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Authors: JOHNSTON, D. H., and BEAUREGARD, M.

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## RABIES EPIDEMIOLOGY IN ONTARIO

D. H. JOHNSTON and M. BEAUREGARD

*Ontario Dept. of Lands and Forests*

*Research Branch, Maple, Ontario, Canada*

and

*Animal Pathology Division, Health of Animals Branch,*

*Canada Dept. of Agriculture,*

*Animal Diseases Research Institute, Hull, Quebec, Canada*

### Abstract

Wildlife rabies invaded Ontario in 1954 along the coasts of Hudson and James Bays. During the period 1954 to 1958, rabies was carried into southern Ontario by red foxes (*Vulpes vulpes*), and in 1958-59 a severe epizootic of rabies occurred. From 1961 to 1969 rabies has remained endemic in southern Ontario. The red fox and striped skunk (*Mephitis mephitis*) are the main wildlife vectors, and account for 43% and 16% of the total rabies cases respectively. Of the domestic species, cattle make up 22%, dogs 7%, and cats 5%. The remaining 7% are in several other wild and domestic species. An annual cycle of rabies occurs each year, with a peak in December and a low in June. Three year cycles of red fox rabies occur in areas of good red fox habitat. During the initiation of the fall-winter rabies peak, the red fox is the species most commonly involved.

Because of the importance of the red fox as a rabies vector, a sample of 244 rabid red fox heads was collected over a one year period to study the sex-age structure of the rabid population. Animals were sexed by the sex chromatin technique and aged by counting tooth cementum layers. Forty percent of the heads showed evidence of ferocious contact as indicated by bites or cuts. Females showed a significantly higher rate of ferocious contact in March during the time of parturition. Animals from high rabies areas showed significantly more evidence of ferocious contact than did animals from areas of isolated cases. Thirty-four percent of the rabid red foxes had attacked porcupines (*Erethizon dorsatum*) as evidenced by porcupine quills in their muzzles. This species is usually avoided by normal red foxes.

During the increase in rabies in late summer and early fall, juvenile males were the predominant sex-age class in the rabid sample. This increase in rabies corresponds to the time of dispersal and reproductive maturation in juvenile males.

The peak of red fox rabies is in March. Yearling females were the most common sex-age class during this time, which also corresponds to the time of parturition. The oldest rabid red foxes were 4 years old. The results of the study indicate apparent relationships between the susceptibility of red foxes to rabies and various physiological changes which occur at different times of the year.

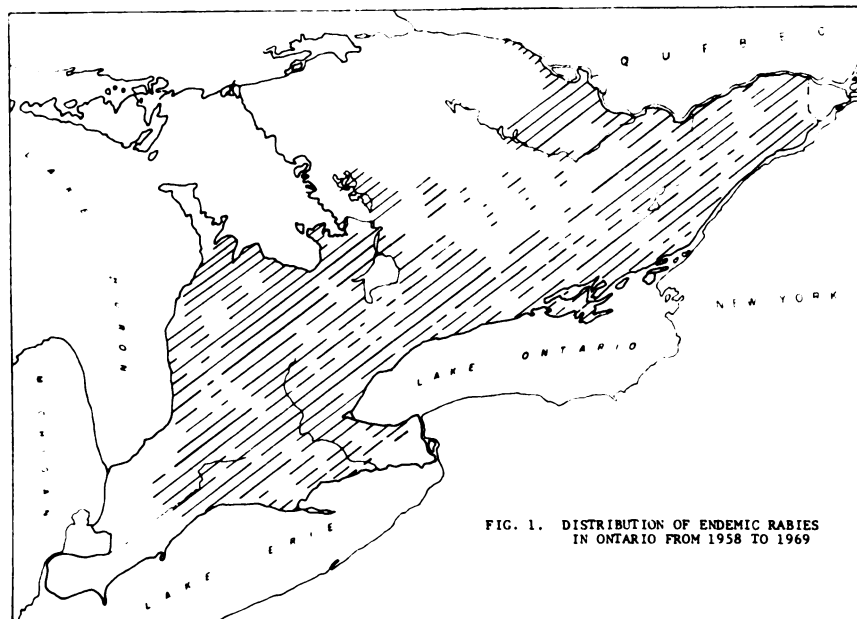


FIG. 1. DISTRIBUTION OF ENDEMIC RABIES IN ONTARIO FROM 1958 TO 1969

### Introduction

Rabies was first diagnosed in Canadian wildlife in 1947 in arctic foxes (*Alopex lagopus*) and gray wolves (*Canis lupus*) from the Northwest Territories (Plummer, 1947). Following this diagnosis, wildlife cases were reported from many parts of the Northwest Territories and the northern parts of the Provinces of Alberta, British Columbia, Manitoba, and Saskatchewan.

In 1951 fox rabies was diagnosed at Churchill, Manitoba and in 1953 at Eastmain, Quebec, on the east coast of James Bay (Plummer, 1954). The first case of wildlife rabies in Ontario was a fox from Fort Albany on the west coast of James Bay in March, 1954. The species of this fox was not recorded but shortly thereafter rabies began to appear in red fox (*Vulpes vulpes*) in the James Bay area. From this focus the disease moved southward in the red fox population, and a severe epizootic developed during 1958 and 1959 in southern Ontario in what is now the present area of endemic rabies (Fig. 1). The high level of rabies during the epizootic is shown in Figure 2. This has remained the highest inci-

dence of wildlife rabies ever diagnosed in the Province.

