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Characterization of an Outbreak of Anthrax in Animals in North Dakota: 243 Cases (2005)

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Abstract

The objective of this study was to characterize an outbreak of anthrax among animals in North Dakota in 2005, and determine characteristics and clinical signs associated with the disease. A total of 243 animals (183 cattle, 32 bison, 11 horses, 11 elk, five sheep and one deer) were diagnosed with anthrax at the Veterinary Diagnostic Laboratory at North Dakota State University, Fargo. Anthrax cases were defined as animals with typical clinical signs and positive results of laboratory culture and polymerase chain reaction (PCR) methods. Data for the study were obtained from veterinary laboratory records and questionnaires mailed to producers of affected animals. Anthrax occurred from July 1 to October 12 of 2005. The cases were located in 16 of 53 counties, specifically in eastern North Dakota, with Ransom, Lamoure and Barnes counties reporting most (71%) of the cases. The number of animals affected per premise varied from one to 40. The first cases were reported in bison. Males from all species infected had a higher attack rate (18/1000) than females (3/1000) with a gender ratio of 6:1, respectively. The predominant clinical signs in all cases were sudden death (38%) and bleeding from orifices (17%). Only 11 producers reported having vaccinated animals before the outbreak. Penicillin and tetracycline antibiotics were administered to animals during the outbreak. Forty producers (37%) burned and buried carcasses using commercial disposal services. Sixty-eight producers (63%) disposed of carcasses themselves by either burial only (11%), burned only (4.6%), or burned and buried (84.4%). Animals affected, clinical signs and final outcome were consistent with a natural anthrax outbreak.

Résumé

L'objectif de cette étude était de décrire une flambée d'anthrax chez des animaux du Dakota du Nord en 2005 et de déterminer les signes cliniques et les caractéristiques associés à la maladie. Un total de 243 animaux (183 bovins, 32 bisons, 11 chevaux, 11 wapitis, cinq moutons et un chevreuil) ont été diagnostiqués avec l'anthrax au laboratoire diagnostic vétérinaire du'*North Dakota State University* à Fargo. Un cas d'anthrax était déclaré lorsque les animaux montraient des signes cliniques typiques et lorsque les animaux testaient positif suite à des cultures en laboratoire et au test de la réaction en chaîne de la polymérase. Les données de cette étude ont été obtenues grâce aux registres du laboratoire vétérinaire et par l'entremise d'un questionnaire envoyé aux producteurs des animaux atteints. La flambée d'anthrax s'est déclarée entre le premier juillet et le 12 octobre 2005. Les cas ont été localisés dans 16 comtés parmi les 53, surtout à l'est du Dakota du Nord. Les comtés de Ransom, Lamoure et de Barnes étaient parmi les plus fréquemment touchés (71%). Le nombre de cas rapportés par site variait de 0 à 40. Les premiers cas ont été rapportés chez les bisons. Le taux d'attaque chez les mâles des espèces affectées (18/1000) était plus élevé que chez les femelles (3/1000) avec un rapport des sexes de 6 :1. Les signes cliniques prédominants dans tous les cas étaient la mort soudaine (38%) et le saignement par les orifices naturels (17%). Seulement 11 des producteurs avaient vacciné leurs animaux avant la flambée. La pénicilline et la tétracycline ont été administrées aux animaux durant la flambée. Un total de 40 producteurs (37%) ont brûlé et enterré les carcasses avec des services commerciaux d'élimination. Les

soixante huit autres producteurs (63%) ont disposé eux-mêmes des carcasses soit en les enterrant (11%), soit en les brûlant (4,6%) ou soit en les brûlant et en les enterrant (84,4%). Les animaux affectés, les signes cliniques et le dénouement étaient tous compatibles avec une flambée naturelle d'anthrax.

Introduction

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. This organism is an encapsulated gram-positive, non-motile, aerobic, spore-forming bacterial rod with a spore size of approximately 1 μm x 8 μm.[18] The three virulence factors of *Bacillus anthracis* are the protective antigen (PA), lethal factor (LF) and edema factor (EF). The three exotoxin components combine to form edema toxin, lethal toxin and, along with the antiphagocytic capsular antigen, are required for full virulence. The toxins are responsible for the primary clinical manifestations of hemorrhage, edema and necrosis.[8,12,18,19] The bacterium thrives naturally in certain types of soils, where it produces resilient spores which can withstand harsh environmental conditions. This form of the bacterium has been known to survive in soil or contaminated objects for several years.[3,6,20] The bacterium is commonly reported in areas with alkaline soils. When soil microecologic conditions are favorable, the spores become viable. Thus, the occurrence of multiple disease outbreaks can be experienced in same locations over consecutive years.[5,6,13,17] However, in unfavorable conditions and over long dormant periods, the spore gradually loses its virulence properties and ability to revert to its vegetative form.[6]

Animals susceptible to anthrax reported in the literature include cattle, sheep, goats, horses, donkeys, pigs, dogs and many warm-blooded domestic animals.[8,13,18,22,27] Wild ruminants such as antelope, gazelles and impalas, and other herbivores and fermenters, including elephants and hippopotami, are equally susceptible.[9,13] Outbreaks of anthrax in livestock are associated with spores that came from other animals dying of the disease. Because these spores do not form until some time after the animal dies, the disease is not reported as contagious.[26]

