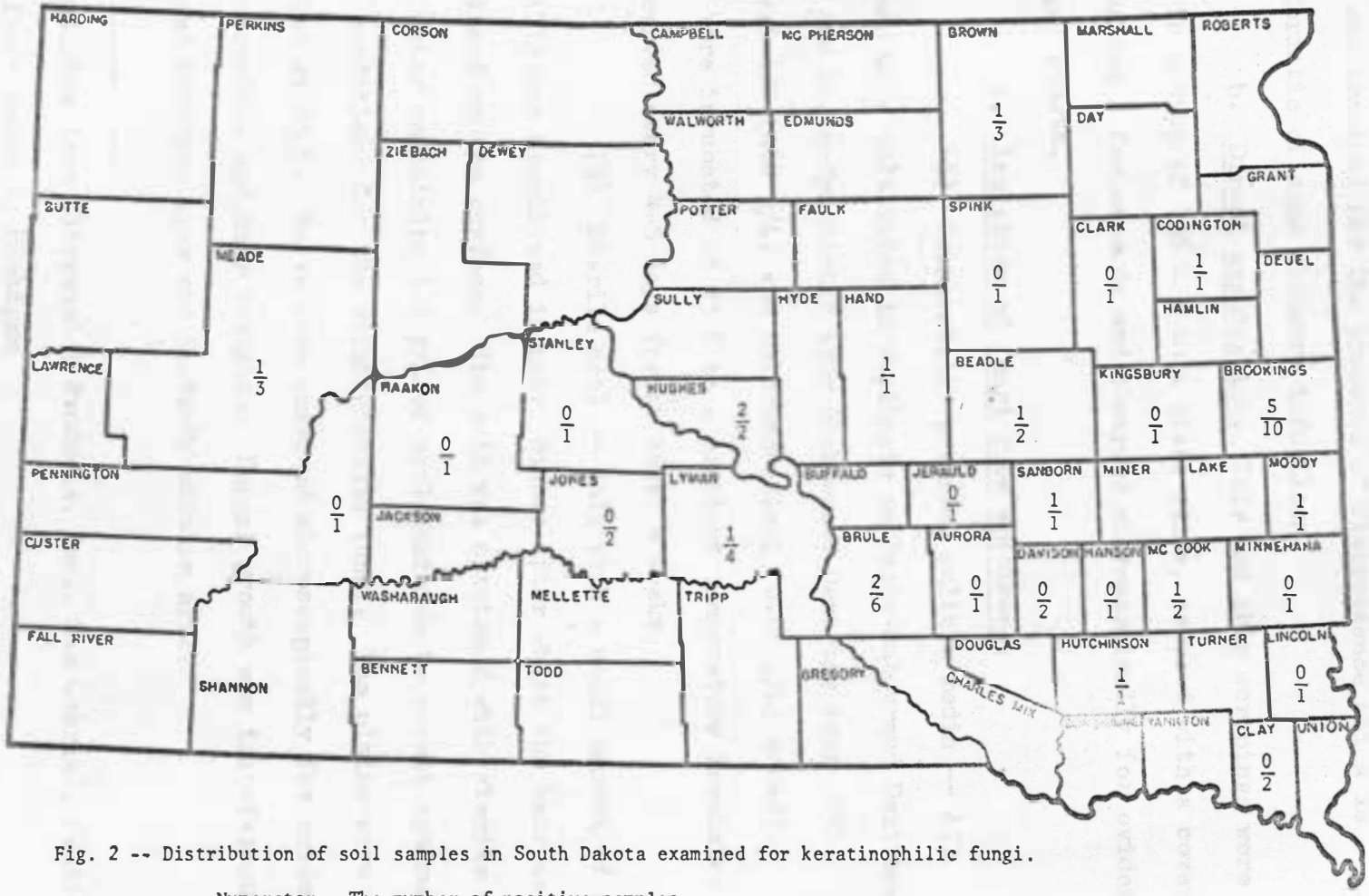

Fig. 2 -- Distribution of soil samples in South Dakota examined for keratinophilic fungi.

Numerator = The number of positive samples.
Denominator = The number of samples tested.

a. Wood's lamp examination (Black-Ray)*. Every hair sample was examined for the presence of fluorescence which is characteristic of some ringworm infections.

b. Direct examination. Hair and skin scrapings were placed in a drop of 10% KOH on a glass slide, covered with a cover slip, heated a few seconds and observed microscopically for evidence of fungal growth.

c. Isolation of fungi from specimens.