Following the initial epizootic, rabies declined to a low level during 1960. In August, 1961, however, the incidence again increased, and since then an annual cycle has developed with a low in June and a peak in December. Superimposed on this cycle are longer-termed fluctuations which are local in nature. The amplitude of the annual cycle has appeared relatively uniform since 1961 (Fig. 2). These statistics are, however, for total animal rabies diagnosed throughout the Province and they tend to mask local trends. In some areas which provide good habitat for red foxes, there is evidence of a 3-year cycle. For example, in Grey County peaks have occurred in 1958, 1961, 1964, and 1967. In areas of poorer red fox habitat, peaks may occur at 4- or 5-year intervals and are usually more diffuse.

The average number of cases per year during the period August, 1961 to March, 1969 is plotted by month for the commonly infected species in Figures 3 and 4. The relative involvement of each species is shown in Table 1.

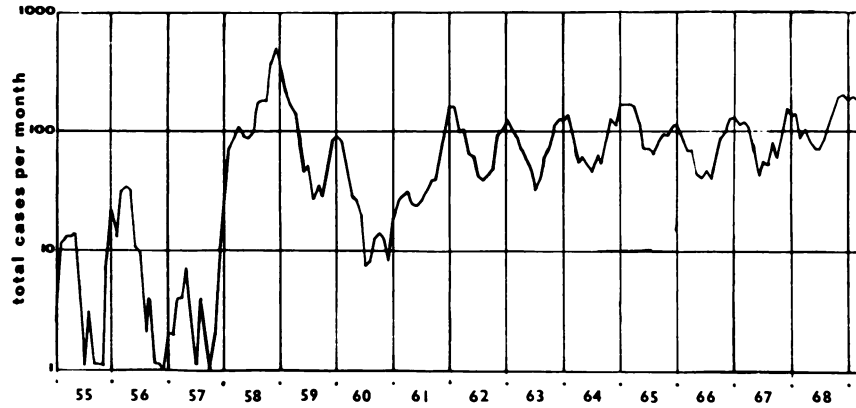


Fig. 2. RABIES IN ONTARIO. Total cases of animal rabies plotted by month during the period January, 1955 to March, 1969.

TABLE 1. Total number of rabies cases by species during the period August, 1961 to March, 1969

Species	No. of Cases	Percentage of Total
Red Fox ( <i>Vulpes vulpes</i> )	3671	42.70
Cow ( <i>Bos taurus</i> )	1910	22.22
Striped Skunk ( <i>Mephitis mephitis</i> )	1344	15.63
Dog ( <i>Canis familiaris</i> )	630	7.33
Cat ( <i>Felis domesticus</i> )	468	5.44
Sheep ( <i>Ovis aries</i> )	195	2.27
Pig ( <i>Sus scrofa</i> )	130	1.51
Horse ( <i>Equus caballus</i> )	119	1.38
Bat ( <i>Vespertilionidae</i> )*	42	.48
Raccoon ( <i>Procyon lotor</i> )	39	.45
Coyote or Wolf ( <i>Canis latrans</i> , <i>C. lupus</i> )	35	.41
Other**	15	.18
<b>TOTALS</b>	<b>8598</b>	<b>100.00</b>
* Includes:		
Big Brown Bat ( <i>Eptesicus fuscus</i> )	—	38
Hoary Bat ( <i>Lasiurus cinereus</i> )	—	1
Red Bat ( <i>L. borealis</i> )	—	1
Eastern Long-eared Bat ( <i>Myotis keenii</i> )	—	1
Eastern Pipistrelle ( <i>Pipistrellus subflavus</i> )	—	1
(Beauregard, M. 1969)		
** Includes:		
Goat ( <i>Capra hircus</i> )	—	5
Woodchuck ( <i>Marmota monax</i> )	—	7
Muskrat ( <i>Ondatra zibethica</i> )	—	3

