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INTRACRANIAL ABSCESSATION IN WHITE-TAILED DEER OF NORTH AMERICA

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ABSTRACT: From January 1996 through April 1997, the geographic distribution, etiology, demographics, seasonality, and prevalence of an intracranial abscessation/suppurative meningoencephalitits syndrome in white-tailed deer (*Odocoileus virginianus*) were evaluated by surveying wildlife disease diagnostic laboratories and by examining both natural mortality and hunter-harvested deer skulls from North America. Intracranial abscesses were diagnosed as the cause of death or illness in 97 of nearly 4,500 (2.2%) white-tailed deer examined from 12 states and four Canadian provinces by the diagnostic laboratories. The bacterium *Arcanobacterium pyogenes* was isolated from 61% of cases; 18 other genera of bacteria also were isolated. The disease was strongly gender-biased ($P < 0.01$) with 87% of cases occurring in males, and the overall prevalence among males was 4.9%. Cases were most common among antlered males (≥1 yr) with few cases among male fawns. Among antlered males, cases were seasonal, primarily occurring from September through April. Four hundred eighteen skulls from deer found dead in the field were examined from southeastern USA, and of the 119 used for further evaluation, 9% had characteristic lesions. Skulls from hunter-harvested males in the southeastern USA had a lesion prevalence of 1.4%. The similarity of disease prevalence among male deer found dead in the field (9.0%) and deer examined as southeastern diagnostic laboratory cases (8.4%) suggests that this disease accounts for slightly <10% of the natural mortality for yearling and adult male white-tailed deer in the southeastern region. The strong bias for occurrence among males suggests this disease may affect quality deer management strategies.

Key words: *Arcanobacterium pyogenes,* case reports, intracranial abscess, natural mortality, *Odocoileus virginianus,* white-tailed deer.

INTRODUCTION

Although an abundance of information exists on cerebral/intracranial abscesses of domestic mammals (Blood and Radostits, 1989), only a few reports are available on the occurrence of intracranial abscesses in white-tailed deer (Erickson et al., 1961; Davidson and Nettles, 1988; Davidson et al., 1990; Forrester, 1992). In the only large study of intracranial abscesses of white-tailed deer, Davidson et al. (1990) examined pathology case records of 683 white-tailed deer from the southeastern United States that were submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS; College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA) for disease diagnosis and reported that a disease complex of intracranial abscessation/suppurative meningoencephalitis was present in 4% of the deer examined. The disease occurred more commonly among males than females, and the cases had a well-defined seasonal pattern, occurring from October through April. Clinical signs reported for affected individuals were incoordination, lack of fear, depression, blindness, weakness, and emaciation, which are similar to clinical signs reported among other species of affected ungulates (Jubb et al., 1985). Characteristic necrosis, erosion, and pitting of skull bones were common among affected deer suggesting that this bone damage would be useful in determining the cause of death when only skeletal remains were found (Davidson et al., 1990).

Arcanobacterium pyogenes, formerly *Actinomyces pyogenes* (Ramos et al., 1997) and *Corynebacterium pyogenes* (Collins and Jones, 1982), is a pyogenic, gram-positive bacterium that has both hemolytic and proteolytic activities (Sneath et al., 1986). This bacterium opportunistically invades damaged or devitalized superficial tissues of many ungulates (Zulty and Montali, 1988) and is found commonly on the exposed mucosal surfaces of cattle, swine, sheep, and other related species. *Arcanobacterium pyogenes* is found frequently associated with bronchopneumonia, endometritis, abortions, abscesses, arthritis, summer mastitis, and other generalized infections of domestic ungulates (Guerin-Faublee et al., 1993; Hagan, 1988). This bacterium also was found to be the most common agent isolated from intracranial abscesses of white-tailed deer (Davidson et al., 1990).

