

Anthrax in Wabessa village in the Dessie Zuria district of Ethiopia

G. Shiferaw

Department of Microbiology, Kombolcha Regional Veterinary Laboratory, P.O. Box 9, Kombolcha, Ethiopia

Submitted for publication: 26 February 2004

Accepted for publication: 7 September 2004

Summary

In 2002 an investigation of sudden death in a goat in Wabessa village in the Dessie Zuria district of Ethiopia was undertaken using fresh blood brought to the Kombolcha Regional Veterinary Laboratory. The sample was examined using standard bacteriological techniques and animal pathogenicity tests were also performed. The laboratory investigation revealed *Bacillus anthracis* as the cause of sudden death. Information gathered from stockowners in the same village revealed other similar recent cases and deaths, both in animals and humans, with farmers clearly describing the clinical signs and necropsy findings of anthrax. The disease occurs annually in this area in May and June, and in the 2002 outbreak mortality rates of 7.7%, 32.7% and 47.1% were observed in cattle, goats and donkeys, respectively. This study indicates that the community of this particular village neither knows of, nor practises, any of the conventional methods for anthrax control. The cutaneous form of the disease in humans and the environmental contamination associated with the practise of opening cadavers are briefly described and the findings are discussed with reference to the epidemiology of anthrax in both Ethiopia and elsewhere. Control strategies are also recommended.

Keywords

Anthrax – *Bacillus anthracis* – Ethiopia – Goat – Outbreak.

Introduction

Ethiopia has a large livestock population and many rural communities depend on animals for food, income and draught power. Disease is one of the major constraints preventing these large livestock resources from being fully exploited. Anthrax is an endemic disease which occurs in May and June every year ('anthrax season') in several farming localities of the country, causing disease both in humans and livestock.

Anthrax is a contagious disease of herbivores, caused by the bacterium *Bacillus anthracis* and characterised by fever and sudden death. Cattle, sheep and goats are the most susceptible species and are frequently found dead, which may lead to the diagnosis being confused with lightning strike, snakebite, or acute poisoning. The disease is less acute, although usually fatal, in camels and horses and

results in extensive swelling of the neck and ventral part of the body. Pigs and dogs are more resistant to the bacterium, displaying swelling in the throat region or intestinal signs, such as diarrhoea or constipation. Scavenger animals tend to show fewer clinical signs and many birds appear resistant to anthrax, although the disease can be a problem in captive birds, such as ranched ostriches (2).

In Ethiopia, although suspected cases of anthrax are reported from several districts, few of these are officially confirmed. This study was therefore undertaken with the objective of examining the impact of the disease in one village in the Dessie Zuria district, by isolating and characterising the causative agent, and by assessing the views and practices of farmers as regards the control and prevention of this zoonotic disease.

Materials and method

Study area

Wabessa village, where the outbreak occurred, is geographically positioned at latitude 11°08'852" north and longitude 39°68'927" east, approximately 7 km northeast of the town of Kombolcha. On the political map, the village lies within the Dessie Zuria district approximately 25 km from the town of Dessie (Fig. 1).

The village has a relatively flat landscape and lies in what is referred to as the Borkena valley/basin. Besides rain-fed cropping, irrigation using perennial rivers is also practised. High levels of soil disturbance from cultivation occur from February to July each year. Since the villagers have reasonably

good access to markets there is also trade in small ruminants.

Case

On 12 June 2002, a farmer from Wabessa Village lost a mature pregnant female goat aged six years. The goat died suddenly without showing any signs of disease. For religious reasons, the flock-keeper nicked the ear of the goat before death and then bled the animal immediately after death. He collected blood and transported it, along with one of the hindquarters, to the laboratory in Kombolcha. The stock-keeper reported that there was an absence of rigor mortis and a failure of the blood to clot, which resulted in a tentative diagnosis of anthrax. He also reported suspected human cases of anthrax among members of a family in the village (some of whom died) that had been exposed to animals that had died of anthrax.