Transmission of the anthrax bacteria occurs through ingestion, inhalation and inoculation. Ingestion of the bacteria, especially by herbivores, is the most common route of infection.[18] Inhalation has been suggested as another way by which infection can occur because of the airborne capacity of the spores.[26] Inoculation by flies, mosquitoes and other blood-feeding insects has been conclusively demonstrated. Turell and Knudsen confirmed that blood-feeding insects could mechanically transmit anthrax, resulting in the cutaneous form of the disease.[25] Based on the length of clinical course of the disease, anthrax can also be classified in three forms.[28] These include a peracute form lasting one to two hours in duration (identified with ruminants), an acute form lasting 24 to 48 hours in duration (common with ruminants and horses), or a subacute to chronic form associated with localized conditions (common with domestic animals like dogs, cats and pigs).[28]

The disease has been reported globally, and occurrence is most common in agricultural regions.[14,18] Developing countries or countries with few veterinary and public health facilities are usually the most affected.[9,18] The worldwide incidence of anthrax is unknown, even though the bacteria *Bacillus anthracis* is present in most parts of the world.[7,9] This lapse in information is attributed to unreliable reporting of cases, which makes it difficult to estimate the true incidence of human and animal anthrax cases worldwide.[4,7,9]

Reported outbreaks in the United States of America (US) have been in North Dakota, South Dakota, Nebraska, Arkansas, Louisiana, Texas and California.[10,11,18,22] These outbreaks were correlated to states with substantial agricultural activity involving animal rearing and open pasture grazing. Incidence of naturally-acquired anthrax is relatively rare for humans and low for animals.[11,18,21,22,23] The relatively low incidence rate in animals and the long period between sporadic outbreaks in the US suggest a decline in the incidence of anthrax and indicate the disease is relatively low in American livestock.[18]

Anthrax is an integral part of North Dakota's ecosystem. Prior to 2005, the state reported an outbreak in 2000 with over 180 confirmed cases spread in 33 premises around the state.[22] In 2004, only one premise in the state reported anthrax. During the summer and early fall of 2005, North Dakota experienced the most extensive anthrax outbreak in recorded state history. The actual number of animal fatalities is unknown but easily totals into the high hundreds.[a] North Dakota further fits the criteria of states with the potential for anthrax outbreaks because of the basic or non-acidic nature of most of its soils. Bovine species and other grazing ruminant animals in the state are particularly susceptible because of frequent contact with the soil during grazing. The objective of this study was to characterize an outbreak of anthrax among animals in North Dakota in 2005 and determine characteristics and clinical signs associated with the disease.

Materials and Methods

Data sources

The data for this study were derived from three sources: North Dakota State University Veterinary Diagnostic Laboratory (VDL), a producer survey sent by

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the North Dakota state veterinarian's office and a supplemental survey mailed to animal producers. Data for affected animals were retrieved in part from 2005 medical records from the North Dakota State University VDL. Samples (whole blood, serum and tissue) from respective premises were forwarded by the local veterinarians to the VDL using specialized anthrax sampling kits for collection (Figure 1). Diagnoses of anthrax in submitted samples were performed by the staff at the VDL, using both bacterial culture and PCR. The use of a single diagnostic laboratory for sampling analyses ensured bias associated with diagnosis was minimized. Additional data were obtained from a producer survey sent by the North Dakota state veterinarian's office to producers in the state that reported cases of anthrax. Also, data were obtained from a supplemental survey mailed to animal producers in the state between the months of May and August of 2006.

Laboratory analyses

Specimens (whole blood, serum and tissue or carcasses) from animals showing clinical signs, and suspected of having anthrax, were submitted to the VDL at North Dakota State University. Initially, laboratory culture alone was used to diagnose *Bacillus anthracis* infections. As the outbreak progressed, additional confirmatory diagnosis of anthrax cases was made by multiplex polymerase chain reaction (PCR).[b] This PCR method characterizes *Bacillus anthracis* isolates and at the same time confirms the species identity independently of the plasmid content.[20]

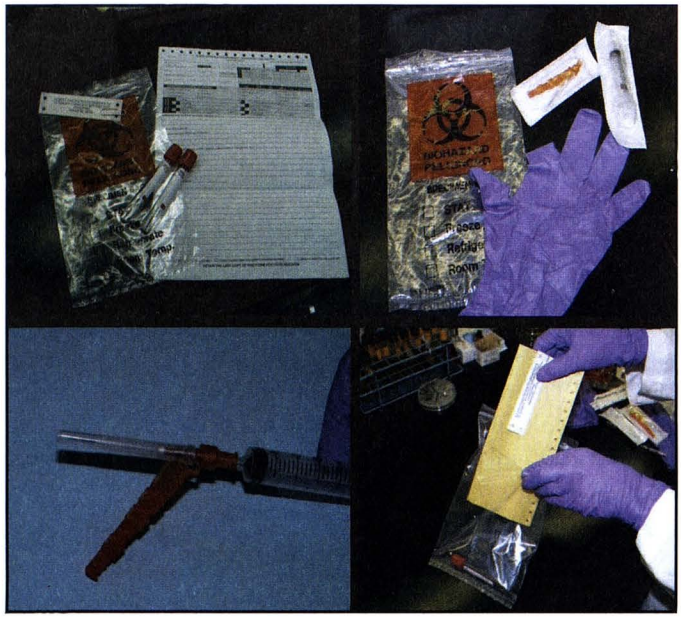

Figure 1. Picture of NDSU-VDL recommended sampling kit used for sample collection during the 2005 anthrax outbreak in North Dakota.