The objective of this research was to better define the epidemiologic characteristics of intracranial abscesses among white-tailed deer throughout their range. Data were obtained from three sources including (1) a survey of case records from wildlife disease diagnostic laboratories; (2) examination of skulls from deer found dead in the field for bone lesions indicative of intracranial abscesses; and (3) examination of skulls from hunter-harvested deer for bone lesions indicative of intracranial abscesses.

MATERIALS AND METHODS

This study was conducted from January 1996 through April 1997. A request for information was sent to 22 wildlife disease diagnostic laboratories throughout North America that were known to conduct diagnostic work on white-tailed deer. General information requested included the total number of white-tailed deer examined, the total number of males examined greater than or equal to 2.5 yr of age, and the total number of males between 1- and 2-yr-old. Specific data were requested for deer infected with intracranial abscesses including the point of origin, date of submission, gender, age, and identity of bacteria isolated from the abscess. Positive cases of intracranial abscesses included those that met the description of the intracranial abscess complex as defined by Davidson et al. (1990).

Collections of skulls from deer found dead in the field (field-collected skulls) from Arkansas, Florida, Georgia, South Carolina, and Texas (USA) were examined for lesions indicative of intracranial abscesses by SCWDS personnel and cooperating biologists. Letters requesting information were sent to state deer biologists from the southeastern United States. The letter briefly explained the purpose of this project, important epidemiologic features of intracranial abscesses, and typical bone lesions associated with intracranial abscesses as described by Davidson et al. (1990). A guide that illustrated the characteristic skull lesions associated with intracranial abscesses also was enclosed with the letter to aid the biologists in identifying skulls with lesions. Specific information requested for individual intracranial abscess cases included date found, location, gender, and age of the infected animal.

Skulls of hunter-harvested deer from South Carolina and West Virginia (USA) also were examined for characteristic lesions. The age of the deer that were examined often could not be determined because mandibles were not always present; thus, each animal was assigned to one of two age classes (adult or yearling) based on the size of the antlers.

Prevalence estimates for this study were defined as the number of positive intracranial abscess cases among the total number of animals surveyed in each of the three data sets. For data obtained from the survey of diagnostic laboratories, differences in prevalence of intracranial abscesses among deer submitted from different regions among male age classes and between genders were evaluated using a Chi-square test (Sokal and Rohlf, 1981). Because of low numbers of white-tailed deer examined by some individual laboratories, regional grouping was necessary to increase sample size. For analysis, diagnostic laboratories were grouped into four loosely defined regions of North America. Diagnostic laboratories in Florida, Georgia, and Mississippi were grouped in the southeastern region. Diagnostic laboratories in New Jersey, New York, and Ontario were grouped in the northeastern region. Diagnostic laboratories in Michigan, Missouri, Oklahoma, Texas, and Wisconsin were grouped in the central region. Diagnostic laboratories in Alberta, Colorado, Idaho, Saskatchewan, Washington, and Wyoming were grouped in the northwestern region. Prevalence data from field-collected skulls and from hunter-harvested deer also were evaluated for differences among male age classes by a Chi-square test (Sokal and Rohlf, 1981). Statistical analysis was done using either Statistical Analysis System (SAS Institute, Inc., 1990) or Epi Info, Version 6 (Dean et al., 1994). Seasonality was evaluated using simple frequency distributions.

RESULTS

Responses were received from 17 of the 22 (77%) diagnostic laboratories surveyed (Table 1). Ten laboratories provided complete information on 86 cases of intracranial abscesses among 3,455 white-tailed deer (1,521 males, 1,591 females, and 342 unknown). Overall, intracranial abscesses accounted for 2.5% of the diagnoses among these deer. In addition to the above, 11 cases of intracranial abscesses were reported among approximately 1,100 deer (1.0%) examined by the other laboratories that did not provide complete information (Table 1). The geographic distribution of these cases included Alberta, British Columbia, Florida, Georgia, Idaho, Michigan, Missouri, New Jersey, New York, North Carolina, Ontario, Saskatchewan, South Carolina, West Virginia, Wisconsin, and Wyoming.

