A contagious bovine pleuropneumonia outbreak on a research farm in Ethiopia, and its dynamics over an eight-month period

This paper (No. 08122016-00088-EN) has been peer-reviewed, accepted, edited, and corrected by authors. It has not yet been formatted for printing. It will be published in December 2016 in issue 35 (3) of the *Scientific and Technical Review*

G. Almaw [(1)*], M. Duguma [(2)], A. Wubetie [(1)], G. Tuli [(1)] & T. Koran [(1)]

(1) National Animal Health Diagnostic and Investigation Center, P.O. Box 04, Sebeta, Ethiopia

(2) Wollega University, P.O. Box 395, Nekemte, Ethiopia

*Corresponding author: tiwawyedegera@yahoo.com

Summary

Contagious bovine pleuropneumonia (CBPP) was recognised on Bako Agricultural Research Farm, in the Oromia region of Ethiopia, for the first time on 5 May 2011. The outbreak was investigated by combining recognition of clinical signs, post-mortem examination, mycoplasma isolation and serological testing using competitive enzyme-linked immunosorbent assay (c-ELISA). The clinical cases were monitored for eight months; sick animals were treated with a range of antibiotics and isolated if necessary. The outbreak of CBPP was confirmed both bacteriologically and serologically and had spread to almost the entire herd (96.7%) within the eight-month observation period. Of the animals that recovered after antibiotic treatment, 12.3% fell sick again, showed typical signs of CBPP and were considered to be carriers. The role of treatment in the prevention of the spread of CBPP was minimal. Newly purchased animals that were not tested and quarantined before begin introduced onto the farm were suspected to have been the most probable source of infection.

Keywords

Contagious bovine pleuropneumonia – Ethiopia – Outbreak.

Introduction

Contagious bovine pleuropneumonia (CBPP) is an infectious disease of cattle caused by *Mycoplasma mycoides* subsp. *mycoides*. According to the World Organisation for Animal Health (OIE), ten of its Member Countries were officially recognised as being free from CBPP in 2014: Argentina, Austria, Botswana, China, Canada, Portugal, India, Singapore, Switzerland and the United States (1). In Ethiopia, according to reports of various outbreaks, national serosurveillance and research results from 1997 to 2010, CBPP was found to be present in almost all regional states (2). The status in East Wollega, where the present outbreak occurred, seemed to be unknown. However, in areas bordering the outbreak area, there were reports of confirmed cases of CBPP (2, 3). During the outbreak investigation, animal health officials were interviewed in two districts, both bordering the outbreak site, and it was learned that no vaccination was carried out and the disease was not known in the area. The first clinical case was observed on 5 May 2011. In August 2011, after repeated failure of treatment with oxytetracycline and penicillin–streptomycin, lung samples were sent by the research centre to the National Animal Health Diagnostic Investigation Centre. This outbreak investigation was carried out with the objective of identifying the primary cause, to enable control measures to be taken. The dynamics of the outbreak were monitored for an eight-month period. The aim of this paper is to report on this case study so that lessons can be learned in order to improve control of this disease.

Materials and methods

Farm description

The animal farm of Bako Agricultural Research Centre is located around 251 km west of Addis Ababa, in the West Shewa Zone of the Oromia Region, on the highway between Addis Ababa and Nekemte.

It has a longitude and latitude of 9°08′N 37°03′E, with an elevation of 1,743 m above sea level. It was established in 1970 with 59 heifers and four bulls of the Horro breed, with the purpose of improving this local breed and distributing animals to farmers (Jemberie, 2012, personal communication). The farm was partially fenced and guards supervised the unfenced area. The husbandry was semi-intensive: cattle were allowed to graze in the large area of the compound under the supervision of animal attendants. The animal feed was supplemented with a mixture of maize and nougcake (a Niger seed oil by-product). Silage was also provided for lactating cows and breeding bulls. The housing was of the loose type. Vaccination was implemented against anthrax, bovine pasteurellosis and blackleg (*Clostridium chauvoei* infection) but not against CBPP. The total population of cattle at the onset of the disease outbreak was 430, of which 36 were calves under six months of age.

