Laboratory Diagnosis of Rabies in Western Canada (1968-1977)

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SUMMARY

The results of laboratory examination of 18,086 specimens for the presence of rabies antigen by the fluorescent antibody and mouse inoculation tests over a ten year period are presented. The submissions were received from British Columbia, Alberta, Saskatchewan, the Yukon and the Northwest Territories. Of those examined, 10.74% were positive; however, the incidence of rabies varied widely in the specimens and species submitted, depending on their origin. The principal wildlife reservoirs of the disease appear to be skunks, foxes and bats. A correlation of almost 99% was obtained between the fluorescent antibody test and the mouse inoculation test, indicating that the diagnostic procedures used were highly reliable in identifying rabies-infected animals.

RÉSUMÉ

Le diagnostic de la rage au laboratoire, dans l'ouest du Canada (1968-1977)

L'auteur rapporte les résultats de l'examen de 18,086 cas suspects de rage, par l'immunofluorescence et l'inoculation de souris, au cours des dix dernières années. Ces cas provenaient de la Colombie Britannique, de l'Alberta, de la Saskatchewan, du Yukon et des Territoires du Nord-Ouest; 10.74% d'entre eux s'avérèrent positifs. Le nombre de cas de rage varia toutefois de façon marquée, selon le lieu d'origine des sujets des diverses espèces animales. Les renards, les moufettes et les chauves-souris représentaient les principaux vecteurs, au sein de la faune. Les résultats de l'immunofluorescence correspondaient à ceux de l'inoculation de souris, dans une proportion de 99%, indice de la grande efficacité de ces deux méthodes de diagnostic.

INTRODUCTION

Rabies is an often fatal infectious disease caused by a neurotropic virus. It may affect all warm-blooded animals and man, although carnivores such as dogs, foxes, coyotes, wolves and skunks appear to be particularly susceptible. With few exceptions, rabies is as widespread throughout the world as it was centuries ago (1) and there are still many aspects of the disease that are not well understood. Although the transmission of rabies was successfully accomplished earlier, Pasteur in 1881 presented the first report on the transmission of the disease by the intracerebral inoculation of rabbits with infected material. In 1903 Negri described inclusion bodies in the cytoplasm of certain nerve cells infected with street virus and in 1958 an immunofluorescence technique was developed (7) that is the most favoured diagnostic test for rabies today.

The history of the Animal Diseases Research Institute (Western), Lethbridge dates back to 1905; however, it was not until 1962 that diagnostic examinations for rabies were initiated on a routine basis. In 1968 the fluorescent antibody technique (FAT) became the standard test for rabies at this laboratory. The mouse inoculation test (MIT) is used to check and confirm FAT results.

This report presents findings on specimens submitted to this Institute for examination during a ten year period (1968-1977).

MATERIALS AND METHODS

A total of 18,299 diagnostic and survey specimens were received from British Columbia, Alberta, Saskatchewan, Yukon and the Northwest Territories (referred to in this report as western Canada). The majority were submitted by the veterinary staff of the Health of Animals Branch, Agriculture Canada, who are primarily responsible for handling this reportable disease in animals. Others were received from public health units, Royal Canadian Mounted Police detachments, provincial veterinary laboratories and other sources. Of these submissions, 213 were unfit for examination and are only included in the data presented in Figure 1. These consisted mainly of dessicated or even headless bats plus other species where the brain had been destroyed, for example, by shooting. Province of Alberta surveys relating to rabies vector control (12) resulted in the examination of 1,941 bats and 1,954 skunks. During an Arctic fox trapline survey in the winter of 1973-1974, 158 fox heads were submitted (3) and 28 fox heads were examined towards the end of 1977 at the commencement of a similar survey.