Twenty genera of bacteria were reported from 66 intracranial abscess cases; isolations were either not obtained or not attempted from 31 cases. Organisms in the genus *Arcanobacterium* were the most common isolates in all regions sampled (Table 1) and were obtained from 40 cases (61% of cases in which isolations were made). In 29 cases (44%), *A. pyogenes* was the only species isolated. Eighteen other genera of bacteria were isolated from 32 cases, including seven cases of mixed infections involving an *Arcanobacterium.* The identity and prevalence of other genera of bacteria included: *Streptococcus* (16%), *Escherichia* (10%), *Pasteurella* (9%), *Proteus* (6%), *Staphylococcus* (6%), *Serratia* (5%), *Bacteriodes* (3%), *Citrobacter* (3%), *Klebsiella* (3%), *Pseudomonas* (3%), *Aeromonas* (2%), *Alcaligenes* (2%), *Bacillus* (2%), *Enterococcus* (2%), *Fusobacterium* (2%), *Hafnia* (2%), *Listeria* (2%), and *Salmonella* (2%).

For the cases in which gender was known, 82 of 94 (87%) intracranial abscess cases occurred in males, and the overall prevalence (4.9%) among males was higher ($P < 0.01$) than the prevalence (0.7%) among females ($\chi^2 = 50.99$, df = 1). Among yearling and adult males, cases had a well-defined seasonality with 42 of 52 (81%) cases among adults and 18 of 18 (100%) cases among yearlings occurring from October through March (Fig. 1). However, the temporal distribution of cases within this period differed between yearlings and older adult males. In yearlings, 89% of the cases occurred during October through December, whereas only 58% of cases in adults occurred during this same period. Cases among adult males tended to extend later in the winter with 33% of the cases in adults occurring from January through April. The few cases of intracranial abscesses in male fawns or females did not have any apparent seasonal patterns of occurrence.

Epidemiologic characteristics of nine captive deer with intracranial abscesses appeared to differ from those of the wild deer. The seasonality of cases was much less definitive with cases in both males and females occurring from July through February. The etiological agents isolated from intracranial abscesses among captive deer also appeared to differ somewhat from the agents involved in wild deer. Seven genera of bacteria were cultured from seven abscesses among these deer. *Eschrichia coli* was the most common bacterium, and it was obtained from three of the seven (42%) cases. The identity and prevalence of the other genera of bacteria isolated from captive deer included *Alcaligenes* (14%), *Arcanobacterium* (14%), *Pseudomonas* (14%), *Serratia* (14%), *Salmonella* (14%), and *Streptococcus* (14%).

The prevalence of intracranial abscesses differed among the regions. The prevalence among deer in the southeastern region (4.8%) was higher than both the northeastern region (1.7%) ($P < 0.01$, $\chi^2 = 14.91$, df = 1) and the northwestern region (1.5%) ($P < 0.01$, $\chi^2 = 26.91$, df = 1). The prevalence among deer in the central region (6.8%) was higher than both

TABLE 1. Summary of intracranial abscess case data for white-tailed deer submitted to 17 diagnostic laboratories in North America.