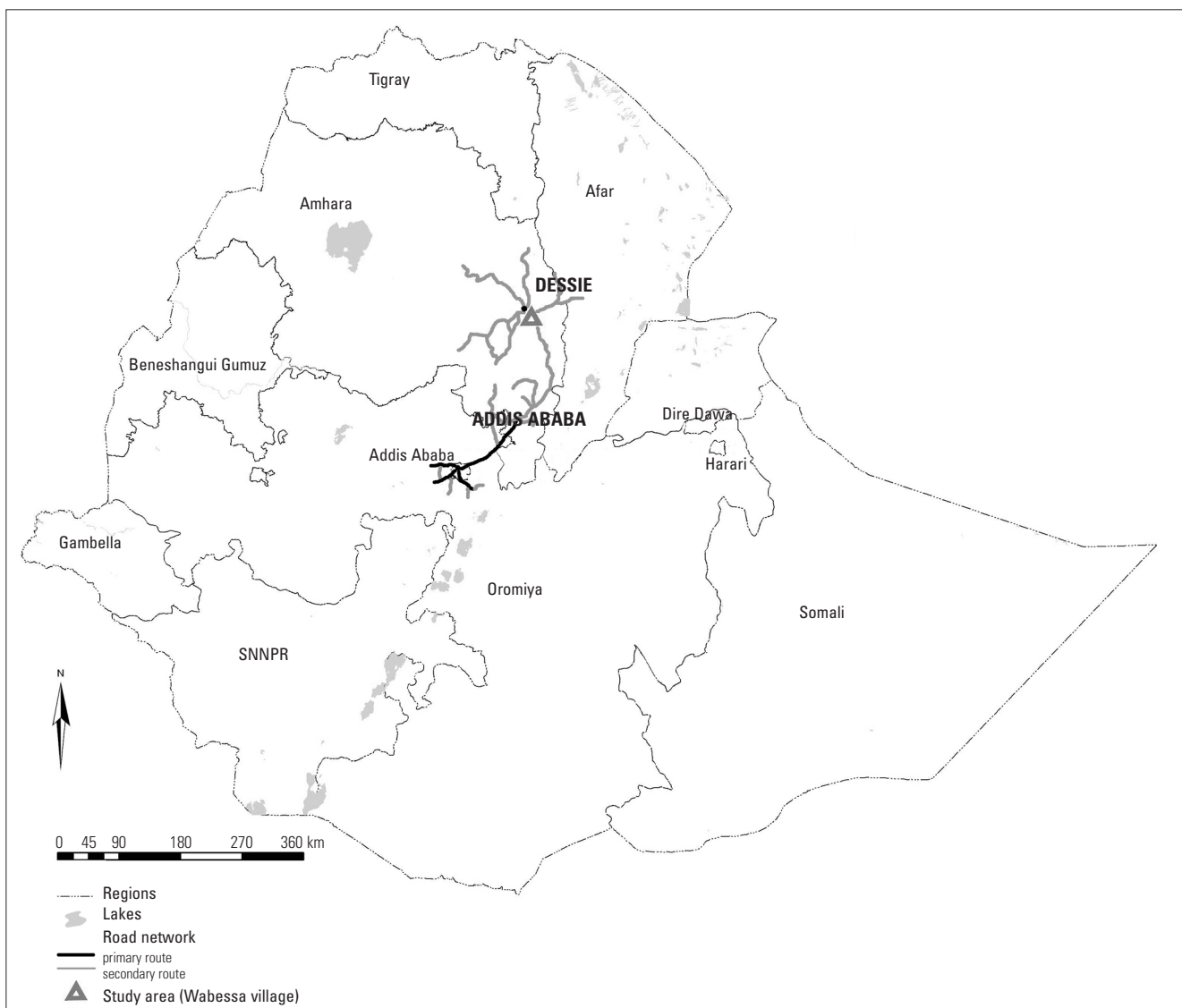


Fig. 1
Map of Ethiopia showing the study area

Physical examination

After taking a history from the owner, a sample of the blood was transferred to a sterile universal bottle and the hindquarters and remaining blood were properly disposed of. The blood sample was visually inspected and then further analysed bacteriologically.

Laboratory examination

The laboratory examination was carried out in a containment level II biohazard safety cabinet.

Primary identification

Direct microscopy

Two direct smears were made from the blood specimen, which were then air dried and fixed by passing through a Bunsen burner flame. One of the slides was stained using methylene blue and the other with a Gram stain. They were examined microscopically under oil immersion magnification.

Culture examination

The sample was inoculated onto blood agar and incubated at 37°C for 24 h. The size and morphology of the bacterial colonies and the rate of haemolysis were observed. The Gram stain reaction and cellular morphology of the blood smears were analysed.

Motility

The motility test was performed using the hanging drop method (7).

Secondary biochemical and animal pathogenicity tests

As the owner had collected the sample himself in a non-sterile bottle, further biochemical and animal pathogenicity tests were conducted to exclude other *Bacillus* spp. The tests performed were as follows

Penicillin susceptibility test

A penicillin (ten unit disc) susceptibility test was carried out using a Mueller-Hinton agar plate.

