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RISK FACTORS FOR HUMAN ANTHRAX AMONG CONTACTS OF ANTHRAX-INFECTED LIVESTOCK IN KAZAKHSTAN

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Abstract. A retrospective cohort analysis was conducted in Kazakhstan to define modifiable risk factors during seven outbreaks of human anthrax. Fifty-three cases and 255 non-ill persons with an epidemiologic link to an infected animal were enrolled. Cases were 58% male and had a median age of 35 years (range = 5–71). Nearly all cases had cutaneous disease (96%). Two patients (4%) were diagnosed with gastrointestinal disease. Although all cases had some contact with an infected animal other than consumption, in multivariable analysis the act of butchering an animal (relative risk [RR] = 3.6, 95% confidence interval [CI] = 1.5–9.6) and the presence of visible cuts on the hands were associated with anthrax (RR = 3.0, 95% CI = 0.9–9.6). Contact with infected livestock, in particular butchering, is associated with developing anthrax. The risk may be exacerbated by the presence of cuts on the hands at the time of contact with the animal or animal products.

INTRODUCTION

Anthrax is a zoonotic illness caused by the spore-forming gram-positive rod *Bacillus anthracis.* Three main clinical forms of anthrax predominate in humans: cutaneous, inhalational, and gastrointestinal. All three forms may progress to sepsis or hemorrhagic meningitis. The most common form, cutaneous anthrax, results from inoculation with *B. anthracis* after handling infected animals or animal products.[1] Cutaneous anthrax has a case-fatality rate < 1% when treated appropriately.[2] In many industrialized countries, human anthrax has been virtually eliminated because of effective control measures including human and animal vaccination, veterinary supervision of animal slaughter, and quality control of animal products.[3] However, in certain parts of the world, anthrax remains an occupational hazard of herdsmen and workers who work closely with animals or who process animal products.[1] Specific practices resulting in human infection have not been fully defined.

In the summer of 1997, a series of outbreaks of human anthrax in the former Soviet republic of Kazakhstan concerned local and state health authorities. The Sanitary and Epidemiology Service (SES), the branch of the Kazakhstan Ministry of Health responsible for disease control, reported that the residents became infected as a result of contact with cattle, horses, and sheep with anthrax. Overall, 73 human cases were reported in Kazakhstan between January 1, 1997 and December 31, 1998.

We identified all patients with a diagnosis of anthrax reported through the public health system in Kazakhstan for two years. We then performed a retrospective cohort study to identify modifiable risk factors for disease.

MATERIALS AND METHODS

The team investigated outbreaks of two or more cases of human anthrax that were epidemiologically linked to sick livestock (one or more domestic animals with laboratory-confirmed anthrax) that occurred between January 1, 1997 and December 31, 1998. A detailed history for each outbreak, including patient demographics, clinical history, known domestic animal exposure, and a list of persons who had exposure to the same animal(s) was obtained by reviewing local health department and medical records.

Case definition and ascertainment. A suspect cutaneous case was defined according to the surveillance definition provided by the SES: a person with a typical skin lesion (a vesicle or pustule that progresses to an ulcer with an eschar surrounded by edema and erythema) or an atypical skin lesion (no eschar formation) and an epidemiologic link to an infected animal. A suspect gastrointestinal case was defined as a person who developed a febrile illness associated with nausea, vomiting, diarrhea, or sepsis within two weeks of exposure to an infected animal. A confirmed case was defined as a suspect case with culture (either blood, cutaneous lesion, or stool), Gram's stained smear (fluid from skin lesion), or serologically (single elevated titer by indirect microhemagglutination[4]) proven disease. Antibiotic susceptibility testing was performed in local laboratories using macrodilution minimum inhibitory concentration testing. Case ascertainment consisted of review of the 1997 and 1998 national surveillance reports provided by the Extremely Dangerous Diseases Branch of the SES.

Contact definition and cohort ascertainment. As part of the routine investigation, the SES investigation team identified all persons who were known to have had contact with anthrax-infected animals associated with outbreaks of human disease that had occurred in Kazakhstan during the investigation period. These contact persons included all those involved in domestic animal husbandry, processing (killing, restraining during killing, gutting, skinning, butchering), cooking, consumption, or other contact with the animal or animal products. Children were not excluded.