Statistical analyses

Descriptive statistics of animals and animal premises that participated in the study were computed using the Statistical Analysis Software (SAS) version 9.[c] The effect of gender on how easily an animal was exposed to anthrax was tested using cattle data in our sample. To determine the influence of gender, we compared the ratio of the gender distribution in our sample of infected cattle, and that of cattle in the entire state. We used state cattle records[15] and the gender ratio of one male to 25 females[d] for the state to make our comparative analysis. The gender ratio difference resulting from the analysis helped determine which gender had the higher attack rate. The attack rate is another way of expressing the incidence rate in a defined population over a specified period of time, such as during the anthrax outbreak. It shows the probability or risk of becoming a case in the defined population.

We used Geographic Information System (GIS)[e] to show the spatial distribution by county of case premises involved in the study. We employed the GIS technique of geocoding to identify each location because of the large surface extent of the study area and the time the study was conducted. Geocoding used location information from street addresses of premises and converted these to longitudinal and latitudinal coordinates which appear as specific points. These geocoded points were then superimposed on administrative and thematic maps layers.

Results

Laboratory diagnosis

The first 218 samples were processed by culture only. The 65 subsequent samples were processed by both culture and PCR simultaneously. After observing excellent agreement between culture and PCR, the remainder of samples (n=293) were processed by PCR only. Of the 65 samples tested by both methods, 64 were positive by culture and 65 were positive by PCR ($K = 0.959$). PCR was able to detect all samples that were positive by culture, while the culture method missed one sample that PCR was able to diagnose.

Temporal distribution of cases

Anthrax occurred from July 1 to October 12, 2005. Of 576 samples submitted to the VDL during the outbreak, 243 were laboratory positive for anthrax. In cases that tested negative for anthrax, no other differential diagnosis was made. Most of them were blood samples that were tested only for anthrax and reported as positive or negative. Figure 2 shows the week with the peak

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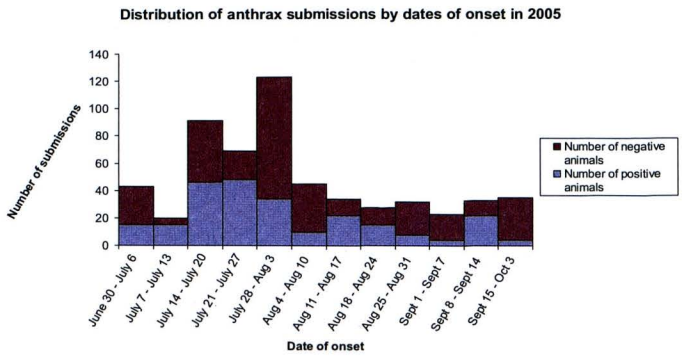

Figure 2. Distribution of all anthrax submissions by date of onset and species: ND, 2005, N=576. Positive cases, N=243.

period (July 28 to August 3) when most submissions (123) occurred. The period with least submissions to the laboratory was the week of September 15 - 21, with only 12 submissions. Figure 2 also shows the distribution of anthrax cases during the same submission period, with cases of anthrax reported from June 30 to September 30. The peak periods with highest incidence of positive cases were from the weeks of July 21 - 27 with 48 positive cases and July 14 - 20 with 46 cases, respectively.

Distribution of cases by county

Geographically, case premises were located in eastern North Dakota, west of the Red River of the North. The cases were reported in counties within the Red River of the North basin. The counties of Ransom, Lamoure and Barnes in North Dakota reported most (n =173; 71%) of cases in the state. The outbreak spread over 16 counties and involved a total of 109 premise locations in the state (mean = 6.8, median = 3, range = 1 - 38). A total of 243 confirmed positive cases were reported during the outbreak. The total distribution of cases in North Dakota extended as far north as Cavalier County, as far west as Kidder County by the Missouri River, and as far east as the counties of Cass, Grand Forks, Traill and Walsh, sharing borders with the state of Minnesota. From a regional scope, the outbreak was much wider as anthrax was diagnosed in adjacent counties in Minnesota and South Dakota, and also in the province of Manitoba, Canada. Figure 3 shows the spatial distribution of positive case premises that submitted samples to the VDL.

Reported species affected

Species affected were herbivorous animals grazing on pastures, including cattle (183; 75%), bison (32; 13%), horses (11; 5%), elk (11; 5%), sheep (5; 2%) and deer (1; 0.6%). Different breeds were reported amongst species: cattle (Angus, Beef Shorthorn, Charolais, Gelbvieh, Hereford, Limousin, mixed breeds, Milking Shorthorn and Simmental), horse (American Quarter Horse) and sheep (Suffolk). The period with the highest number of positive sample submissions involved four species of animals (cattle, horse, bison and elk), while the period with the least number of positive sample submissions involved only two animal species (cattle and bison).

Premises affected during the outbreak had herd sizes ranging from six to 2000 head of animals (mean = 193.4, median = 136). The number of animals affected on a premise during the outbreak ranged from one to 40 animals (mean = 5.1, median = 2). When age was reported, the distribution of animals affected by age groups were seven (9.6%) less than one year old, 19 (26%) 1 - 5 years of age and 47 (64.4%) animals greater than five years old. The remaining records (n = 170; 69%) did not report an age. Distribution by gender of reported cases during the outbreak showed that 56 female animals (81%) were affected as compared to only 13 male animals (19%). The comparative analysis by gender using the attack rate calculation indicated that males (attack rate = 18/1000) had the greater attack rate during the outbreak as compared to females (attack rate = 3/1000). The gender ratio based on the attack rate was about six males to one female, or 6:1, respectively. The first cases reported during the outbreak were bison, specifically from bulls in the pasture. Figure 4 shows the temporal distribution of positive submissions by animal species.