Nutrient gelatine stab inoculation

A vaccine strain was used as a positive control and uninoculated nutrient gelatine as a negative control.

Animal pathogenicity test

The animal pathogenicity test was conducted using two guinea pigs that were subcutaneously injected with 0.25 ml of broth suspension (5). The results were established on the basis of the course of the disease, the type of bacteria isolated from the guinea pigs that died, and the time that elapsed between injection and death (deaths that occur within

24 h, or after a period of four to five days, are attributed to non-specific causes, while deaths occurring within 24 h to 72 h of inoculation are considered to be caused by anthrax). Samples of unclotted blood from the guinea pigs were also tested for the presence of *Bacillus* spp.

Farmer experience

The villagers were interviewed as a group. Participatory approaches were employed to gain information on the magnitude of the problem, the villager's knowledge of the disease, and the control measures practised.

Results

Physical examination

Visual inspection of the sample revealed that the blood was unclotted, showed no colour change and appeared similar to whole blood taken using anticoagulant.

Laboratory examination

Primary identification

Direct microscopy

Direct microscopy revealed the presence of Gram-positive large rods, occurring in short chains. The methylene blue stained smears showed blue rods in short chains, with an amorphous purplish material between the bacteria (M'Fadyean reaction).

Culture examination

The bacterium grew readily on nutrient and blood agar as pure culture. On blood agar, the colonies were non-haemolytic, flat, dry and greyish. Smears made from the culture growth showed Gram-positive rods in long chains. On tryptose soy broth, the colonies resembled interwoven threads (cotton) on the bottom with no surface growth.

Motility

The bacterium was found to be non-motile.

Secondary biochemical and animal pathogenicity tests

Penicillin susceptibility test

The isolate was sensitive to penicillin with a wide zone of inhibition (30 mm) on Mueller-Hinton agar.

Nutrient gelatine stab inoculation

Gelatine liquefaction occurred belatedly (after ten days) compared to that induced by the vaccine strain (after seven days).

Animal pathogenicity test

Both guinea pigs were found dead three and four days after subcutaneous challenge with 0.25 ml of broth suspension. Local swelling in the injection area and generalised signs of illness were observed before death. *Bacillus anthracis* was recovered both from unclotted blood and oedema fluid.

Farmer experience

The major diseases mentioned during the group discussion with the farmers were foot and mouth disease, mange, anthrax and blackleg. Clinical signs and necropsy findings of anthrax were clearly described. The total number of animals in the herd where the outbreak occurred was 82 (13 cattle, 17 donkeys and 52 goats) and the farmers indicated that seven households in the village were affected. The number of infected animals was 1 cow, 8 donkeys and 17 goats and the fatality rate in all cases was 100%, giving mortality rates of 7.7% (cattle), 47.1% (donkeys) and 32.7% (goats).

The outbreak also gave rise to three unconfirmed human cases (one in a 10-year-old child and the others in adults aged 24 and 42) and three unconfirmed deaths (two children under ten years old and one 82-year-old adult). The cutaneous form of anthrax was observed in five of the patients, while three of the patients complained of abdominal pain. The human cases were related to direct contact with infected material and the consumption of infected meat. The farmers neither knew of, nor practised, any of the conventional methods for anthrax control.

Discussion

In laboratory tests *B. anthracis* was isolated from the goat blood sample, and these results, together with the clinical history, resulted in a diagnosis of anthrax as the cause of sudden death in the goat. The biochemical characteristics, antibiotic susceptibility and animal pathogenicity of the isolates were similar to those reported in the literature, although Aydin *et al.* (1) reported isolates from four cattle and one sheep that showed weak haemolytic activity and Patra *et al.* (6) detected penicillin-resistant strains and non-virulent bacilli related to *B. anthracis*.

The study in this village indicated that anthrax was a cause of illness and death among humans and livestock. A retrospective study of national district disease outbreak reports for 2001 (reporting rates were no higher than 65%) revealed 456 anthrax outbreaks for that year (Ministry of Agriculture, personal communication). This figure would probably be higher if the district-reporting rate were good.