Portions of brain tissue were examined by the FAT prescribed by the World Health Organization for diagnosis of rabies (7). The FAT-negative specimens involving human contact (and domestic animal contact until 1973) were further examined biologically by the standard MIT (15). The brains of mice dying within 30 days postinoculation were

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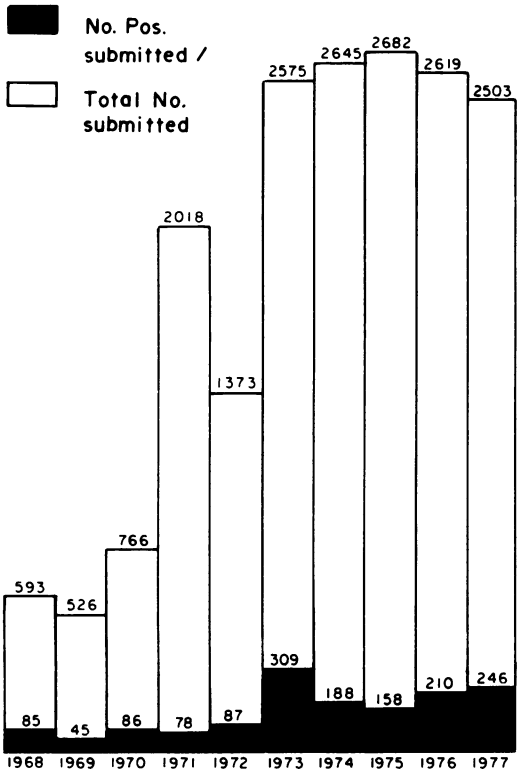

FIGURE 1. Rabies submissions to Animal Diseases Research Institute (Western), 1968-1977.

examined by FAT. In February 1976 an additional process for the early detection of rabies antigen was introduced whereby, starting on the fifth day after intracerebral injection, one weanling mouse was sacrificed each day for ten days and the brain examined (15).

RESULTS AND DISCUSSION

Table I lists the results of the examination of 18,086 submissions during the ten year period. The decision as to whether there was human contact, other than that associated with collecting the specimen, was dependent on the judgment of the submittor or deduced from the history accompanying the specimen. Tables II to VI provide results and number of submissions by regions. Table VII gives comparative results of the MIT and FAT in 4,466 cases where both tests were conducted and Figure 1 shows the total submissions received together with the total number of positive diagnoses per year.

The data presented should not be construed as indicating definitively the incidence of rabies in the animal populations of western Canada. From time to time this aspect has been discussed elsewhere for various species and regions (2, 3, 4, 6, 9, 10, 11, 13, 16, 17). However, based on the submissions received (Table I), one can generalize that there are wildlife reservoirs of rabies in western Canada and that the most common vectors of the disease in wildlife appear to be the skunk, the bat and the fox. Complete histories of domestic animal submis-

TABLE I

RESULTS OF ALL RABIES EXAMINATIONS BY SPECIES 1968-1977
(WESTERN CANADA)

Species	Human Contact		No Human Contact		Total	% Pos.
	Neg.	Pos.	Neg.	Pos.		
Bat	182	10	2356	143	2691	5.69
Caribou	0	0	0	1	1	100.00
Cat	1083	32	645	17	1777	2.76
Cattle	582	50	718	68	1418	8.32
Coyote	120	2	765	17	904	2.10
Dog	1395	29	916	37	2377	2.78
Fox	90	14	157	72	333	25.83
Horse	60	2	69	1	132	2.27
Pig	11	1	39	13	64	21.89
Raccoon	13	0	26	1	40	2.50
Sheep	10	0	12	1	23	4.35
Skunk	91	56	1615	921	2683	36.41
Weasel	7	2	12	0	21	9.52
Wolf	10	1	23	1	35	5.71
Others	764	0	642	0	1406	0.00
Subtotal	4418	199	7995	1294	13,905	10.74
Arctic Fox[a]			272	14	286	4.90
Bat[a]			1908	33	1941	1.70
Skunk[a]			1934	20	1954	1.02
Total	4418	199	12,109	1360	18,086	8.62

[a]Survey specimens

TABLE II

RABIES SUBMISSIONS 1968-1977
(BRITISH COLUMBIA)

Species	Human Contact		No Human Contact		Total
	Neg.	Pos.	Neg.	Pos.	
Bat	40	7	346	35	428
Cat	75	0	27	0	102
Cattle	2	0	3	0	5
Coyote	4	0	15	0	19
Dog	128	0	61	0	189
Fox	1	0	9	0	10
Skunk	3	0	5	0	8
Others	125	0	49	0	174
Total	378	7	515	35	935

TABLE III

RABIES SUBMISSIONS 1968-1977
(ALBERTA)

Species	Human Contact		No Human Contact		Total
	Neg.	Pos.	Neg.	Pos.	
Bat	98	1	1659	84	1842
Cat	363	1	251	0	615
Cattle	195	6	270	7	478
Coyote	56	0	556	14	626
Dog	541	2	451	7	1001
Fox	5	0	23	0	28
Horse	16	0	39	1	56
Pig	4	1	11	0	16
Skunk	25	1	741	28	795
Wolf	7	1	13	0	21
Others	295	0	325	0	620
Total	1605	13	4339	141	6098

sions which prove positive on laboratory examination often indicate previous contact with one of these species.