The predominant clinical sign reported was sudden death (38%) followed by bleeding from orifices (17%). Table 1 shows the frequency distribution of clinical signs observed both antemortem and postmortem, and reported during the 2005 anthrax outbreak, for positive anthrax cases. Table 2 shows the frequency distribution of clinical signs for negative anthrax cases during the outbreak. Figure 5 shows the distribution of reported clinical signs by the animal species involved in the 2005 outbreak. For positive cases, most clinical signs were reported across most species. For instance, sudden death was reported in all species affected. Bleeding from orifices, bloating and swelling, ataxia, recumbency and dystocia were signs identified in most animal species. Ventral edema was only reported in horses.

Only 11 producers out of 269 surveyed reported having vaccinated their animals against anthrax before the outbreak. The number of vaccinated premises increased to 111 during the course of the outbreak, based on data from the state veterinarian's office and the supplemental survey. Some producers augmented the vaccine with antibiotics – penicillin and tetracycline – during prophylactic treatment, on recommendation of their veterinarians.

Method of disposal differed among the 109 premises. Data indicates producers either buried, or burned, or burned and buried carcasses found on their properties.

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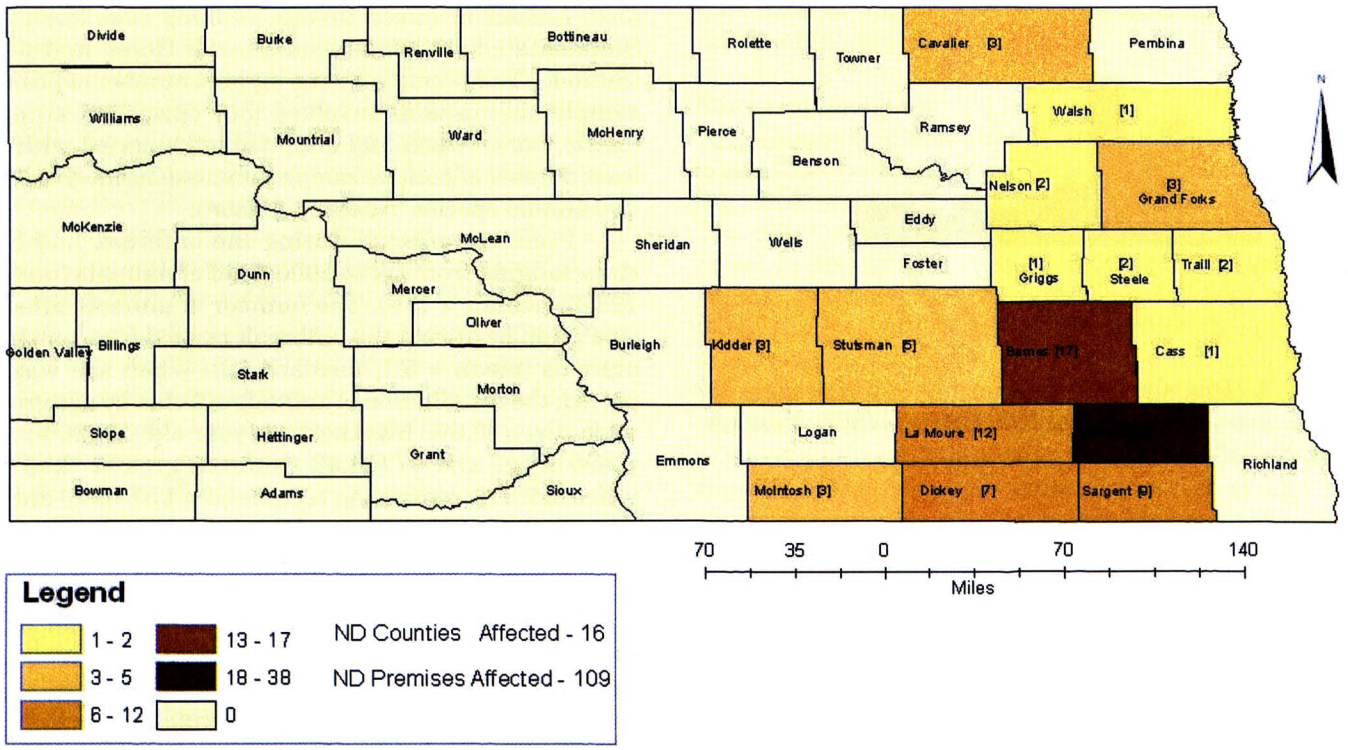

Figure 3. Spatial distribution of case premises that submitted samples to the VDL-NDSU in 2005, N=109.

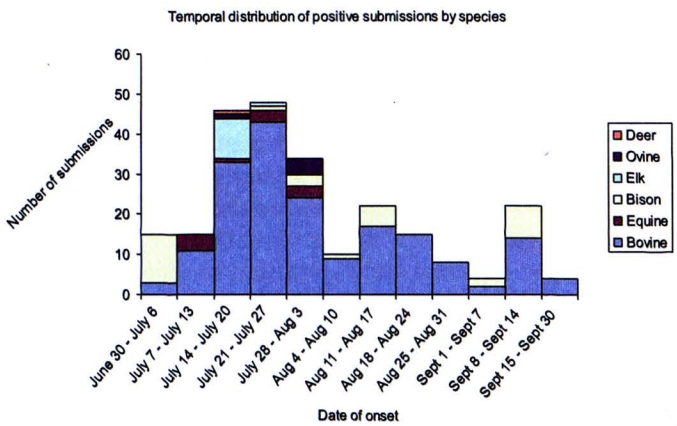

Figure 4. Temporal distribution of positive submissions from the various animal species.