The outbreak reported in this paper gave rise to a total of 6 human and 26 animal cases and 3 human and

26 animal deaths. The highest incidence of disease was in donkeys (n = 8, 47.1%), followed by goats (n = 17, 32.7%) and cattle (n = 1, 7.7%). Retrospective data from the national district disease outbreak reports for 2001 indicated that the highest number of cases occurred in sheep, followed by cattle, equines and goats. A 1997 report by Vaissaire *et al.* (9) recorded a higher incidence of anthrax in cattle than other domestic species in France. In Wabessa, five of the six human cases were cutaneous anthrax. This is consistent with a study by Nikolove *et al.* (4). The cutaneous form of anthrax accounts for 95% to 99% of human cases of the disease worldwide (3). The human cases are significantly associated with the animal disease (10). Humans are moderately resistant to anthrax (3). The disease is of the point source type and direct human-to-human transmission is exceedingly rare; direct animal-to-animal transmission within a species (excluding the case of carnivores feeding on meat from anthrax-infected carcasses) is likewise very rare (3). The farmer who collected the samples in Wabessa did not contract the disease even though he was exposed to the infectious agent from the goat without any protection. This could have been due to naturally acquired immunity following recent recovery from the cutaneous form of the disease.

The outbreak occurred during the hot and humid transition period between the dry season and the wet season. During this period temperatures are high both day and night and the soil temperatures are higher than usual. The soil was also significantly disturbed during this time (cultivation season), which possibly disturbed old anthrax grave sites. This may have contributed to the multiplication of the bacteria and the subsequent soil-borne infection of animals.

The community of this particular village neither knew of, nor practised, any of the conventional methods for anthrax control. The farmer affected by the disease collected the goat blood samples for laboratory confirmation at his own initiative. A common practice (constituting a serious hazard to human health and the environment) in many rural communities is to nick the ear of animals that are dying from disease or injury after invoking a religious blessing. This is done to meet religious requirements so that the carcass can be bled and the meat consumed if the animal does not recover. Although probably not a concern for human health when performed in cases of injured animals, this practice does have human health implications when carried out in cases of animals dying of a zoonotic disease such as anthrax. If the effects of anthrax outbreaks are to be minimised the animal health delivery system must be strengthened and public awareness must be improved so that farmers/villagers are able to participate in combating the disease.

Conclusions and recommendations

This study and the retrospective national district disease outbreak reports revealed that anthrax is still a serious public health and economic concern for farming communities in Ethiopia. The key to reducing environmental contamination, preventing public health issues and controlling the disease is to have appropriate handling and removal practices for dead animals, but these do not exist in Ethiopia at the present time.

The following actions are recommended as control strategies for anthrax:

- improving the animal health delivery system
- identifying anthrax areas and seasons of occurrence
- introducing targeted annual vaccination of livestock (for animals over six months old) based on the level of disease endemicity

- increasing public awareness as regards public health concerns and participation in anthrax control practices (for example, by the use of visual aids)

- implementing an early detection, early warning and early reaction system to limit the effects of an outbreak.

Acknowledgements

The author highly appreciates the technical assistance of W/zo Zewdie Abebe and other members of the Microbiology Department at the Kombolcha Regional Veterinary Laboratory. The author would also like to thank Mr Noah Kebede of the International Livestock Research Institute for providing the map and Dr Amsalu Demisse and Dr Fekadu Kebede for their encouragement.



Un foyer de fièvre charbonneuse dans le village de Wabessa, district de Dessie Zuria, en Éthiopie

G. Shiferaw

Résumé

Suite à la mort soudaine d'une chèvre dans un village du district de Dessie Zuria en 2002, un prélèvement de sang frais de l'animal a été soumis au Laboratoire vétérinaire régional de Kombolcha pour examen. Les techniques bactériologiques classiques ainsi que diverses épreuves de pouvoir pathogène pour l'animal ont été appliquées. Ces examens ont mis en évidence la responsabilité de *Bacillus anthracis* dans la mort de l'animal. Des réunions organisées avec les propriétaires de bétail ont permis de recueillir des informations sur les cas similaires, suivis ou non de mort, survenus chez des animaux et des habitants du village. Les signes cliniques et les observations *post-mortem* rapportés par les éleveurs correspondaient manifestement à la fièvre charbonneuse. La maladie survient chaque année dans cette région aux mois de mai et de juin. Les taux de mortalité observés lors de ce foyer précis étaient de 7,7 % chez les bovins, de 32,7 % chez les caprins et de 47,1 % chez les ânes. La communauté villageoise concernée ne connaît et n'applique aucune méthode conventionnelle de prophylaxie de la fièvre charbonneuse. L'étude fait état de formes cutanées de fièvre charbonneuse chez l'homme ainsi que des risques de contamination environnementale liés à l'ouverture des cadavres. L'auteur examine les résultats de l'étude à la lumière de l'épidémiologie de la fièvre charbonneuse en Éthiopie et ailleurs dans le monde, et recommande quelques stratégies de prophylaxie.