(1) Commercially prepared culture media -- All specimens were cultivated in duplicate on Bacto-Sabouraud Dextrose Agar** and Bacto-Mycobiotic agar (Sabouraud Dextrose Agar with cycloheximide (0.4 g/L) and chloramphenicol (0.05 g/L) added). Plates were incubated at 25 C in a constant temperature incubator and observed every 4-5 days for at least 4 weeks.

(2) Sterile soil -- Soil with a small amount of sawdust added (7l) was sterilized in petri dishes after which the hair samples were placed on the surface. The soil was moistened with sterile distilled water containing 1.0 g/L of cycloheximide to permit optimal growth conditions for the keratinophilic fungi. The plates were incubated at 25 C. Hairs were examined microscopically for evidence of macroconidia and hair invasion. Fungal growth was transferred to Sabouraud Dextrose Agar and Bacto-Mycobiotic agar.

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* Available from Ultraviolet Products, Inc., San Gabriel, California

** Difco - Detroit, Michigan

3. Soil specimens

The soil baiting procedure of Vanbreuseghem (80) was utilized to facilitate the isolation of the keratinophilic fungi from the soil. The soil sample was placed in a petri dish to half the depth of the dish. The soil was moistened with sterile distilled water, avoiding excessive wetting. Small tufts (1 to 2 cm) of sterilized human and horse hair were placed on the soil surface. The covered plate was incubated at 25 C and observed at 4-6 day intervals for the appearance of fungus growth on the hairs. Hairs which showed fungal growth were examined in lacto-phenol cotton blue mounts and transferred to Bacto-Sabouraud Dextrose agar and Bacto-Mycobiotic agar in an attempt to isolate the keratinophilic fungi in pure culture.

4. Identification procedures

All organisms were identified on the basis of gross and microscopic morphology. If teased mount preparations in lacto-phenol cotton blue (11) were not satisfactory, the slide culture preparation was utilized. The ability of dermatophytes to perforate hair *in vitro* was also used to identify the organisms. The test was performed as follows: short strands of human hair (1 cm) were placed in petri dishes and sterilized by autoclaving them at 120 C for 10 minutes. Twenty-five ml of sterile distilled water and 2 or 3 drops of 10% sterilized yeast extract were added to the plates. The plates were then inoculated with several small fragments of the test fungi. The plates were incubated at 25 C and examined at regular intervals over a 4 week period. For examination, hairs were removed from inoculated plates with sterile

forceps, placed in a drop of lacto-phenol cotton blue and examined under the microscope for perforations (11).

The teased mounts were prepared by removing a small portion of the colony and placing it in a drop of lacto-phenol cotton blue. The material was teased apart by using 2 steel needles before adding a cover slip to the slide. Sterile technique was used in all instances of teased mount preparation.

The slide culture technique (11) allows for the observation of fungal structures without disrupting them. The sequence (Figure 3) for preparation of the slide culture was as follows:

1. A microscope slide was placed on a bent glass rod in the bottom of a petri dish (100x20 mm) covered and sterilized at 121 C for 15 minutes.
2. Potato dextrose (Difco) agar plates were prepared with 20 ml per plate. After solidification, 1 cm square blocks were cut in the agar using a sterile scalpel.
3. Using sterile technique, the agar blocks were placed on the slide in the petri dish.
4. All four sides of the agar blocks were inoculated with the fungi.
5. The inoculated agar blocks were covered with a sterile cover slip.
6. Eight ml of sterile distilled water was added aseptically and the plate incubated at 25 C.
7. When spores appeared, or abundant growth was evident, the cover slip was carefully removed with the fungal growth upward.
8. The agar block was removed from the slide and placed in disinfectant.