Twelve (11%) producers buried only, five (4.6%) burned only and 92 (84.4%) burned and buried carcasses, respectively. Also, 40 (37%) producers used commercial disposal services, and 68 (63%) disposed of carcasses themselves, without the assistance of a commercial service.

Discussion

All anthrax samples submitted to the VDL came from dead animals, including the 243 that tested positive for anthrax. The most frequently observed clinical sign is usually sudden death.[24,28,29] Reported clinical signs exhibited by affected animals were similar to those reported in previous anthrax outbreaks in North Dakota and in the US.[2,24,29] It is possible that the number of animal deaths reported either to the VDL or the local veterinarians was underestimated, since our sample size

Table 1. Distribution of clinical signs reported for positive cases during the 2005 anthrax outbreak in North Dakota (N=243).

Clinical signs	Frequency (%)
Sudden death	116 (38)
Bleeding from orifices (nasal, oral, anal, ocular, ear)	51 (17)
Bloating, swelling	18 (6)
Ataxia, lameness, wobbly rear legs, weakness, lethargy	15 (5)
Swollen abdomen, enlarged spleen/liver	11 (4)
Subcutaneous ecchymosis, gas under skin	4
Ventral edema	2
Rigor mortis	2
Muscle pallor	2
Dystocia	2
Recumbency/laying down	2
Injected sclera	1

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Table 2. Distribution of clinical signs reported for negative cases during the 2005 anthrax outbreak in North Dakota (N=333).

Clinical signs	Frequency (%)
Sudden death	105 (32)
Bleeding from orifices (nasal, oral, anal, ocular, ear)	28 (8)
Bloating, swelling	14 (4)
Ataxia, lameness, wobbly rear legs, weakness	9 (3)
Swollen organ or limb	7 (2)
Enlarged spleen	4 (1)
Blood in chest and abdominal cavity	3 (1)
Ventral edema	3 (1)
Gas under skin	3 (1)
Foaming from nose and mouth	2 (1)
Liver flukes	2 (1)
Muscle pallor	2 (1)
Rigor mortis	2(1)
Larger thymus	1
Injected sclera	1

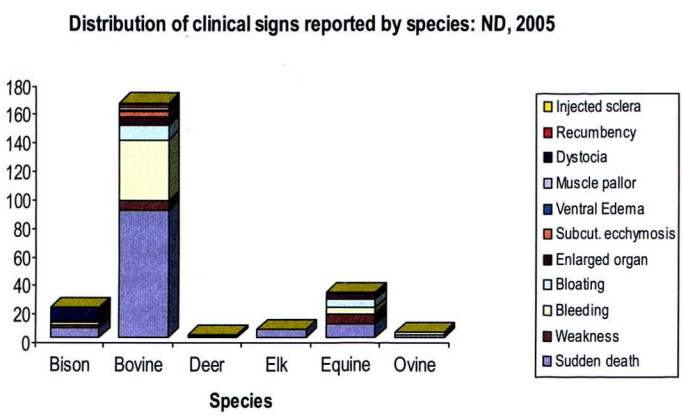

Figure 5. Distribution of reported clinical signs for various animal species in 2005 in North Dakota.

comprised only animals that were tested at the NDSU-VDL. Another possible reason for underestimation of affected animals was the lag time between death of the animal and sample submission to the VDL for an optimal bacterial culture. Samples should be sent within 48 hours of animal death in order to obtain a significant laboratory diagnosis.[f] Beyond this time frame, a positive laboratory culture is not likely due to carcass decomposition and associated death of the bacteria. Given the nature of most pastures in North Dakota, most producers do not routinely check their livestock on a daily basis. Some producers indicated animal carcasses were discovered more than three days postmortem. Obtaining useful bacterial samples from these carcasses in summer is almost impossible. However, a PCR[b] procedure used by the staff at the NDSU-VDL has made it possible to get a positive diagnosis up to 72 hours after death.[19] Interestingly, the distribution of clinical signs in both positive (Table 1) and negative cases (Table 2) were very similar, leading to the suspicion that the negative cases could have possibly been false-negative. Later in the study, when both PCR and bacterial culture were conducted simultaneously, PCR picked up one sample that tested negative on bacterial culture.

Animal species affected during the anthrax outbreak of 2005 were similar to those reported elsewhere and in previous outbreaks.[2,29] Reports indicate mainly herbivores or grazing animals are susceptible to the bacteria; a similar pattern was noted in this study. Interestingly, bison were the first species reported to be affected by anthrax. This was also noted in South Dakota and in northern Canada. The outbreak in South Dakota was in Sully County on July 20, 2005 in a pasture containing 300 unvaccinated bison and rodeo bulls. About 40 head of bison and two rodeo bulls were diagnosed with anthrax.[g] Dragon *et al* also reported bison were first affected in an anthrax outbreak in the Lake Claire Delta region of northern Canada in 2001, after an outbreak in the region the previous year that killed over 100 bison.[5] Length of exposure and bison behavior on pastures possibly account for the above finding. Bison on pasture, especially male bulls, usually stomp and roll on exposed soil and mud patches. The rolling behavior serves as a means to clean out pests on their skin surfaces. The presence of cuts on skin surfaces makes it easier and possible for anthrax infection to occur via the cutaneous route. Also, these animals often graze pastures very short, predisposing them and other grazing animals with similar behavior to easily contact and consume the bacteria, resulting in the gastrointestinal route of infection. Within species, there was a reported diversity among cattle breeds only. Beef breeds (Angus, Beef Shorthorn, Charolais, Gelbvieh, Hereford, Limousin, mixed breeds, Milking Shorthorn and Simmental) were mainly reported as compared to dairy breeds. This is likely associated with the different management practices utilized by the two production systems, as well as total numbers of beef and dairy cattle in North Dakota.[15]

The spatial distribution of animal cases by county in North Dakota matched that of known areas of concentration of previous outbreaks, along the southern and eastern regions of the state.[2,26] Spatial similarities between the 2005 outbreak and previous outbreaks have interesting ecologic and environmental implications. It indicates possible seeding of soils with bacteria spores in these counties and confirms that anthrax is endemic to North Dakota.