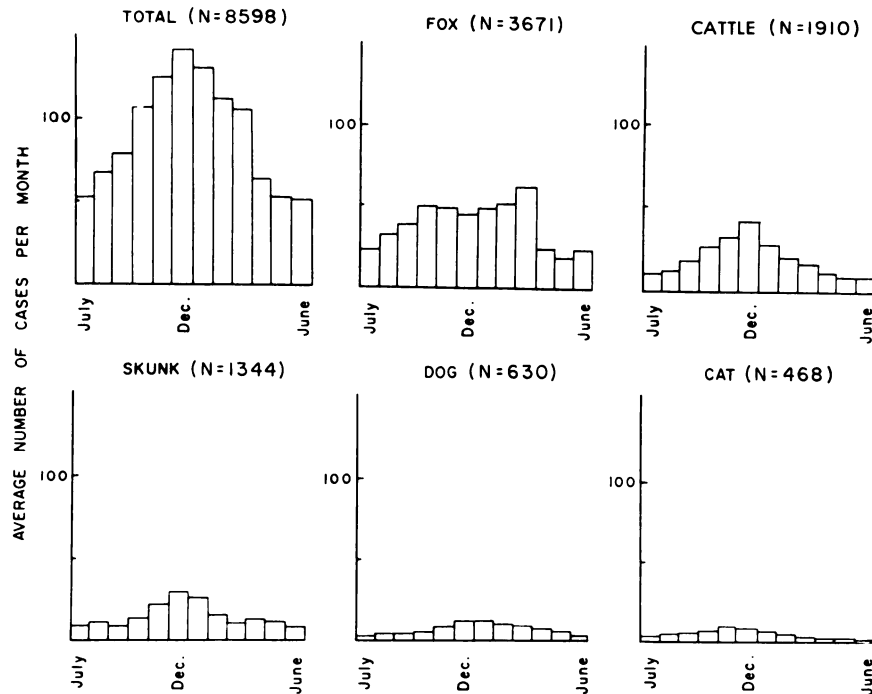


Fig. 3 AVERAGE ANNUAL RABIES INCIDENCE BY SPECIES DURING THE PERIOD AUG. 1961 TO MAR. 1969

Figures 1, 2, 3, and 4 and Table 1 were compiled from statistics supplied by the Canada Department of Agriculture for all laboratory diagnosed animal rabies cases and for a small number of clinically diagnosed domestic animal cases.

The red fox is the most important wildlife vector, accounting for approximately 43% of all animal rabies cases and 71% of the total wildlife cases. The other important wildlife vector is the striped skunk (*Mephitis mephitis*), which accounts for approximately 16% of all animal rabies cases and 26% of the total wildlife cases. In all other wildlife the occurrence of rabies is very low. Other species involved include bats (*Vespertilionidae*), woodchuck (*Marmota monax*), muskrat (*Ondatra zibethica*), coyote (*Canis latrans*), timber wolf (gray wolf), and raccoon (*Procyon lotor*).<sup>1</sup>

Woodchucks, muskrats, coyotes, timber wolves, and raccoons are species which usually appear as incidental cases and are probably not important as reservoirs of wildlife rabies in Ontario. Woodchucks, muskrats, and raccoons are very common throughout the endemic rabies area yet the level of rabies diagnosed in these species is exceedingly low.

Cases of coyote and wolf rabies are combined in the statistics on diagnosed rabies cases in Ontario (Fig. 4), but only two cases have been positively identified as timber wolves. The combined incidence in these two species is, however, very small and they account for only 0.41% of the total animal rabies cases in the Province.

Bats of the family Vespertilionidae have been found rabid in Ontario but they only account for 0.48% of the total animal rabies cases. The commonest bat

<sup>1</sup> Taxonomic nomenclature throughout the text follows Peterson (1966).

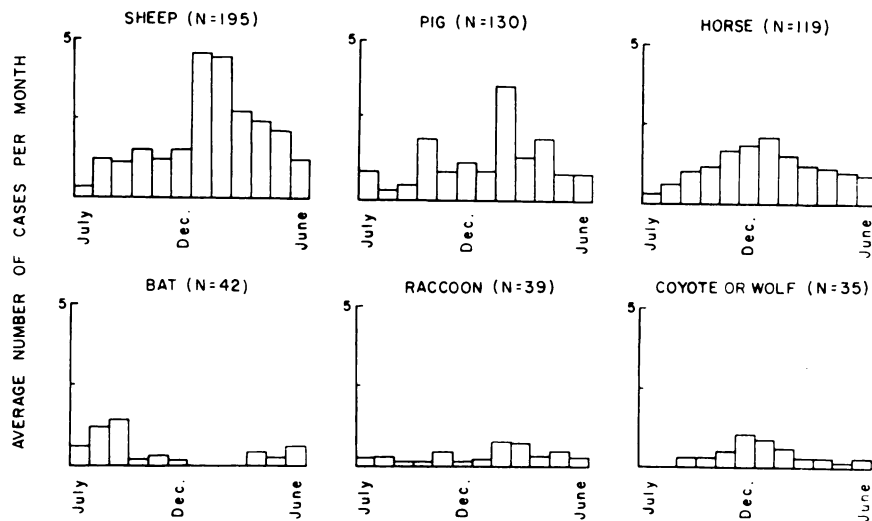


Fig 4 AVERAGE ANNUAL RABIES INCIDENCE BY SPECIES DURING THE PERIOD AUG 1961 TO MAR 1969

species infected is the big brown bat (*Eptesicus fuscus*) (Table 1). The extent to which bats are involved with terrestrial mammal rabies in Ontario is not known. The possibility of rabies transmission by bats to terrestrial mammals has been extensively discussed by Beaugard and Stewart (1964) and Beaugard (1969). It appears very unlikely, however, that vespertilionid bats are an important source of infection for terrestrial mammals. The fact that bat rabies has been diagnosed in areas of North America where terrestrial mammal rabies does not occur, may be considered as evidence of a lack of effective transmission by bats of this family (Girard et al., 1965; Friend, 1968). Avery and Tailour (1960) first diagnosed bat rabies in Canada from British Columbia in 1957, and since that time bat rabies has persisted there in the absence of rabies in other terrestrial mammals.