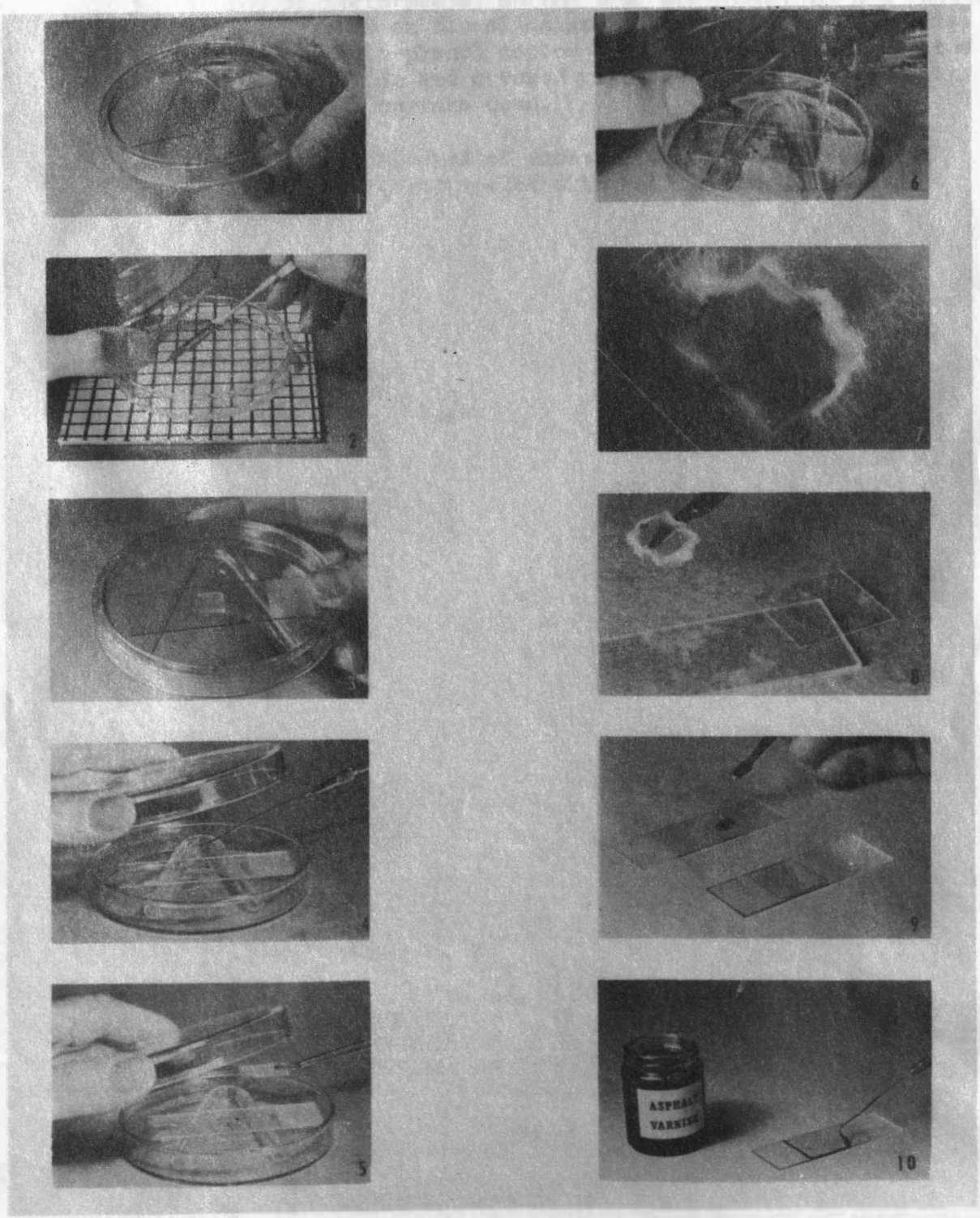

Fig. 3 -- The sequence for the preparation of the slide culture (11).

9. A drop of lacto-phenol cotton blue was placed on the fungal growth present on the slide and covered with a cover slip. A drop of lacto-phenol cotton blue was placed near one end of a clean slide and covered with the original cover slip with mycelial surface down.

10. The slide was cleaned of excess stain, and the edges of the cover slip were sealed with nail polish or asphalt tar varnish.

Results and Discussion

Hair samples from 217 wild animals of 32 species were examined. The animals and the fungi isolated from them are listed in Table 5. The scientific names of the mammals listed are from Walker (81). Keratinophilic fungi were isolated from 61 specimens (28.1%). Animals harboring dermatophytes (21.6%) were free of ringworm lesions, except for 2 bison samples. The direct microscopic and Wood's lamp examination were negative for all specimens. The keratinophilic fungi recovered were as follows: 1 isolate of Trichophyton gloriae, 16 of T. mentagrophytes, 30 T. terrestre, 2 of Keratinomyces ajelloi and 14 of Chrysosporium spp. Other genera of fungi observed were Alternaria, Aspergillus, Cephalosporium, Geotrichum, Mucor, Nigrospora, Paecilomyces, Penicillium, Rhizopus, Scopulariopsis, Sporotrichum, Stemphylium, Streptomyces, Trichoderma and Verticillium.