Laboratory[a]	Number of years reported	Total abscess cases among all deer	Abscess cases among male deer: Fawn	Abscess cases among male deer: Yearling	Abscess cases among male deer: Adult	Number (%) of cases attributable to *Archanobacterium* spp.[e]
Colorado Division of Wildlife (CDW)[1]	32	0/NR[b]	NR	NR	NR	NR
University of Florida (UF), College of Veterinary Medicine[2]	21	5/38 (13.2%)[c]	0/11 (0%)	2/6 (33.3%)	3/11 (27.3%)	4/4 (100%)
Southeastern Cooperative Wildlife Disease Study (SCWDS)[2,3,10,12,14]	26	37/810 (4.6%)[d]	1/145 (0.6%)	8/105 (7.6%)	22/142 (15.5%)	18/26 (69%)
Idaho Fish and Game Department (IFG), Wildlife Health Laboratory[4]	3	2/11 (18.2%)	0/1 (0%)	0/4 (0%)	1/3 (33.3%)	0/2 (0%)
Michigan Department of Natural Resources (MDNR), Rose Lake Wildlife Disease Laboratory[5]	10	2/720 (0.3%)	NR	NR	NR	0/0 (0%)
Mississippi State University (MSU), College of Veterinary Medicine[6]	NR	0/NR	NR	NR	NR	NR
University of Missouri (UM), Missouri Veterinary Medical Diagnostic Laboratory[7]	3	3/46 (6.5%)	0/8 (0%)	1/1 (100%)	1/5 (20%)	1/3 (33%)
New Jersey Division of Fish, Game and Wildlife (NJDFGW)[8]	19	7/289 (2.4%)	0/82 (0%)	7/32 (21.9%)	0/16 (0%)	1/3 (33%)
New York Department of Environmental Conservation (NYDEC)[9]	4	10/495 (1.2%)	1/111 (0.9%)	1/38 (2.6%)	2/17 (11.8%)	8/8 (100%)
Oklahoma State University, College of Veterinary Medicine (OKVM)[11]	20	0/300+ (0%)	NR	NR	NR	NR
Texas Veterinary Medicine Diagnostic Laboratory (TVMD)[13]	NR	est. 3+/NR	NR	NR	NR	NR
Washington State University (WSU), College of Veterinary Medicine[4]	20	1/<20 (5%)	NR	NR	NR	NR
Wisconsin Department of Natural Resources (WDNR)[15]	8	2/28 (7.1%)	0/3 (0%)	0/11 (0%)	1/7 (14.3%)	1/2 (50%)
University of Wyoming (UW), College of Agriculture[16]	11	1/44 (2.3%)	0/8 (0%)	0/4 (0%)	1/13 (7.7%)	1/1 (100%)
Alberta Natural Resources Service (ANRS), Fish and Wildlife[17]	6	4/66 (6.1%)	0/0 (0%)	1/1 (100%)	3/32 (9.4%)	0/1 (0%)

TABLE 1. Continued.

Laboratory[a]	Number of years reported	Total abscess cases among all deer	Abscess cases among male deer: Fawn	Yearling	Adult	Number (%) of cases attributable to *Archanobacterium* spp.[c]
Ontario Ministry of Natural Resources (OMNR), Wildlife Research[19]	24	2/55 (3.6%)	NR	NR	NR	1/1 (100%)
University of Saskatchewan, Western College of Veterinary Medicine (WCVM)[18,19,20]	29	21/1442 (1.3%)	5/264 (1.9%)	1/102 (1.0%)	8/208 (3.8%)	5/16 (31%)

[a] Numeric superscripts denote states/provinces from which intracranial abscess cases were submitted to each laboratory: Colorado,[1] Florida,[2] Georgia,[3] Idaho,[4] Michigan,[5] Mississippi,[6] Missouri,[7] New Jersey,[8] New York,[9] North Carolina,[10] Oklahoma,[11] South Carolina,[12] Texas,[13] West Virginia,[14] Wisconsin,[15] Wyoming,[16] Alberta,[17] British Columbia,[18] Ontario,[19] Saskatchewan.[20]

[b] NR = No value reported.

[c] Includes data published by Forrester (1992).

[d] Includes data published by Davidson et al. (1990).

[e] Denominator represents number of cases with bacterial cultures.