The number of specimens received for examination varies from year to year and area to area. In the northern parts of the provinces and the Territories the low human population density probably has a bearing on the number received. The native trappers in the Territories have lived with "crazy fox" and "crazy caribou" disease for many years. They recognize the clinical syndrome in these animal species and in wolves and sled dogs. In fact, the single caribou in Table VI was submitted with confidence that it was rabid, as was another received in 1978. Collection of specimens for confirmation has not always been considered necessary by these people. Also, the occurrence of rabies in areas where none has occurred in the recent past will generate a flurry of activity from an anxious public resulting in periodic increases in submissions.

The numbers in Tables II to VI do not include any of the survey data collected in Alberta (skunks and bats) or the Northwest Territories (Arctic fox). There appears to be a very low incidence of rabies (at least in the settled areas) in British Columbia and the Yukon; however, there have been relatively few submissions from the latter area during the years reported here. Of 1,559 positive cases diagnosed during the ten year period, 977 (62.7%) were skunks, 948 of which originated in the southern part of the Province of Saskatchewan. There is little doubt that rabies is endemic in skunks in that portion of the province to the south of a line drawn through (and including) Prince Albert. Only 66 dogs and 49 cats were positive during the same period from all areas served by the laboratory.

The survey of 258 trapline foxes caught on Banks Island in the winter of 1973-1974 revealed a 5.4% incidence of rabies positive submissions (3). This one-season study together with one initiated in the 1977-1978 season (unpublished) are not definitive enough to establish an overall picture of rabies in the Canadian north. However, they do provide substantive evidence of its existence and the probability that it has been present in the Arctic fox population for many years.

Fortunately cases of human rabies are rare in Canada, however, the potential for exposure and infection is present. During the years under study only one person is known to have died from rabies in western Canada. It was only after extensive investigation that it became apparent that the source of infection was a bite from a bat some three weeks earlier (8). In the same period however, a minimum of 4,617 people had been exposed to a potentially rabid animal to a degree that engendered concern (Table I). In many of these cases more than one individual was involved. This laboratory does not have records of human involvement, other than those required to assist in laboratory diagnosis of the submissions received. The responsibility for human prophylaxis lies with the medical profession, but it is safe to assume that treatment is initiated in a large percentage of these cases of potential exposure. Subsequently, 199 or 4.31% of the animals involved were diagnosed rabies-positive. Of the 764 human contact submissions in which the species is not identified ("others" in Table I), 633 were from the order *Rodentia*. No case of human rabies has ever been traced to a rodent in the United States (5) or in Canada. Rabies can occur in rodents, but is extremely rare and certainly not endemic in either country.

Fluorescent antibody techniques are now the most widely accepted tests in rabies diagnostic laboratories (7, 14). These, combined with animal inoculation where the FAT is negative and there is human exposure, provide a highly reliable diagnosis of the presence or absence of rabies antigen in the tissue examined. The FAT, when performed by a competent laboratory worker, identifies at least 98% of rabies-infected brain specimens submitted

TABLE IV

RABIES SUBMISSIONS 1968-1977
(SASKATCHEWAN)

Species	Human Contact		No Human Contact		Total
	Neg.	Pos.	Neg.	Pos.	
Bat	44	2	351	24	421
Cat	628	28	359	17	1032
Cattle	385	44	445	61	935
Coyote	60	2	194	3	259
Dog	661	15	347	22	1045
Fox	81	7	98	8	194
Horse	44	2	30	0	76
Pig	7	0	28	13	48
Raccoon	13	0	26	1	40
Sheep	10	0	12	1	23
Skunk	63	55	869	893	1880
Weasel	7	2	12	0	21
Others	333	0	243	0	576
Total	2336	157	3014	1043	6550