Two isolates of T. mentagrophytes, 7 of T. terrestre, and 8 of Chrysosporium spp. were recovered from 88 deer hair samples. The isolation of fungi pathogenic to man from deer would indicate that deer could be a reservoir for the transmission of fungal diseases. One of the samples yielded T. mentagrophytes and T. terrestre from a soil plate but no isolates were recovered from the duplicate Bacto-Sabouraud Dextrose or Bacto-Mycobiotic agar plates.

Trichophyton terrestre was isolated on soil baited with hairs from an antelope (Antilocapra americana). No keratinophilic fungi were isolated from antelope hair cultured on the agar plates. A search of

TABLE 5 - Keratinophilic Fungi Isolated from Wild Animals in South Dakota

Animals	Number Examined	Number Positive	Fungi Isolated																								
			Trichophyton gallinae	T. gallopavum	T. gloriae	T. indicum	T. mentagrophytes	T. persicolor	T. rosaceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serratus	Chrysosporum sp.	Arthroderma curreyi	A. curreyi	A. tuberculatum			
ARTIODACTYLA																											
CERVIDAE																											
Odocoilcus hemionus (Mule deer)	13	3										1									2						
O. virginianus (White-tailed deer)	53	13					2					6									6						
ANTILOCAPRIDAE																											
Antilocapra americana (Antelope)	2	1										1															
BOVIDAE																											
Bison bison (American buffalo)	9	2					1												1								
CARNIVORA																											
CARIDAE																											
Canis latrans (Coyote)	10	4					1					3															
Urocyon cinereoargenteus (Gray fox)	2	1																			1						
Vulpes fulva (Red fox)	3	2										2									1						
PROCYONIDAE																											
Procyon lotor (Raccoon)	17	1					1																				
MUSTELIDAE																											
Mephitis mephitis (Striped skunk)	4	2					1														1						
Mustela frenata (Long-tailed weasel)	3	1			1																1						
M. nigripes (Black-footed ferret)	1	1					1																				
M. vison (Mink)	3	1										1															
Taxidea taxus (American badger)	7	4					1					3															
CHIROPTRA																											
VESPERTILIONIDAE																											
Myotis sp.	2	0																									
LAGOMORPHA																											
LEPORDIAE																											
Lepus americanus (Snowshoe rabbit)	18	5					4					1															
Sylvilagus floridanus (Cottontail rabbit)	3	0																									

TABLE 5 (Continued) - Keratinophilic Fungi Isolated from Wild Animals in South Dakota

Animals	Number Examined	Number Positive	Fungi Isolated: Trichophyton gallinae	T. gallopavum	T. gloriae	T. indicum	T. mentagrophytes	T. persicolor	T. rosaceum	T. schoenleinii	T. simii	T. terrestre	T. verrucosum	T. violaceum	Microsporum canis	M. cookei	M. gypseum	M. vanbreuseghemii	Keratinomyces ajelloi	Ctenomyces serratus	Chrysosporum sp.	Arthroderma curreyi	A. cuniculi	A. tuberculatum
MARSUPIALIA																								
DIDELPHIDAE																								
Didelphis virginianus (Opossum)	2	1																			1			
RODENTIA																								
CASTORIDAE																								
Castor canadensis (Beaver)	1	0																						
CRIETIDAE																								
Microtus pennsylvanius (Field vole)	5	3					1					1							1		2			
M. ochrogaster (Field vole)	1	1										1												
Oncynomys leucogaster (Grasshopper mouse)	5	2										2												
Perognitnus flouescons (Plains pocket mouse)	2	0																						
Peromyscus maniculatus (Prairie white-footed deer mouse)	23	8					3					4									1			
ERETHIZONTIDAE																								
Erithizon dorsatum (North American porcupine)	1	1										1												
MURIDAE																								
Mus hudsionus (House mouse)	1	0																						
M. musculus (House mouse)	1	1										1									1			
Ratus ratus (Brown rat)	2	1										1												
ONDATRAE																								
Ondatra zibethica (Muskrat)	4	0																						
SCIURIDAE																								
Citellus tridecemlineatus (Striped ground squirrel)	4	0																						
Sciurus niger (Fox squirrel)	16	2										2												
GALLIFORMES																								
TETRAONIDAE																								
Pedioecetes phasianellus (Sharp-tailed grouse)	1	1					1																	
Total	217	61			1		16					31							2		18			
Isolation rate, (%)		28.1			0.5		7.4					14.3							0.9		8.3			

the literature failed to reveal any previous isolations of dermatophytes from antelope.