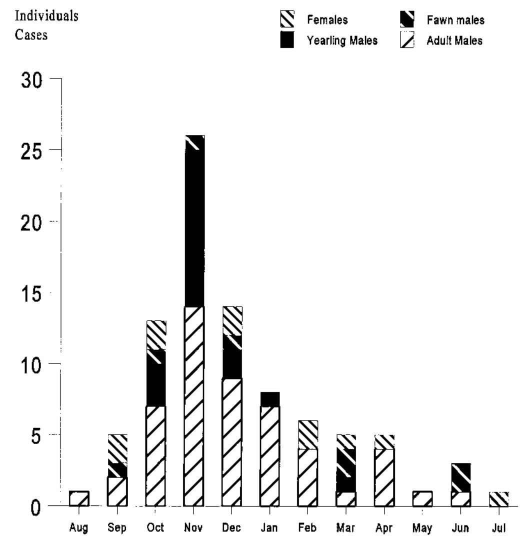

FIGURE 1. The seasonal distribution of intracranial abscess cases among white-tailed deer as compiled from surveys returned by wildlife disease diagnostic laboratories in North America.

the northeastern region ($P < 0.01$, $\chi^2 = 9.51$, df = 1) and the northwestern region ($P < 0.01$, $\chi^2 = 11.80$, df = 1). The prevalence among deer in the southeastern and central regions was not different ($P > 0.05$, $\chi^2 = 0.46$, df = 1). The prevalence among deer in the northeastern and northwestern regions was not different ($P > 0.05$, $\chi^2 = 0.01$, df = 1).

Skulls from 418 male deer found dead in five states (Arkansas, Florida, Georgia, South Carolina, and Texas) were examined for lesions that were characteristic of intracranial abscesses. Of the 418 deer skulls examined, 299 (71.5%) were from Texas, 104 (24.9%) were from South Carolina, 11 (2.6%) were from Georgia, 3 (0.7%) were from Florida, and 1 (0.2%) was from Arkansas. Characteristic lesions were not noted in any skulls from Texas or Arkansas. The prevalence of lesions in the skulls from Texas was dramatically less ($P < 0.01$) than the prevalence among skulls from the other southeastern states ($\chi^2 = 15.30$, df = 1); thus, the skulls from Texas were excluded from final analyses. Excluding the Texas data, 11 of 119 (9%) skulls from the southeastern states had lesions as described by Davidson et al. (1990). Seven of 95 (7%) adult males and four of 24 (17%) yearling males had characteristic lesions. There was not a difference ($P > 0.05$) in the prevalence of lesions between adult and yearling males ($\chi^2 = 2.15$, df = 1). All cases occurred in males that were in hard antler.

Four hundred ninety-two skulls from hunter-harvested male deer, comprised of 465 from South Carolina and 27 from West Virginia, were examined for characteristic bone lesions associated with intracranial abscessation. Typical lesions were identified in skulls from six of 434 (1.4%) adults and in one of 58 (1.7%) yearlings. There was no difference ($P > 0.05$) in the prevalence between the adult and yearling animals ($\chi^2 = 0.08$, df = 1).

Additional reports of suspected intracranial abscesses in hunter-harvested deer were provided by state biologists or hunters from Georgia, Kentucky, North Carolina, Pennsylvania, Virginia, and Wisconsin. The suspected cases reported by both biologists and hunters either had characteristic lesions or exhibited abnormal behavior associated with intracranial abscesses, but none were ever confirmed by a diagnostic laboratory.

DISCUSSION

By combining results from the surveys of diagnostic laboratories, field-collected skulls and hunter-harvested deer skulls, this research has verified that intracranial abscesses occur among white-tailed deer throughout much of the species' range. Based on this study and previous reports, intracranial abscesses are now known to occur among white-tailed deer in Florida (Davidson et al., 1990; Forrester, 1992), Georgia (Davidson et al., 1990; Newberry, 1994), Idaho, Kentucky, Michigan, Minnesota (Erickson et al., 1961), Missouri, North Carolina (Davidson et al., 1990), New Jersey, New York, Pennsylvania, South Carolina (Davidson et al., 1990), Texas, Virginia, Washington, Wisconsin (Trainer and Hale, 1965), West Virginia, and Wyoming in the United States and in the provinces of Alberta, British Columbia, Ontario (Fletch and Anderson, 1965), and Saskatchewan (Thornberry, 1995) in Canada. Although a report was received that a few intracranial abscesses had been observed in deer submitted to a diagnostic laboratory in Texas, the absence of lesions in 299 skulls from deer found dead in southern Texas suggests that the disease may be less frequent in this generally more arid location.