Anthrax cases peaked in August 2005. The temporal distribution of animal cases in ND in 2005 matched

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that of anthrax outbreaks from other US states where data were available, with onset of illness starting from May/June to October and peaking in mid-July/early August.[6] The onset of dusty drought conditions, with grasses already over-grazed, and bare soil surfaces in most pastures could account for this observed peak period leading to exposure.[6] Outbreaks of anthrax in temperate regions generally occur during late spring or early summer to the mid-fall season.[24,28,29] This period is associated with flooding and drought cycles implicated in the sub-soil vertical migratory and settling pattern of the bacteria spores coming to the surface. A possible association between premise sites and proximity to constantly flooded, low-lying areas with fresh and abundant pasture which is grazed short during drought periods was reported by USDA.[29]

Generally, anthrax epizootics occur during periods of dry spells punctuated by prolonged periods of intense rain.[6,27] A proposed role of water in anthrax epizootics is the aggregation and concentration of spores in storage areas.[6] Prolonged rainfall promotes runoff and pooling of standing water. It has been reported that the surface of *B. anthracis* spores is hydrophobic, making the spores resistant to dissolution by water.[6] Spores are then possibly transported in clumps of organic matter by runoff to standing pools of water. Dry weather causes evaporation of standing water and concentration of floating anthrax spores as water pools shrink. The high buoyant density of *B. anthracis* spores provides an opportunity for spores to adhere to vegetation as the vegetation resurfaces during evaporation.[6]

Weather conditions in North Dakota prior and during the anthrax outbreak were consistent with the expected cycle of very wet conditions followed by very dry conditions. The median rainfall estimate between the months of April to September 2005 was 2.5 inches (6.4 cm). The rainfall estimate before the outbreak in early June was at a high of 7.2 inches (18.3 cm); but rainfall estimates during the outbreak peak in July showed a significant decrease at 1.9 inches (4.8 cm) which was the lowest estimate during the outbreak. This significant correlation demonstrates an important relationship between the anthrax outbreak and weather conditions. Despite the above insight, further studies into the role of flood and drought conditions during outbreaks are warranted to better understand the mechanism of exposure in pastures.

There were generally more female animals (82%) reported with anthrax than males (18%). Animals most affected were five years of age and older. Pasture forage is the primary diet for adult animals, unlike young animals that get much of their nourishment from their mothers. Thus, contact with contaminated pasture soil and grass is greater for adults, specifically when the grass is grazed very low. While grazing and feeding habits of adults possibly increased the likelihood of anthrax infection on pasture, young animals could possibly become infected from roaming and coming into contact with adult carcasses in the field.

The sex disparity can be correlated to the number of female and male animals on pasture. Producers generally own more female than male animals because of reproduction benefits. Our study indicates that males had a six-times higher attack rate than females, though they accounted for less than 10% of the livestock population in North Dakota. Males therefore had a higher probability or risk of becoming an anthrax case as compared to females. It will be interesting to further determine if the increased attack rate is as a result of male behavior on the pasture or general susceptibility because they are males. An increased attack rate for males also has vaccination implications for producers. Producers with larger herds of livestock should be encouraged to first vaccinate male animals before the outbreak period. If financial constraints exist, they are encouraged to at least vaccinate males because of their higher attack rate.

Only 11 producers reported they vaccinated their animals against anthrax prior to the outbreak. This finding may be attributed to several factors, including the nature of vaccine used. The Sterne vaccine provides protection for about eight months to one year, implying that annual revaccination is required to boost and maintain immunity against the disease.[1,24] More likely because of the sporadic nature of anthrax infection, most producers become complacent after the first few years of vaccination, especially if there is no outbreak on their premises in subsequent years. Vaccination usage is often a response to outbreaks and their frequency of occurrence. Another possible reason for the low vaccination rate before the outbreak may be the low degree of awareness among producers about the vaccine. This emphasizes the need for additional public media information and awareness campaigns. There is a possibility the subsequent reported anthrax cases in 2006 (five premises in four counties affected) were low because of the intensive public information and awareness campaigns carried out in the state. These campaigns stressed the benefits of vaccination before the outbreak season.

Type of disposal method used is an important control strategy for reducing exposure to unwanted agents. Efficient carcass disposal, vaccination, antibiotic treatment, relocating uninfected animals to uninfected pastures and quarantine are essential for cleaning up infected pastures. In North Dakota, our data indicated that producers used either burial, burning, or burning and burial as the three main methods of disposal. Burning followed by deep burial is the recommended method of disposal.[24] Burning alone leaves surviving spores on the soil surface, and burial alone does not destroy spores or vegetative organisms found on the carcass and im-

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mediate soil surface. If the carcass is not burned, cautious application of quicklime (calcium oxide) on the carcass before deep burial is recommended.[24] Burning combined with deep burial offers the advantage of not only destroying spores on the surface, but any surviving spores are buried deep below the soil surface, thereby decreasing the likelihood of ingestion by animals in subsequent grazing seasons.