In general, vespertilionid bats appear to be relatively poor transmitters of rabies virus by the bite route. Attempts to infect terrestrial mammals by the bites from big brown bats, hoary bats (*Lasiurus cinereus*), red bats (*L. borealis*), and silver-haired bats (*Lasionycteris noctivagans*) in various experiments have

failed to produce fatal rabies (Constantine and Woodall, 1966; Constantine, Solomon and Woodall, 1968). In these experiments the only response to rabies virus of vespertilionid origin was a low serum anti-rabies titre in some animals. On the other hand, Bell et al. (1962) was able to produce fatal rabies in mice bitten by rabid big brown bats. Although the above evidence is contradictory it does appear that the vespertilionid bats involved with rabies in Ontario are relatively poor bite transmitters compared to insectivorous species from other families such as the leaf-nosed bat (*Macrotus waterhousii*), and the Mexican free-tailed bat (*Tadarida brasiliensis*) (Constantine, 1966; Constantine et al., 1968).

The peak of bat rabies in Ontario occurs during August and September (Fig. 4) and it is during this time that the colonial vespertilionid bats migrate from nursery colonies occupied during the spring and early summer, to hibernation sites. The bats fly through the hibernacula in what is referred to as "swarming" behavior (Fenton, 1969). At this time of year all sex and age groups of several species come together. Also associated with the "swarming" period are some records of long flights

by "swarming" little brown bats (*Myotis lucifugus*) ranging from 32 to 800 km. From these observations, it appears that the peak of bat rabies in Ontario corresponds to a time when the populations of various species of colonial bats are undergoing extensive movements, and all sex and age groups are in contact prior to mating and hibernation. The extent of rabies transmission to terrestrial species during this time remains unknown, however.

In considering the wildlife species involved with rabies in Ontario, it appears from present information that bats, woodchucks, muskrats, coyotes, wolves, and raccoons are only of incidental significance, and that the red fox and striped skunk are the primary wildlife vectors. The annual cycle of rabies in the Province provides an excellent opportunity to examine the time at which these two vector species become involved with rabies each year. From Figure 3 it can be seen that the monthly incidence of rabies begins to increase in red foxes before it rises in any other species involved in the fall-winter peak. Red fox

rabies begins to increase in August, whereas the increase in other species is one or more months later.

Because of the apparent involvement of red foxes during the initiation of this peak, the current study was undertaken particularly to examine the structure of the red fox population contracting rabies at this time.

As the red fox is the wildlife vector most commonly implicated in rabies transmission to domestic species in Ontario, there is undoubtedly a great deal of ferocious contact between red foxes and other animals. In the present study evidence of ferocious contact, e.g., bites or cuts, was recorded and the data examined for significant trends with regard to the sex and age of the animals involved. The seasonal and geographical distribution of animals showing evidence of ferocious contact was investigated as well.

The peak of red fox rabies in Ontario is in March (Fig. 3). As this peak occurs during the period of reproduction, the sex of the animals contracting rabies during this time was also of interest.

#### Materials and Methods

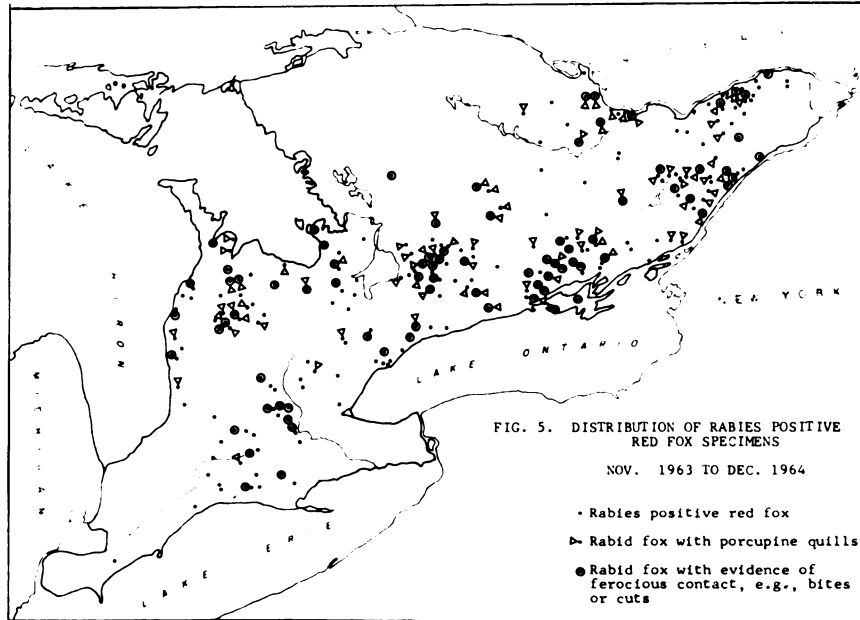
**Collection of Sample.** A sample of 244 rabid red foxes was collected during the period November, 1963 to December, 1964 inclusive, for the purposes of determining the sexes and ages of the population contracting rabies at various times of the year.