The overall prevalence (2.2%) of intracranial abscesses among diagnostic laboratory cases was low in comparison with other natural mortality factors of white-tailed deer, such as hemorrhagic disease (Couvillion et al., 1981) or winter starvation (Halls, 1984). Intracranial abscesses were more common among white-tailed deer of the southeastern and the central regions, and this tendency for higher prevalence suggests that deer in these areas are at a higher risk of developing this disease. However, because conclusions for some regions were based on a relatively small sample size and because the data were not collected randomly, caution is indicated in interpreting regional differences.

Although 18 other genera of bacteria were isolated from intracranial abscesses, *A. pyogenes* was clearly the predominate bacterium involved, occurring in 61% of all cases in which isolations were made. This bacterium commonly inhabits the mucosal membranes and skin of many species of animals and is characterized as an opportunistic pathogen that readily invades devitalized superficial tissues (Zulty and Montali, 1988). The present study along with the previous report by Davidson et al. (1990) verifies that *A. pyogenes* should be considered the principal agent involved in intracranial abscesses in white-tailed deer.

In this research, we revealed a gender-related bias with 87% of the cases occurring in males. Intracranial abscesses were significantly more common among males (4.9%) than females (0.7%). The conformity in these results with those of Davidson et al. (1990) reaffirms that this disease is a much more important source of natural mortality for males than females. The current research also verifies that adult males are more commonly affected than male fawns. However, the difference in infection rates between adult males and yearling males noted by Davidson et al. (1990) was not evident in the present study. The difference in prevalence between antlered males (adults and yearlings) and nonantlered males (fawns) seems to further substantiate the role of antlers and/or behaviors associated with reproduction as risk factors in the development of intracranial abscesses. We believe that this disease may hinder some white-tailed deer management strategies, such as quality deer management, that attempt to balance male to female ratios and to increase male age structure.

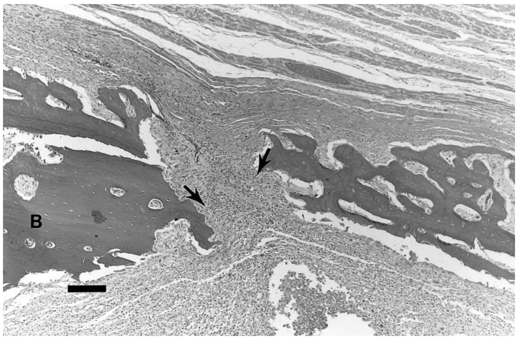

FIGURE 2. Affected portion of the skull overlying an intracranial abscess in a 4.5-yr-old male white-tailed deer submitted the SCWDS in 1996. The frontal bone (B) contains an area of complete lysis (marked by arrows) that is infiltrated by numerous neutrophils, lymphocytes, macrophages and periosteal fibrosis. These inflammatory cells extend from the superficial surface of the bone and adjacent skeletal muscle (top of photograph), throughout the area of lysis and into the underlying meninges. H&E. Bar = 1.1 mm.