Conclusions

This study enabled us to characterize anthrax cases in the 2005 outbreak that occurred in North Dakota. The outbreak was the largest epizootic in recorded state history and involved no human cases. Bison were the first cases seen during the outbreak. Males had a six-times higher attack rate than females, though they were estimated to be less than 10% of the livestock population in North Dakota. The characteristics of animals affected and reported, and final outcome of the animals in terms of survival, were consistent with anthrax outbreaks reported in the literature.

Early bison die-off during the anthrax outbreak was another important finding, because bison can possibly serve as an indicator of an impending outbreak. Further research to determine if bison are more susceptible to the disease or if their behavior on pasture is the main cause for the increase in risk is needed to establish their indicator status.

This study reports the control characteristics observed during the outbreak. Information gathered could help veterinarians and public health agencies plan appropriate control and prevention measures for future anthrax outbreaks, including safe sample handling submission procedures, carcass handling, vaccination programs, antibiotic administration, herd handling and premise use.

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Endnotes and Personal Communications

[a] North Dakota State Veterinarian's office, personal communication, March 2006.

[b] *Bacillus anthracis* PCR Standard Operating Procedures, University of Nebraska Veterinary Diagnostic Center.

[c] SAS Institute Inc, SAS/STAT® User's Guide, Version 8, Cary, NC: SAS Institute Inc, 1999, Chapter 28: pp 1298-1299.

[d] Greg Lardy, PhD, North Dakota State University, Extension Animal and Range Science. Mail post communiqué sent and received on January 18, 2007.

[e] Geographic Information Systems (GIS), ArcInfo™8, ESRI, 380 New York Street, Redlands, CA 92373-8100 (www.esri.com).

[f] North Dakota State University Veterinary Diagnostic Laboratory Communiqué sent to local veterinarians in the state.

[g] Sam Holland, DVM, South Dakota State Veterinarian. Mail post communiqué sent and received on January 22, 2007.

References

1. Advisory Committee on Immunization Practices (ACIP). Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on immunization practices. *Morbidity and Mortality Weekly Report* 49(RR15):1-20, Dec 15, 2000.

2. Bales ME, Dannenberg AL, Brachman PS, Kaufmann AF, Klatsky PC, Ashford DA: Epidemiologic response to anthrax outbreaks: field investigations, 1950–2001. *Emerg Infect Dis* 8:1163-1174, 2002.

3. Chunsun R, Kyunghee L, CheonKwon Y, Seong WK, Oh H: Sensitive and rapid quantitative detection of anthrax spores isolated from soil samples by real-time PCR. *Microbiol Immunol* 47:693-699, 2003.

4. Dixon TC, Meselson M, Guillemin J, Hanna PC: Anthrax. *N Engl J Med* 341:815-826, 1999.

5. Dragon DC, Bader DE, Mitchell J, Wollen N: Natural dissemination of *Bacillus anthracis* spores in north Canada. *Appl and Environ Microbiol* 71:1610-1615, 2005.

6. Dragon DC, Rennie RP: The ecology of anthrax spores: tough but not invincible. *Can Vet J* 36:295-301, 1995.

7. Friedlander AM: Microbiology: tackling anthrax. *Nature* 414:160-161, 2001.

8. Hanna PC, Ireland JA: Understanding *Bacillus anthracis* pathogenesis. *Trends Microbiol* 7:180-182, 1999.

9. http://www.fao.org/ag/magazine/0112sp.htm. Accessed on 02/14/2007

10. Inglesby TV, Henderson DA, Bartlett JG, *et al*: Anthrax as a biological weapon: medical and public health management. *J Am Vet Med Assoc* 281:1735-1745, 1999.

11. Kassenborg H, *et al*: Human ingestion of *Bacillus anthracis* – contaminated meat – Minnesota, August 2000. *Morbidity and Mortality Weekly Report* 49:813-816, Sept 15, 2000.

12. Koch R, Gaffky G, Loeffler F: The virulence of cultivated anthrax virus. Experimental studies on the artificial attenuation of the infectious properties of the bacillus of anthrax by means of cultivation. *Science* 4:276-277, 1884.

13. Lindeque PM, Turnbull PC: Ecology and epidemiology of anthrax in Etosha National Park, Namibia. *Onderstepoort J Vet Res* 61:71-83, 1994.

14. Madigan M, Martinko J (eds): *Brock Biology of Microorganisms*, ed 11. Prentice Hall, 2005, pp 817-846.

15. National Agriculture Statistic Services (NASS): 2002 Census. Accessed 02/14/07 at: http://www.nass.usda.gov/Census/Pull_Data_Census

16. Stoltenow CL, Hammer CJ (eds): *Anthrax. V-561* (revised). Fargo, ND: NDSU Extension Service, March 2006, pp 2-3.

17. North Dakota Department of Agriculture (NDDoH): Map of 2006 anthrax cases. Accessed 02/14/07 at: http://www.agdepartment.com/Programs/Livestock/BOAH/2006Anthrax.pdf

18. Parkinson R, Rajic A, Jenson C: Investigation of an anthrax outbreak in Alberta in 1999 using a geographic information system. *Can Vet J* 44:315-318, 2003.