The specimens were collected under the routine rabies surveillance program maintained in the province by the field staff of the Canada Department of Agriculture, Health of Animals Branch.

The locations from which the specimens were taken is shown in Figure 5. In most cases the specimens consisted only of heads, which were submitted to the Animal Disease Research Institute, Hull, Quebec for rabies diagnosis. There were also five specimens taken near Englehart, Temiskaming District, Ontario, where a small focus of infection has persisted since the epizootic period of 1958-59.

**Rabies Diagnosis.** Rabies diagnosis on the red fox sample was carried out by Beauregard using the following methods. The first group of 113 specimens, collected from November, 1963 to June, 1964 were diagnosed by the William's staining method. The remaining group of 131 specimens, collected up to the end of December, 1964 was diagnosed by the fluorescent antibody method. Both techniques have been previously described by Beauregard, Boulanger and Webster (1965). Following diagnosis, the specimens were fixed in 10% formalin and shipped to Johnston for sex and age determination.

**Determination of Sex.** In most cases the specimens consisted of heads only. As the sex of the animal was not recorded in the field at the time of submission for rabies diagnosis, histological examination of tissues for the presence of sex chromatin was necessary to determine the sex (Hay and Moore, 1961; Moore, 1966).



Smears of buccal epithelium were prepared by the method described by Barr (1965), with the exception that the buccal mucosa was fixed and stored in 10% formalin rather than 95% ethyl alcohol.

In a few cases where the oral mucosa was unsuitable for histological examination, thin sections of the frontal part of the cerebral hemispheres were cut and neurons examined for sex chromatin by the method described by Barr (1965). *Ferocious Contact.* The heads of the rabid red foxes were examined for evidence of bites or cuts indicating some type of ferocious contact.

Many red foxes also had porcupine (*Erethizon dorsatum*) quills in the muzzle, which provided additional evidence of contact with a species which is normally avoided. The occurrence of quills in rabid specimens was noted. The approximate age of the puncture wounds associated with the quills was determined by skinning the heads and examining the underlying tissue.

*Age Determination.* Many techniques for aging mammalian species have been compiled in an annotated bibliography

by Madsen (1967). Four techniques were investigated for their suitability in aging the sample of rabid red foxes.

(a) Eye lens weight. The dry weight of crystalline lenses was used by Friend and Linhart (1964) to separate juvenile and adult red foxes. Our method of lens preparation was essentially the same as they described, except that some lenses were prepared for drying with the surrounding capsule intact and some were removed from the capsule. Lenses were air-dried on blotting paper and placed in vials for oven drying.

A sample of 20 pairs of lenses was prepared initially and it was obvious, from this sample, that there were inconsistencies in paired lens weights which far exceeded expected variation. As the sample of rabid animals was collected over a whole year, there was great variation in the initial handling of specimens before fixing in the laboratory. Hot summer temperatures promoted rapid decomposition of some specimens and winter freezing caused exfoliation of lens layers. Similar results have been noted by Montgomery (1963). The technique was abandoned.



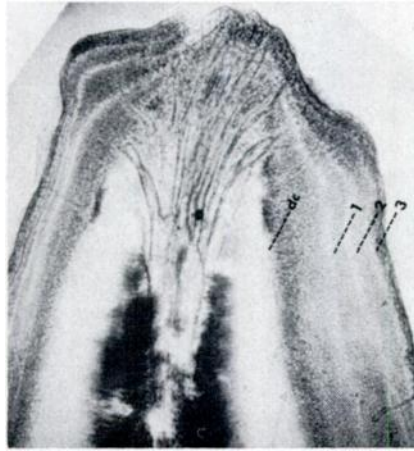


FIGURE 6. *Sagittal section of the root of an upper canine from a 45 month old red fox. X 30. dc—dentino-cemental junction, 1, 2, 3—outer edge of annual cementum layers.*

(b) Closure of cranial sutures. Skulls were cleaned by boiling following fixation and storage in 10% formalin. The degree of suture closure was noted for the basioccipital-basisphenoid and presphenoid-basisphenoid sutures, and for the palatal portion of the premaxillary-maxillary suture lateral to the anterior foramen (Churcher, 1960). A series of 188 known age crania, used by Churcher, was made available by the Royal Ontario Museum, Toronto as a reference for age determination.

(c) Mandibular periosteum. Klevezal (1965) has stated that the age of arctic fox can be determined by counting the number of layers in the mandibular periosteum. He also noted that the periosteum was thickest on the lingual side of the mandible.

As this method was determined by Klevezal (1965) using arctic fox, it was necessary to evaluate the validity of the technique for the red fox. Initially serial sections of known-age mandible were cut to determine the area of thickest periosteum and to evaluate the number of periosteal layers and cement lines in

relation to age. The lingual side of the mandible in the region of the first and second molar proved to be the best location.

Thin undecalcified sections of mandible were prepared by the grinding method described by Frost (1958). Transverse sections of mandible were cut with a fine jeweller's saw and polished on wet emery paper to a thickness of 30 to 50  $\mu$ .