The strong seasonality exhibited by the cases in this study closely resembles the results reported by Davidson et al. (1990), who noted cases only during October through April. This well-defined period of infection among both age classes represent the period in which velvet shedding, sign post/rut behavior, and antler shedding occur (Halls, 1984). Davidson et al. (1990) suggested that the sexually dimorphic characteristic of males possessing antlers and behaviors associated with antlers were key risk factors in the higher rate of infection among males. During this time period antlered males are involved in the rubbing of the antlers and the head (Halls, 1984). Cutaneous and subcutaneous lesions of the head noted in the present and in the earlier study (Davidson et al., 1990) suggest that these lesions incurred by males are a key risk factor. The repeated injuries to the skin may result in cutaneous and/or subcutaneous bacterial infections. Certain bacteria, particularly *A. pyogenes,* produce proteolytic enzymes (Sneath et al., 1986) capable of eroding through the skull sutures and bone (Fig. 2) which can lead to the formation of intracranial abscesses. Alternate mechanisms for pathogenesis including spread by lymphatic or hematogenous routes also are possible (Jubb et al., 1991).

In the current study, we disclosed a difference between the temporal patterns of cases in yearling and adult males similar to the study by Davidson et al. (1990). The present data indicate that although cases in both adults and yearlings peaked in October, 89% of cases in yearlings occurred from October through December, whereas just 58% of cases among adult males occurred during the same period. Cases among adult males tended to taper off more slowly and were more evenly distributed through April. Potential explanations for the temporal difference between yearling and adult males include (1) adult males may have a longer period of susceptibility than yearlings because of greater post-rut stress, (2) the infection in adult males may take longer to develop or infiltrate the skull, possibly because of denser bone structure of the skull or other physiological differences, or (3) yearlings are more susceptible and have a more rapid disease course.

The period of infection among the antlered males coincides with the period of high male mortality reported among several studies from different areas of North America (Gavin et al., 1984; Nelson and Mech, 1986; DeYoung, 1989). Although each of these studies attributed much of the losses to predation, Gavin et al. (1984) and Nelson and Mech (1986) also suggested that the increased rate of mortality among adult males was caused by post-rut stress associated with breeding behavior exhibited by males during this period. Herein, we offer an additional explanation of this increased seasonal mortality and suggests that intracranial abscesses may lead to increased vulnerability of affected animals to predation or other mortality factors (vehicle collisions). The possibility of intracranial abscesses also must be considered when male deer are found in poor physical condition since Davidson et al. (1990) indicated that affected animals also often resemble deer suffering with simple malnutrition.

The lower rate of infection in females and fawns and the predominance of bacteria other than *A. pyogenes* among females and fawns suggest there may be a different pathogenic process involved in the infection of nonantlered deer. Review of reports from diagnostic laboratories (data not presented) disclosed that many of the intracranial abscesses among females and fawns developed following injury to some region of the body other than the head. Infection of these wounds and hematogenous spread from the infected wounds may be important in intracranial abscess formation in these animals.

There also may be differences in the factors affecting the formation of intracranial abscesses between captive and wild animals. There were three key epidemiological differences noted between infections in captive and wild deer. First, infection among captive deer involved primarily females or fawns, which was the reverse of what was occurring among wild animals. Second, the agents isolated from abscesses in captive deer generally were different from those involved with wild deer in which *A. pyogenes* was predominant. Third, infections among captive deer did not have any apparent seasonal patterns. All these differences suggest that different risk factors may be responsible for intracranial abscesses among captive deer.

The prevalence of characteristic bone lesions in field-collected deer skulls (9%) from the southeastern United States was exceptionally close to the prevalence (8.4%) of intracranial abscesses in males submitted to diagnostic laboratories in that region. It is important to note that there may be biases that contribute to some of the inconsistencies between the two types of data. For the field-collected deer, the prevalence of disease in yearling males appeared to be somewhat higher than in adult males, yet the diagnostic laboratory data showed the opposite to be true. One possible explanation for these differences may be that bias exists as to whether certain deer are submitted to laboratories, such as lower submission rates of yearlings by hunters and biologists. A lower submission rate of yearling males could be a result of individual concern over what is happening to the larger antlered, older males of a population and apathy over what is happening to yearling males. Another possible explanation may be that the prevalence determined from the low number of field-collected skulls examined may not truly represent the amount of disease that is occurring because of such a small sample size.

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