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19. Pfaller MA, Murray PR, Rosenthal KS: *Medical Microbiology*, ed 5. St. Louis, Mosby, 2005, pp 265-269.
20. Ramisse V, Patra G, Garrigue H, Guesdon J, Mock M: Identification and characterization of *Bacillus anthracis* by multiplex PCR analysis of sequences on plasmids pX01 and pX02 and chromosomal DNA. *FEMS Microbiol Let* 145:9-16, 1996.
21. Ryan KJ, Ray CG (eds): *Sherris Medical Microbiology*, ed 4. McGraw Hill, 2004, pp 305-307.
22. Shafazand S, Doyle R, Ruoss S, Weinadker A, Raffin TA: Inhalational anthrax: epidemiology, diagnosis, and management. *Chest* 116:1369-1376, 1999.
23. Shireley L, *et al*: Human anthrax associated with an epizootic among livestock – North Dakota, 2000. *Morbidity and Mortality Weekly Report* 50:677-680, Aug 17, 2001.
24. Sternbach G: The history of anthrax. *J Emerg Med* 24:463-467, 2003.
25. Turell MJ, Knudson GB: Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). *Infect Immunol* 55:1859-1861, 1987.
26. Turnbull PCB, Lindeque PM, Le Roux J, Bennett AM, Parks SR: Airborne movement of anthrax spores from carcass sites in the Etosha National Park, Namibia. *J Appl Microbiol* 84:667-676, 1998.
27. USDA: Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), Centers for Epidemiology and Animal Health (CEAH), Center for Emerging Issues (CEI). *Epizootiology and Ecology of Anthrax*. Accessed on 02/14/07 at: http://www.aphis.usda.gov/vs/ceah/cei/taf/emerginganimalhealthissues_files/anthrax.text.pdf
28. USDA: Animal and Plant Health Inspection Service (APHIS). *Fact sheet: Anthrax. 1999*. Accessed on 04/14/07 at: http://cahfs.ucdavis.edu/disease_pdfs/aphisanthrax.pdf
29. World Health Organization (WHO): *Guidelines for the Surveillance and Control of Anthrax in Humans and Animals 2003*. Accessed on 02/14/07 at: http://www.who.int/emc-documents/zoonoses/docs/whoemczdi986.html#_Hlk436471631.

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Abstract

Evaluation of laboratory tests for SAT serotypes of foot-and-mouth disease virus with specimens collected from convalescent cattle in Zimbabwe

D.J. Sawmmin, D.J. Paton, S. Parida, N.P. Ferris, *et al*
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During a field study in Zimbabwe, clinical specimens were collected from 403 cattle in six herds, in which the history of foot-and-mouth disease (FMD) vaccination and infection appeared to be known with some certainty. Five herds had reported outbreaks of disease one to five months previously but clinical FMD had not been observed in the sixth herd. A trivalent vaccine (South African Territories [SAT] types 1, 2 and 3) had been used in some of the herds at various times either before and/or after the recent outbreaks of FMD. The primary aim of this study was to evaluate the performance of serological tests for the detection of SAT-type FMD virus infection, particularly ELISAs for antibodies to non-structural proteins (NSPs) of FMD virus and solid phase competition ELISAs (SPECs) for serotypes SAT1 and SAT2. Secondary aims were to examine NSP seroconversion rates in cattle that had been exposed to infection and to compare virus detection rates by virus isolation and real-time reverse transcriptase-PCR (rtRT-PCR) tests on both oesophagopharyngeal fluids and nasopharyngeal brush swabbings. In addition, the hooves of sampled animals were examined for growth arrest lines as clinical evidence of FMD convalescence. Laboratory tests provided evidence of FMD virus infection in all six herds; SAT2 viruses were isolated from oesophagopharyngeal fluids collected from two herds in northern Zimbabwe, and SAT1 viruses were isolated from three herds in southern Zimbabwe. Optimized rtRT-PCR was more sensitive than virus isolation at detecting FMD virus persistence and when the results of the two methods were combined for oesophago-pharyngeal fluids, between 12 and 35 percent of the cattle sampled in the convalescent herds were deemed to be carriers. In contrast, nasopharyngeal swabs yielded only two virus-positive specimens. The overall seroprevalence in the five affected herds varied with the different NSPs from 56 percent to 75 percent, compared with 81 percent and 91 percent by homologous SPCE and virus neutralisation tests respectively. However, if serological test results were considered only for the cattle in which persistent infection with FMD virus had been demonstrated, 70 to 90 percent scored seropositive in the different NSPs.

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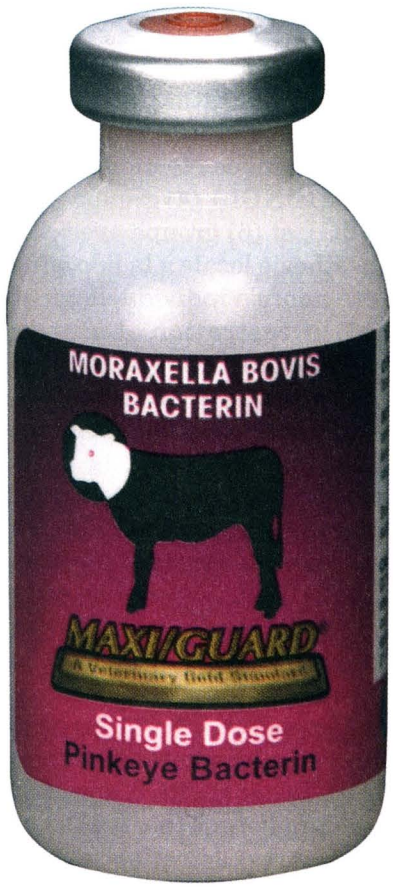

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