After grinding, the sections were washed with water, air dried and washed with ethyl ether to remove fat and other organic debris from the osteocyte lacunae and canaliculi. Sections were thoroughly air dried and temporary mounts made with glycerine for immediate viewing. This method leaves any air spaces in the bone matrix clean and filled with air so that they are highly refractile. Any liquid substance, e.g., fat, water, or mounting media, which penetrates and fills the lacunae and canaliculi tends to clear the section and make the periosteal layers and cement lines less distinct. Sections were viewed by both bright-field and dark-field illumination.

(d) Tooth cementum. The observation of cementum layers corresponding to annual growth patterns has been widely used to age mammals (Madsen, 1967). In the Canidae it has been used by Linhart and Knowlton (1967) to age coyotes, and by Kleinenberg and Klevezal (1966) to age arctic fox. Both of these studies employed decalcification and staining procedures to demonstrate cementum lines.

To test the usefulness of the cementum technique for aging red fox, sections were prepared from known-age material by the methods described above for mandibular periosteum. Sagittal and transverse sections of all teeth were cut to determine the tooth with the widest cemental deposition and clarity of annual layers. The posterior lingual margin of the upper canines proved to be the best location. Subsequently, the upper left canine from all specimens was sectioned sagittally and used for age determination.

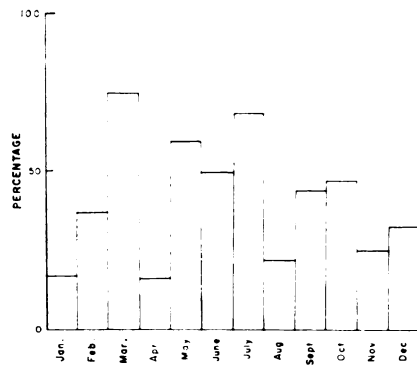


Fig. 7. PERCENTAGE OF RABID RED FOX SPECIMENS WITH EVIDENCE OF FEROCIOUS CONTACT, e.g., BITES OR CUTS

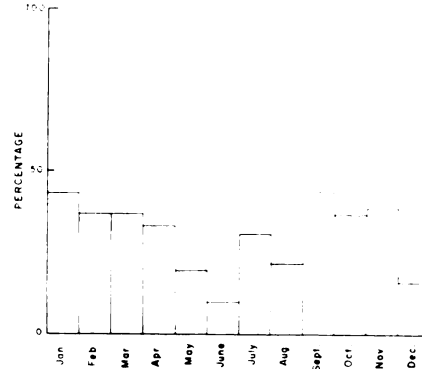


Fig. 8. PERCENTAGE OF RABID RED FOX SPECIMENS WITH PORCUPINE QUILLS

### Results

A total of 235 of the 244 red foxes diagnosed positive for rabies proved suitable for both sex and age determination. The sample included 136 males and 99 females. The percentage of males and females in various age classes for each month is presented in Figure 9.

The "juvenile class" noted in Figure 9, includes young animals up to 9 months of age, based on a mean parturition date around mid-March. The "first year breeding class" includes young animals 10 to 21 months old which have just reached reproductive maturity and are breeding for the first time. The "subsequent years breeding class" noted in Figure 9, includes all animals 22 months and older. The oldest rabid animal recorded was a female 51 months old.

During the period March to June, females were the predominant sex and yearling females the predominant age class. During the period July to December, males were the predominant sex and juvenile males the predominant age class.

In January and February rabies incidence was about equal in both sexes. The highest monthly mortality in older animals was observed in February when 60% of the specimens were 23 months or older.

The sexing of heads by the presence or absence of sex chromatin in buccal

epithelial cells, or in cerebral neurons was successful except in specimens which had begun to decompose. In the nuclei of buccal epithelial cells, the sex chromatin mass was usually located adjacent to the nuclear membrane. In neuronal tissue, the sex chromatin mass was located adjacent to the nucleolus. Similar positioning of the sex chromatin was recorded by Hay and Moore (1961).

Of the four aging techniques tested, only the tooth cementum method gave consistently accurate results to the nearest year. The suture closure technique was excellent for distinguishing animals during the first 2 years of life, but there was no way of accurately separating 3 and 4 year old animals by this method. None of the animals showed fusion of the maxillary-premaxillary suture which would indicate an age of 5 years or older.

The mandibular periosteum showed cement lines which increased in number with the age of the animal, but the number of lines was not consistently proportional to age. In areas of the mandible where the periosteal layers were thick, usually two cement lines were observed per year. In areas where the periosteum was thinner, however, usually only one cement line was seen per year. Between these two extremes there were areas where two lines "joined" to become a single line.