Informed consent and the collection of patient information. All participants provided signed consent and received a review of the purpose of the study and the risks and benefits posed by participation. Parents provided informed consent for the children less than 14 years of age (49 children, 14% of total cohort). All individuals enrolled in the study were asked to complete a detailed standardized questionnaire requesting demographic, clinical, and exposure information. Interviews were performed in the patient's native language, either Rus-

sian or Kazakh. A single attempt was made to interview each person. If the person was unavailable for interview, the questionnaire was completed by review of the data obtained at the time of the initial SES investigation (60% of the cohort was available for second interview). Parental proxies were used if children less than 14 years of age were unable to complete the questionnaire. Multiple members of the same family may have been interviewed. This project was reviewed by the Human Subjects Coordinator of the National Center for Infectious Diseases, Centers for Disease Control and Prevention, and determined to be a public health response that did not require Institutional Review Board review.

Statistical analysis. Data collected during the investigation were double entered into a computerized database (EpiInfo 6.0; Centers for Disease Control and Prevention, Atlanta, GA). Bivariable analyses of all demographic and exposure variables were performed. Subsequent analyses used a composite variable called "slaughter" that was created from several variables representing participation in the actual killing and immediate processing of the animal (gutting, skinning, restraining, or other handling during the event). Butchering, the process of cutting up the carcass into smaller pieces appropriate for cooking, was often performed at a later time and, therefore, remained separate for analytic purposes. The analysis was performed using the GENMOD procedure in SAS (SAS Institute, Cary, NC). Poisson regression was used with a generalized estimating equations algorithm to control for the clustered nature of the data to examine the relationship of several exposure factors to case/non-case status. A multivariable model was constructed using variables with an outcome associated P value < 0.20 on bivariable analysis of age, sex, and other potential confounding variables. The model was reduced by excluding non-significant, non-confounding variables. A second model was created using the composite variable "slaughter" in place of its individual components. Two-way interactions between main effects were investigated and deleted from the model when they were shown to be non-significant. Because of the potential for an individual to perform many of the tasks under investigation, multivariable models were examined for the presence of multicollinearity to see if one or more of the variables correlated with other factors in the model.[5]

RESULTS

Outbreak identification. Between January 1, 1997 and December 31, 1998, we identified nine outbreaks of two or more cases of human anthrax resulting from exposure to infected animals. Of the 73 cases reported during these two years, 65 (89%) were associated with outbreaks. We investigated seven of these outbreaks that included 53 cases (Figure 1 and Table 1). All investigated outbreaks, with the exception of Ornek, occurred in areas that were not previously described areas of anthrax activity. All source animals were privately owned and unvaccinated, and six of the seven animals associated with these outbreaks were ill at the time of slaughter.

FIGURE 1. Map of Kazakhstan showing the nine sites of human anthrax outbreaks between 1997 and 1998. The asterisks indicate outbreaks that were not investigated as part of the cohort study.

Results of cohort analysis. A total of 53 (82%) outbreak-associated suspect cases and 255 contacts of infected animals were enrolled in the retrospective cohort study. Cases were 58% male and had a median age of 35 years (range = 5–71). The median incubation period was seven days (range = 1–14). Symptoms began a median of two days before diagnosis (range = 0–4).

Fifty-one patients (96%) had cutaneous disease, and 29 (57%) of these were microbiologically confirmed. All confirmed cases had culture-positive ulcers and 16 also had positive rods seen on a Gram's stain smear. Forty-four (83%) persons had a single ulcer, six persons (11%) had two ulcers, and one (2%) person had three ulcers. Ulcers were distributed on exposed areas, 33 (63%) on the hand, 10 (19%) on the forearm, and seven (13%) on the lower extremity. Two were found on the trunk and one on the face. Edema (43%) and lymphadenopathy (58%) frequently accompanied ulcers. Healed lesions demonstrated scarring consistent with a deep ulcer. Additionally, 29 (55%) of patients had measurable fever with a median maximum temperature of 39°C. Headache (25%) and myalgias (43%) were also frequently reported.