for diagnosis (14). The figures in Table VII show a correlation between FAT and MIT of 98.93%, where the initial examination by FAT was negative. Apart from the care and discipline required to maintain a high degree of accuracy in laboratory diagnoses, other factors may interfere with achieving a perfect performance. For example, 28 or 58.33% of the MIT positive specimens in Table VII had not died from rabies, but were killed or died from other known causes before the brain was submitted for examination. A substantial proportion of these, especially in bats and cats, were not yet showing overt clinical signs of disease. It is possible that the disease had not reached the stage where rabies virus antigen was present in the brain in sufficient quantity for demonstration by FAT. Also, sometimes there is a temptation to examine specimens that are unfit because of the high degree of reliability of the test. For example, a fox brain included in this data was examined by FAT one month after it had been originally collected in the Northwest Territories. It is not surprising that the rabies antigen would be more difficult to find considering the amount of postmortem autolysis that had occurred. It has been shown that there is a threshold of viral antigen required in infected dog brains before detection by FAT can be assured (14).

A recent check at this laboratory of 44 FAT-positive Arctic fox survey specimens received in the winter of 1977-1978 resulted in a 100% correlation with the results obtained by MIT.

The results presented here, together with the widely held view that fresh specimens negative by FAT do not present any danger of infection for man, even if subsequently positive by MIT (14), provide substantive evidence of the reliability of the diagnostic procedures described in identifying

TABLE V

RABIES SUBMISSIONS 1968-1977
(YUKON TERRITORY)

Species	Human Contact		No Human Contact		Total
	Neg.	Pos.	Neg.	Pos.	
Cat	1	1	0	0	2
Dog	7	1	12	1	21
Fox	2	1	10	0	13
Wolf	1	0	2	0	3
Others	4	0	11	0	15
Total	15	3	35	1	54

TABLE VI

RABIES SUBMISSIONS 1968-1977
(NORTHWEST TERRITORIES)

Species	Human Contact		No Human Contact		Total
	Neg.	Pos.	Neg.	Pos.	
Caribou	0	0	0	1	1
Cat	16	2	8	0	26
Dog	58	11	45	7	121
Fox	1	6	17	64	88
Wolf	2	0	8	1	11
Others	7	0	14	0	21
Total	84	19	92	73	268

TABLE VII

COMPARISON OF FLUORESCENT ANTIBODY AND MOUSE INOCULATION TESTS

Species	No. examined	FAT Neg MIT Neg	FAT Neg MIT Pos
Bat	188	182	6 (3.20)[a]
Cattle	588	582	6 (1.02)
Cat	1097	1082	15 (1.37)
Coyote	120	120	0 (0.00)
Dog	1405	1395	10 (0.71)
Fox	97	92	5 (5.15)
Horse	61	61	0 (0.00)
Pig	13	11	2 (15.38)
Raccoon	20	20	0 (0.00)
Sheep	13	13	0 (0.00)
Skunk	95	91	4 (4.21)
Weasel	16	16	0 (0.00)
Wolf	13	13	0 (0.00)
Others	740	740	0 (0.00)
Total	4466	4418	48 (1.07)

[a]Percent of total examined

rabies infection in a wide range of mammalian species.

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LETTER TO THE EDITOR

Cat and Small Dog Insulin Diluent

DEAR SIR:

Undoubtedly, other veterinarians have experienced some frustration in attempting to administer two to four units of NPH insulin to a diabetic cat or toy dog using the highly concentrated form of insulin (100 units/mL) available in Canada. Diluting NPH insulin with sterile water or a sodium chloride solution leads to unsatisfactory responses due to the instability of insulin in such a diluent.

A suitable diluent formula recommended by Dr. Shirley E. Johnson, medical director at Connaught Laboratories Limited, consists of:

Disodium phosphate, anhydrous	0.2% (w/v)
Sodium chloride	0.42% (w/v)
Glycerin	0.64% (w/v)
Metacresol acetate	0.2% (v/v)

In sterile, pyrogen-free distilled water, adjusted to pH 7.1-7.4. Sterilize the mixture by passing through a sterilizing filter (Millipore membrane, 0.22 micron). Refrigeration is required and the quantity prepared should be dated and utilized within two to three months.

Ms. Mary Rose Stang, Central Supply Pharmacy Unit at the Western College of Veterinary Medicine suggests that diluted preparations of insulin should not exceed a 1:10 dilution as dilute insulin lacks stability and may adsorb to plastic. The diluent may be purchased from the WCVM pharmacy.

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