Two of the 9 samples from bison (Bison bison) were from cutaneous lesions. Repeated attempts to culture dermatophytes from these lesions were unsuccessful. Keratinomyces ajelloi was isolated from hair which surrounded the lesion. Histopathologic examination of tissue sections of the cutaneous lesions revealed the presence of fungal elements in the keratin layer of the skin. One isolate of T. mentagrophytes was recovered from a hair specimen of a bison which showed no clinical signs of a dermatophyte infection.

Dermatophytes were isolated from 40% of the family Canidae examined. Coyotes had a high incidence of T. terrestre on their hair (30%). One isolate of T. mentagrophytes was recovered from the hair of a coyote. No isolates of T. mentagrophytes were recovered from 5 foxes. Trichophyton mentagrophytes was isolated from 1 of 17 raccoons. The high incidence of dermatophytes found on animals in the family Canidae is probably due to their close association with the soil.

Members in the family Mustelidae were examined and found to harbor dermatophytes. Trichophyton mentagrophytes was obtained from 1 of 4 striped skunks and 1 of 7 badgers. Fifty-seven percent of the badger samples harbored fungi which are pathogenic to man. Three samples of hair from weasels were cultured and 1 isolate of Chrysosporium spp. and 1 T. gloriae were recovered. Trichophyton gloriae, a geophilic dermatophyte adapted to semi-arid soils, was recently reported by Ajello (10). This would appear to be the first reported isolation of this organism from a mammal.

Two hair samples from bats were cultured and found negative for keratinophilic fungi. Since only 2 samples from this order (Chiroptera) were obtained, no definite conclusions could be drawn from these negative results.

Hair samples from 18 hares (Lepus americanus) were cultured and 5 isolates of T. mentagrophytes and 1 of T. terrestre were recovered. No keratinophilic fungi were isolated from 3 cottontails (Sylvilagus floridanus) examined.

One isolate of K. ajelloi was obtained from 2 opossum hair samples. No other keratinophilic fungi were recovered from the Marsupialia.

Thirty-six members of the family Cricetidae (mice and voles) were examined for keratinophilic fungi and 14 isolates (38.9%) were recovered. Six field voles were found to harbor 3 of the 4 genera of keratinophilic fungi isolated during this investigation. One isolate of T. mentagrophytes, 2 of T. terrestre, 1 of K. ajelloi and 2 of Chrysosporium spp. were recovered. Samples from 28 white-footed deer mice (Peromyscus maniculatus) were examined and 3 isolates of T. mentagrophytes, 4 of T. terrestre and 1 of Chrysosporium spp. were recovered. The grasshopper mouse (Oncynomys leucogaster) was found to harbor only 1 species of keratinophilic fungi, the geophilic dermatophyte T. terrestre. No isolations of keratinophilic fungi were made from 2 plains pocket mice (Perogritnus flouescons).

Hairs of 4 animals in the family Muridae of the order Rodentia were examined for keratinophilic fungi. One isolate of T. terrestre

and a Chrysosporium spp. were recovered from a house mouse (Mus musculus). Keratinophilic fungi were not isolated from a sample of hair collected from another species of house mouse (Mus hudsionus). Two hair samples from brown rats were cultured and 1 isolate of T. terrestre was recovered.

The small rodents examined from the families of Cricetidae and Muridae had a high incidence of dermatophytes harbored on their coats (32.5%). These animals are a possible reservoir of pathogenic fungi for other wild and domestic animals.

Keratinophilic fungi were not isolated from 4 ground squirrel specimens (Citellus tridecemlineatus), but 2 isolates of T. terrestre were recovered from 18 fox squirrels (Sciurus niger).

The isolation of T. terrestre from a North American porcupine (Erithizon dorsatum) was of special interest in this study. It was from the specimen of this animal that the value of using sterile soil as a medium for culturing keratinophilic fungi was demonstrated. Duplicate plates of Bacto-Sabouraud Dextrose agar and Bacto-Mycobiotic agar, used during this investigation were inoculated with quills from the porcupine. Within 7 days, the plates were overgrown with a yeast. Trichophyton terrestre which was probably inhibited by the rapid growth of the yeast, did not grow on the agar plates, but did grow in abundance on the soil plate. It is of interest to note that in 10 cases, keratinophilic fungi were isolated on the sterile soil plates, but not on the agar plates.