TABLE 1
Characteristics of seven outbreaks of human anthrax in Kazakhstan, 1997–1998

Site	Date	Source animal	Cutaneous cases	Gastrointestinal cases	Non-ill contacts
1. Zhambyl Oblast/Lugovskoy Rayon/Ornek	July 1997	Sheep	7		15
2. Zhambyl Oblast/Lugovskoy Rayon/Ornek	August 1997	Horse	8		59
3. East Oblast/Targobataisky Rayon/Akzahr	August 1997	Cow	15		72
4. Atyrau Oblast/Atyrau Rayon/Atyrau	September 1997	Horse	10	2	85
5. Zhambyl Oblast/Juvalensky Rayon/Respek	October 1997	Horse	6		11
6. Akmola Oblast/Arshalynsky Rayon/Nikholayevka	August 1998	Horse	3		6
7. Zhambyl Oblast/Korday Rayon/Solotor	September 1998	Horse	2		7
Total			51	2	255

Only two patients were diagnosed with gastrointestinal disease. Each had a positive indirect hemagglutination titer, but stool cultures and pathologic specimens were not available. Neither of these patients had concomitant skin lesions. No patients complained of oral, respiratory, or hemorrhagic symptoms. No patients progressed to sepsis or death.

In accordance with Kazakhstan law, all patients were hospitalized (median = 13 days, range = 1–40). Patients were treated with either oral or intravenous antibiotics depending on disease severity and availability of medications. Although the isolates from the 29 culture-confirmed patients were not available at the time of investigation, all were reported to be penicillin and tetracycline sensitive. Local physicians used penicillin and tetracycline as the antibiotics of choice with nearly equal frequency (54% versus 46%).

Most of the persons with cutaneous disease (76%) were directly involved in the procedures related to the slaughter or the butchering of an infected animal. The attack rate for each of these activities was very high (slaughter = 67%, butcher = 60%). The remainder of the cases either cooked with the meat (10%) or handled the animal carcass or raw meat for another purpose (10%). Two persons whose only exposure was eating contaminated meat (one barbequed, one boiled) developed cutaneous lesions. Each of the two patients developing gastrointestinal illness had multiple exposures to infected animals including butchering the animal and cooking and eating infected meat. Human-to-human transmission does not appear to have occurred among any of the cases reported from the recent outbreaks in Kazakhstan.

Risk factors for the development of disease are shown in Table 2. In Kazakhstan, men are more likely to kill (67%), skin (67%), and butcher (62%) the animal, whereas the women are generally responsible for meat preparation and cooking (78%). However, no significant difference was identified for age or sex distribution between cases and contacts. "Slaughter", a composite variable including killing, gutting, skinning, restraining or other handling of livestock during the slaughter event was strongly associated with development of cutaneous disease (relative risk [RR] = 8.3, 95% confidence interval [CI] = 4.8–14.4). Each of the variables comprising slaughter was also significant on bivariable analysis. Butchering was also strongly associated with developing anthrax (RR = 7.7, 95% CI = 4.4–13.4). Eating (RR = 0.6, 95% CI = 0.3–1.1) and cooking (RR = 1.2, 95% CI = 0.7–2.1) and any consumption of infected meat (RR = 0.6, 95% CI = 0.3–1.1) were not significantly associated with disease. Meat is usually boiled for an extended period or grilled on a stick (shaslik) prior to consumption. Eating boiled meat was associated with a lower risk of cutaneous disease (RR = 0.5, 95% CI = 0.3–0.9), whereas eating shaslik was associated with a higher risk (RR = 3.3, 95% CI = 1.8–6.0).

Only eight of the 42 cases with memory of the event recall having cuts on their hands or forearms at the time of exposure. However, in contrast, only one of 127 contacts answering the question recalled cuts on their hands (RR = 4.2, 95% CI = 1.9–9.0).

In the first multivariable model, taking part in either the butchering (RR = 3.1, 95% CI = 1.3–7.6) or slaughtering (RR = 2.9, 95% CI = 1.2–6.9) of an infected animal remained associated with cutaneous disease. However, when the slaughter variable was reduced to its individual components, only butcher remained statistically significant (RR = 3.6, 95% CI = 1.5–9.6). The presence of cuts was associated with a nearly significant trend toward development of disease (RR = 3.0, 95% CI = 0.9–9.6). A variance-covariance matrix for non-linear regression demonstrated that the individual activities were not highly collinear. Consumption of boiled or grilled meat did not remain significantly associated with disease.

DISCUSSION

This study examined exposures among a cohort of 308 persons identified as contacts of anthrax-infected livestock or animal products that were associated with seven outbreaks of human disease. Our analysis confirms the long-held assumption that participation in the slaughter or butchering of infected livestock is a risk factor for developing cutaneous anthrax.[6,7] The risk may be exacerbated by the presence of cuts on the hands at the time of contact with the animal or animal products.