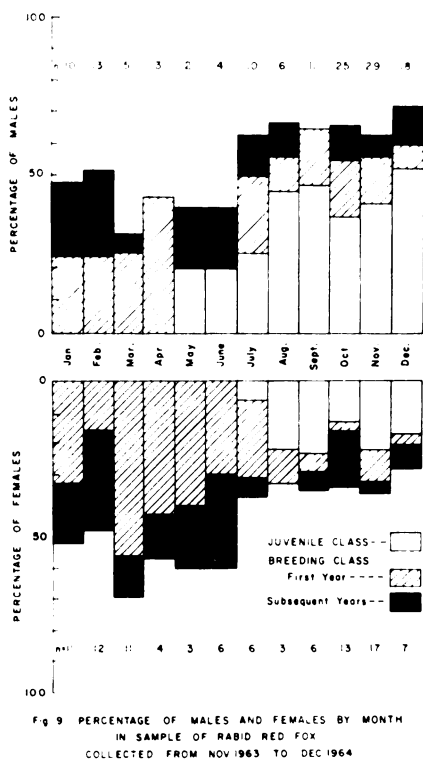


FIG. 9. PERCENTAGE OF MALES AND FEMALES BY MONTH IN SAMPLE OF RABID RED FOX COLLECTED FROM NOV. 1963 TO DEC. 1964.

Because the number of lines varied with the location from which the section was cut, it was difficult to be certain that the total number of lines counted in a section was the actual maximum number of lines laid down in the periosteum. This technique gave a general separation of young and old animals, but the results were not consistent enough to be used for accurate aging.

The tooth cementum technique proved to be the most accurate aging method tested. The layers observed in sagittal sections of canine tooth root correspond directly to age. In Figure 6 the bands of clear cementum marked 1, 2 and 3 correspond to the end of seasonal growth during the first, second and third years. A fourth clear band is being formed on the outer edge of the cementum, but it is not clear in Figure 6 because of edge refraction. In most teeth sectioned, the outer clear band is not well defined from the edge of the cementum until about

the following June when new cementum has been laid down outside the clear band. The root section in Figure 6 is undecalcified and unstained, and was photographed by bright-field illumination. The tooth cementum layer technique was used as the standard technique for aging all specimens from the sample of rabid red foxes.

From the sample of 235 rabid animals, for which sex and age were determined, 93 (40%) showed evidence of ferocious contact. The geographical distribution of animals with evidence of ferocious contact is given in Figure 5. Animals which were collected from high density rabies areas had significantly more evidence of ferocious contact than did isolated cases ( $P < 0.05$ ).

The seasonal distribution of rabid red foxes showing evidence of ferocious contact is given in Figure 7. The distribution appears to be random, except in March when there was a significantly higher percentage (80%) of females showing evidence of ferocious contact ( $P < 0.05$ ).

Eighty (34%) of the rabid red foxes had porcupine quills in the muzzle. Only one animal showed evidence of old porcupine quill wounds which had healed, and this animal had again encountered a porcupine prior to being collected as a rabid specimen. Porcupine quill wounds in all other foxes were recent. The geographical distribution of animals showing evidence of porcupine contact was within the known range of porcupines in Ontario (Fig. 5). The seasonal distribution of rabid red foxes showing evidence of porcupine contact is given in Figure 8.

Of particular interest is the common occurrence of rabid animals containing porcupine quills during the winter months when porcupines at this latitude are using winter dens. The contact rate between porcupines and rabid red foxes appears to be quite high, and yet there has never been a case of rabies diagnosed in a porcupine in Ontario.

In addition to the above evidence of contact with other animals, there were many specimens which smelled strongly of skunk scent. Unfortunately, an accurate count was not possible as several specimens were stored in the same container.

### Discussion

In northern temperature regions of North America and Europe where red fox rabies is endemic, a fall-winter peak in mortality is a common phenomenon. Red fox rabies begins to increase in the late summer, rises to a high in October and November and peaks in March (Fig. 3). This pattern has been noted not only in Ontario, but also in Czechoslovakia by Kral (1969), in Denmark by Müller (1966), and in Germany by Pitzschke (1966) and Ulbrich (1967). Friend (1968) reported fox rabies peaks of comparatively low amplitude in New York State in March and April and in October and November. In New York State, however, the vector system is more complex, as the gray fox (*Urocyon cinereoargenteus*) is also a wildlife vector. This species is found only rarely in Ontario and does not occur in Europe.

Another aspect of red fox rabies common to both Ontario and Europe is the presence of a 3-year cycle. Distinct 3-year peaks in fox rabies have been recorded in Ontario from areas of good habitat for this species. These are areas which were known to have supported high red fox densities prior to the invasion of wildlife rabies into Ontario during the period 1954 to 1958. In areas where good red fox habitat is more diffuse, rabies peaks may occur at 4- or 5-year intervals and the number of animals involved is usually smaller.

Three year peaks in fox rabies have been reported in Germany by Kauker and Zetl (1960), and Friend (1968) has reported 4- or 5-year rabies peaks in New York corresponding to peak populations in foxes. Müller (1966) has noted in Denmark that peaks in red fox rabies also coincide with peaks in the red fox population.

Several authors working on red fox rabies have commented on the fall-winter peak in rabies in relation to the ecology of this species. (Friend, 1968; Kral, 1969; Müller, 1966; Pitzschke, 1966; Ulbrich, 1967). Most have identified the rise in incidence during the autumn with the time of dispersal and establishment of new territories by juvenile red foxes.