Initial isolation attempts using sterile soil as a culture medium were unsuccessful. Mucor spp. and Rhizopus spp. overgrew the

hair obscuring any keratinophilic fungi which might have been present. The addition of cycloheximide (1.0 g/L) in the sterile water used to moisten the soil plates inhibited the growth of the Phycomycetes, thus facilitating the isolation of the keratinophilic fungi.

The head and neck from a sharp-tailed grouse (Pedioccetes phasianellus) were submitted for examination. The neck had dry, yellow, lesions 0.4 cm to 1.3 cm in diameter. The nictitating membrane of the eyes had similar but smaller lesions. Representative portions of the lesions were cultured and T. mentagrophytes were isolated. Pataila (65) isolated T. gallinae from favic grouse in Finland. This is apparently the first reported isolation of T. mentagrophytes from sharp-tailed grouse which showed signs of favus.

The seasonal distribution of keratinophilic fungi in wild animals examined is shown in Table 6. The seasonal high (50.0%) occurred in the winter months (December, January, February). The seasonal low (23.3%) occurred in the fall months when most of the samples were collected for this study. Spring (28.6%) and summer (30.1%) had approximately the same percent positive samples.

Fourteen of 57 (24.6%) samples collected in the western third of South Dakota were positive for keratinophilic fungi. The eastern third of the state had an isolation rate of 28.6% (32 of 112). Forty-eight samples from Central South Dakota were examined and 21 isolates (43.8%) of keratinophilic fungi were recovered. The large number of positive samples from coyotes and mice inhabiting the central area was responsible for this higher recovery rate.

TABLE 6

Seasonal Variation in Percent Positive Specimens of Keratinophilic Fungi

*Season	+Number Examined	Number Positive	Percent Positive	Trichophyton gloriae	T. mentagrophytes	T. terrestre	Keratinomyces ajelloi	Chrysosporium sp.
Winter	20	10	50%	-	4	5	-	1
Spring	14	4	28.5%	-	1	2	-	1
Summer	73	20	30.1%	1	6	12	1	6
Fall	103	24	23.3%	-	4	12	1	9
Total	210	60	28.6%	1	15	31	2	17

* Months included: winter, December to February; spring, March to May; summer, June to August; and fall, September to November.

\+ Samples 1, 3, 12, 83, 109 not included because date of collection was unknown

Eighteen of 54 (33.3%) soil samples collected from various areas of South Dakota were positive for the isolation of keratinophilic fungi. Keratinomyces ajelloi was isolated from all 3 regions of the state. Trichophyton terrestre was isolated (7 of 18) from soil samples collected from the central and eastern two-thirds of South Dakota. Trichophyton mentagrophytes was isolated only from soils near the Missouri River in Central South Dakota. Samples collected at Oakwood State Park, Brookings County, yielded T. gloriae. This is apparently the first recorded isolation of this geophilic dermatophyte from non-arid soils.

Keratinophilic fungi and dermatophytes were found in all areas of South Dakota (Figure 1 and 2). Keratinophilic fungi (exclusive of Chrysosporium spp.) were isolated from 21.6% of all specimens examined in this study as contrasted with a 12.0% average as reported in the literature. Improved culturing techniques (sterile soil plus cyclohexamide) and a larger spectrum of animal species contributed to this success.

Conclusions

1. Keratinophilic fungi are widely distributed in the soil and wild animal populations in South Dakota.

2. Trichophyton terrestre and Chrysosporium spp. were the most common keratinophilic fungi isolated from wild animal hairs. These geophilic organisms are of no public health significance.

3. Trichophyton mentagrophytes, an etiological agent of ringworm, was isolated from 15 of 217 wild animal hair specimens.

4. Trichophyton gloriae was isolated from a weasel and from non-arid soil.

5. Microsporum gypseum was not isolated from soil samples or wild animal hair samples from South Dakota.

6. The etiological agent of favus in a sharp-tailed grouse was isolated and identified as Trichophyton mentagrophytes.

7. Increased emphasis on outdoor recreation in South Dakota will result in more contacts between people and wild animals. As reservoirs of infection, the wild animals may be a factor in the epidemiology of ringworm infections to man.

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