Official worldwide estimates of human anthrax range between 2,000 and 20,000 cases annually and most cases are cutaneous.[8] Human disease is most frequent in countries where animal disease is endemic, including various Asian,

TABLE 2
Demographic and exposure variables analyzed in cohort study of human anthrax, Republic of Kazakhstan, 1997–1998*

Variable	Ill (n = 53)	Not ill (n = 255)	Relative risk	95% CI	*P*
Males	30/52 (57)	126 (49)	1.3	0.8–2.3	0.3
Median age, years (range)	36 (5–71)	31 (3–110)			0.09
Cook	17 (34)	74/250 (30)	1.2	0.7–2.1	0.6
Eat meat	38 (76)	215/253 (85)	0.6	0.3–1.2	0.16
Raw	4/46 (9)	0/250 (0)	Undefined	5.6–Undefined	<0.001
Grilled	29/46 (63)	71/249 (29)	3.3	1.8–6.0	<0.001
Boiled	21/46 (46)	168/249 (69)	0.5	0.3–0.9	0.01
Slaughter animals	31 (60)	15 (6)	8.3	4.8–14.4	<0.001
Kill	11/50 (22)	11/250 (4)	3.6	1.8–7.0	<0.001
Gut	12/50 (24)	6/251 (2)	5.0	2.6–9.5	<0.001
Skin	19/50 (24)	11/251 (4)	5.5	3.1–9.8	<0.001
Restrain	6/50 (12)	3/251 (1)	4.4	1.9–10.3	<0.001
Butcher	32 (62)	20 (8)	7.7	4.4–13.4	<0.001
Cuts on the hands	8/42 (19)	1/127 (1)	4.2	1.9–9.0	<0.001

* CI = confidence interval.

African, South American, and Caribbean countries.[8] Consistent with current worldwide surveillance, all but two of the patients we identified had cutaneous anthrax. Cutaneous lesions were usually singular and were distributed on the exposed areas of persons who came in direct contact with these animals. Although up to 20% of persons with cutaneous anthrax may progress to sepsis and death without treatment,[9,10] all patients in this study received early treatment and subsequently, none of these cases were fatal.

Agricultural anthrax outbreaks are usually cutaneous or, rarely, gastrointestinal. Previous reports have suggested that direct contact with uncooked meat has resulted in cases of cutaneous anthrax.[10,11] However, only a single case-control study of an outbreak in Paraguay related to the slaughter of an infected cow attempted a formal analysis.[12] Although the investigators demonstrated an uncontrolled association with handling raw beef, the case-control study was underpowered to evaluate specific activities related to the slaughter and distribution of infected meat. In Kazakhstan, we were able to evaluate many specific activities that are performed during the slaughter and preparation of animals killed for consumption. Although the composite variable slaughter was strongly associated with disease, the most important single risk activity was butchering an infected animal. This is likely because of the time required for the procedure and the high likelihood of skin to be cut by bone shards or a loosely handled meat cleaver. Additionally, butchering is usually performed several hours after the killing and cleaning of the animal and this may allow for the accumulation of infectious endospores from the vegetative cells that were circulating in the animal's blood.[13]

In most reports, males are disproportionately affected with anthrax, usually because of increased exposure to infected animals.[1] Attack rates for high-risk activities in Kazakhstan were similar regardless of sex. Importantly, although the preparation of food and eating well-cooked meat from infected animals were not associated with a significant risk for development of anthrax, cases of cutaneous disease did occur among persons claiming only those exposures. In contrast, gastrointestinal anthrax is usually associated with consumption of raw or undercooked meat and occurs among people who cannot afford to waste a protein source or fuel.[14–16] In Kazakhstan, malnutrition is not a common problem and consumption of raw or undercooked meat is infrequent, but each person reporting eating raw meat developed cutaneous anthrax, possibly self-inoculating while handling the food.

Although guidelines exist for the slaughter, disposal, and quarantine of domestic animals suspected to have anthrax and for those that have been exposed to anthrax, it is apparent that resources in Kazakhstan are currently insufficient for livestock vaccination, supervised herd health and meat inspection from federal veterinary services, and public food-safety education (Chorba T, unpublished data). Domestic animal health programs and routine animal vaccination in affected areas should be revitalized in Kazakhstan. Until a comprehensive animal prevention program is implemented, directed public health interventions aimed at the distal points of exposure identified in this study may help reduce the incidence of human anthrax. Additionally, because of the frequency of lesions on the hand, a simple intervention would be to wear gloves during the high-risk activities of slaughtering and butchering livestock.

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