This relation is also true in Ontario, but analysis of the sex-age structure of the red fox population contracting rabies in the late summer and fall (August to December), indicates that 66% of the rabid animals are males and 65% of the males are juveniles (Fig. 9). This sex differential corresponds to the findings of Phillips and Storm (1968) who have shown that male juveniles disperse earlier in the fall than female juveniles. They state that male juveniles begin to disperse away from their natal home range in late September and early October. Females, however, do not disperse until November and December. The fact that male juveniles begin to move earlier than females increases their chance of contacting other animals on neighboring territories, and this may contribute to the higher incidence of rabies in this group. On the other hand, female juveniles probably encounter males dispersing through their natal home ranges during the same period, yet the percentage of rabid females is comparatively low during the fall. The degree to which behavioral isolating mechanisms may influence rabies transmission between the sexes at this time is unknown.

Another factor which should be mentioned in connection with the different times of dispersal is the variation in the time of reproductive maturation. Male foxes are sexually mature in late November or December, whereas females do not begin estrus until late January or early February (Venge, 1959).

A factor which may also contribute to an increased level of rabies in juvenile males is the stress associated with the period of dispersal. Stress has been suggested as a contributing factor in the activation of latent rabies infections (Soave, 1964; Sanderson, Verts and Storm, 1967). Soave (1962) also reported reactivation of latent rabies in a guinea pig (*Cavia porcellus*) with adrenocorticotrophic hormone, and Sanderson, et al (1967) using cortisone, reported reactivation of latent rabies in guinea pigs and raccoons.

That juvenile male red foxes are under more stress than juvenile females during the dispersal period, is suggested by the fact that they are moving earlier and also travelling further through unfamiliar territory. Phillips and Storm (1968) report that the mean dispersal for 65 male juvenile red foxes was 42 km (range 0.8 to 336 km); whereas the mean dispersal for 47 juvenile females was only 11.4 km (range 0.8 to 77 km). The longest movement on record for a red fox was a juvenile male which moved 392 km (Ables, 1965). During the fall it appears, therefore, that earlier maturation and stress associated with dispersal may contribute to a higher level of rabies in juvenile males than in juvenile females.

The additional stress which presumably accompanies high population levels, may also be a significant factor in the 3-year cycles that have been observed in red fox rabies. Prior to these outbreaks the red fox populations are known to be high. In Russia, Kantorovich (1964) has associated reactivation of a latent form of rabies in arctic fox with high population levels.

Our data showed a significantly higher number of animals with evidence of ferocious contact in areas of high rabies density. This presumably indicates a higher contact rate with other animals in these areas, and probably a higher initial population than in areas where only isolated cases occur. Conversely, it is known from aerial survey data in Ontario (Johnson unpublished), that local red fox populations are reduced to very low levels following rabies epizootics. More data on population fluctuations over the course of these 3-year cycles are needed, but it does appear that there is a direct relationship between the degree of increase in the population and the subsequent level of rabies mortality.

The peak of red fox rabies in March has been related to intraspecific exposure during the mating period (Pitzschke, 1966; Kral, 1969). However, it should be noted that the peak of rabies in March also coincides with the time of parturition. Analysis of the sex ratio of rabid red foxes, collected during March, revealed that approximately 70% of the

animals involved were females and 80% of the females were juveniles. Although the sample during March and subsequent months is small compared to the number of males collected during the fall peak, it appears that more females than males are becoming rabid during the time of parturition.

The exact association which may exist between rabies and pregnancy is difficult to determine because the actual incubation periods of naturally transmitted red fox rabies are unknown. It is not clear, therefore, whether the peak during the time of parturition corresponds to the red fox gestation period or to a natural rabies incubation period of the same length. However, the fact that more females than males become rabid at this time does suggest a link with pregnancy.

In relation to the hormonal changes occurring during estrus and pregnancy, Thiery (1959) has noted that folliculin has an enhancing effect on incubating rabies, whereas pregnancy has an inhibiting effect. Sanderson, et al (1967) have suggested that the stress associated with parturition and lactation may be important in the reactivation of latent rabies in female skunks in Illinois. They reported that during April, May and June females were 15 times more frequently infected with rabies than males.

Field observations of red foxes, near full term gestation, by Johnston, have revealed several instances of females becoming clinically rabid within a few days of parturition, or actually during parturition. Four cases of rabid females attacking their litters following parturition were observed. This field evidence is in agreement with the data on ferocious contact, from the sample of rabid red foxes, in which 80% of the females collected in March showed evidence of ferocious contact. Further investigation is necessary to clarify the exact mechanisms influencing the appearance of rabies in females during March, but it does appear that this peak in rabies is connected with the time of parturition in red foxes in Ontario.

In conclusion, the results of the investigation into the sex and age classes of red foxes, dying of rabies at various

times of the year, indicate that mortality is not uniformly distributed among these classes. Juvenile males form the commonest sex-age class contracting rabies during the fall, and this appears to be linked with the stresses of dispersal and with reproductive maturation. In March, rabies is relatively more common among females, and there appears to be a correlation with the time of parturition. However, further experimentation is

necessary to verify the exact means by which these physiological conditions affect the susceptibility of wild red foxes to rabies.

Any future experimental studies on rabies in red foxes should take into account the sex, age and reproductive status of the animals, and the possible effect which these factors may have on apparent susceptibility and length of incubation period.

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