Mots-clés

Bacillus anthracis – Chèvre – Éthiopie – Fièvre charbonneuse – Foyer.



Carbunco bacteridiano en el pueblo de Wabessa, distrito de Dessie Zuria en Etiopía

G. Shiferaw

Resumen

Una investigación sobre la muerte repentina de una cabra en un pueblo del distrito de Dessie Zuria en 2002 comenzó con la llegada al Laboratorio Veterinario Regional de Kombolcha de una muestra de sangre fresca. Además de las técnicas bacteriológicas de rigor, se llevaron a cabo pruebas de patogenicidad en animales. El trabajo de laboratorio reveló que el microorganismo responsable de la súbita muerte del animal era *Bacillus anthracis*. A raíz de conversaciones mantenidas con grupos de ganaderos de la aldea se obtuvo información sobre otros casos y fallecimientos recientes de personas y animales en circunstancias parecidas. Los granjeros describieron lo que a todas luces correspondía a los síntomas clínicos y el estado *post-mortem* que provoca el carbunco bacteridiano. La enfermedad se presenta con periodicidad estacional en esta región, en los meses de mayo y junio. En el caso del brote aquí descrito se observaron índices de mortalidad en bovinos, caprinos y asnos del 7,7%, 32,7% y 47,1% respectivamente. Del estudio se desprende que los habitantes de esa aldea en concreto no conocen ni aplican ninguno de los métodos convencionales de lucha contra el carbunco. Se mencionó la forma cutánea de la enfermedad en el hombre, así como la contaminación ambiental resultante de la práctica de abrir los cadáveres. El autor, tras exponer las conclusiones del estudio y relacionarlas con la epidemiología del carbunco en Etiopía y otras partes, recomienda una serie de estrategias de control.

Palabras clave

Bacillus anthracis – Brote – Caprinos – Carbunco bacteridiano – Etiopía

References

1. Aydin F, Atabay H.I., Genc O., Atahan H. & Boluk M. (2000). – The epizootiology and epidemiology of anthrax in Kars district, assessment of anthrax cases recorded between 1995 and 2000, some characteristics of *B. anthracis* strains isolated from various sources. *Kafkas Univ. vet. Fak. Dergisi*, **6** (1/2), 55-59.
2. Hine R.S. (1988). – Anthrax. In *Concise veterinary dictionary*. Oxford University Press, Melbourne, Toronto, 40-41.
3. Logan N.A. & Turnbull P.C.B. (1999). – *Bacillus* and recently derived genera. In *Manual of clinical microbiology* (P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover & R.T. Tenover, eds), 7th Ed. American Society of Microbiology, Washington, DC, 357-369.
4. Nikolove G., Ivonova I. & Nacheva T. (1998). – Epizootic and epidemic aspects of anthrax in the region of Silistra during the period of 1982-1996. *Vet. Med. (Bulgaria)*, **4** (2), 87-90.
5. OIE (World Organisation for Animal Health) (2000). – Anthrax. Chapter 2.2.1. In *Manual of standards for diagnostic tests and vaccines*. OIE, Paris, 233-244.
6. Patra G., Vaissaire J., Weber-Levy M., le Doujet C. & Mock M. (1998). – Molecular characterization of *Bacillus* strains involved in outbreaks of anthrax in France in 1997. *J. clin. Microbiol.*, **36** (11), 3412-3414.
7. Quinn P.J., Carter M.E., Markey B. & Carter G.R. (1994). – *Bacillus* species. In *Clinical veterinary microbiology*. Mosby-Year Book, St Louis, 178-183.
8. Radostitis O.M., Gay C.C., Blood D.C. & Hinchcliff K.W. (2000). – Diseases caused by *Bacillus* species. In *Veterinary medicine*, 9th Ed. W.B. Saunders Company Ltd, Philadelphia, 747-751.
9. Vaissaire J., Mock M., Patra G., Valognes A., Grenouillat D., Pion I., Gauthier D., Ricart J., Doujet C., Weber M., Pedaille F., Patty R., Prudhomme C. & Game Y. (1997). – Outbreaks of anthrax in France in 1997 in different animals and man. Applications of new diagnostic techniques. *Bull. Acad. vét. Fr.*, **70** (4), 445-456.
10. Vaissaire J., Mock M., le Doujet C. & Levy M. (2001). – Anthrax: epidemiologic study in France. Colloque sur le contrôle épidémiologique des maladies infectieuses (CEMI). *Epidémiologie, surveillance et prévention des zoonoses. Méd. Mal. infect.*, **31** (2), 257-271.