Clinical observation

Animals were first observed from a distance while standing and moving. Those animals showing depression, standing apart and lagging behind the travelling group were separated and examined in detail clinically. This included assessment of rumen motility, temperature and respiratory rate, auscultation, percussion, and evaluation of posture and exercise intolerance or coughing during exercise. The whole herd was monitored for clinical signs for an eight-month period (May to December 2011). Sick animals were isolated and treated with the drugs available on the farm, and their improvement was monitored. Before the primary cause of the outbreak was confirmed to be CBPP, sick animals were treated generally for bacterial pneumonia and, because of this, penicillin–streptomycin was among the drugs used. Those cured were monitored for relapse. For the purpose of this study, a ‘relapsed case (carrier state)’ was defined as an animal that showed clinical signs for a second time after a minimum of four weeks of apparent health. This period was selected by considering the nature of the disease: under natural infection a period of up to four weeks is required for an animal to recover from the disease (4). Another assumption was that

recovered animals will be immune to new infection for at least six months, and therefore if an animal fell sick during this period it was considered to be a relapse, not new infection.

Post-mortem examination

Post-mortem examination (PME) was conducted on only two cows, at different times. The first cow was found dead without having been treated. The second PME was conducted purposely on a cow that was on the verge of death and showing typical signs of CBPP. Detailed PME was conducted following slaughter. The thoracic cavity was explored for the presence of fluid, adhesions and/or fibrinous deposits. The lungs were examined for consolidation and a typical marbling appearance.

Bacteriology

Lung samples were collected from the two cows examined post mortem. A block of tissue containing an active lesion from the interior of the lung was cut and smeared over the surface of a plate containing semi-solid mycoplasma medium with supplement (Oxoid, United Kingdom) (5). The inoculated agar was incubated at 37°C in a carbon dioxide incubator at 10% CO_2 tension. The plates were examined for growth daily under a stereoscopic microscope with transmitted light.

Serology

For each animal sampled, a history of CBPP vaccination was ruled out and about 9 ml of blood was drawn from the jugular vein using a sterile 21 gauge needle and plain vacutainer tube, and labelled with the animal identification number. The blood was left slightly inclined at room temperature for 24 h and the serum was collected into cryovials and stored at –20°C until tested. The serum samples were analysed using a competitive enzyme-linked immunosorbent assay (c-ELISA) (IDEXX, France) based on a monoclonal anti-MmmSC (anti-*Mycoplasma mycoides* subsp. *mycoides* small colony) antibody (the Mab 117/5 test). A two-round c-ELISA test was conducted with a 68-day interval to identify false-negative cases that might have been in

the early stage of infection at the first round. The test was conducted with the intention of culling all seropositives and vaccinating those animals found to be negative on double testing, in accordance with the recommendation for the control of CBPP at herd level (6). To this end, 355 serum samples were collected and tested in the first round, and 120 (100 negatives in the first round and 20 others not tested in the first round) serum samples were collected in the second round. Calves (less than six months old) were not sampled, to avoid confounding by maternal antibody.

Antibiotic treatment

A total of 235 new (excluding relapsed) cases developed signs of respiratory disease during the eight-month period, and received antibacterial treatment. One hundred and twenty-one of the cases were treated with oxytetracycline 10%, 57 with penicillin–streptomycin, 30 with tylosin 20% and 27 with oxytetracycline 20%. The treated cases were monitored for cure and relapse during the eight-month observation period. Whether treatment could minimise the spread of CBPP was assessed from the serology results, although this was not a controlled experimental study.

Data collection and analysis

During serum sample collection, data regarding the animals' sex and age were collected and their association with CBPP prevalence was tested using the Pearson chi square test. For relapsed cases, the differences between types of drug in the time interval to relapse were tested with a two-sample t-test. In addition, the association of relapse with breed, sex and treatment was tested using the Pearson correlation coefficient. The data were analysed using the Stata 8 package (StataCorp, Texas, USA).

Results

Trend in clinical cases and the role of treatment

The first case of CBPP in the outbreak was observed on 5 May 2011. The major signs noticed were dyspnoea, fever (up to 41°C),

inappetence, depression, rapid respiration, coughing, mouth breathing, bloat, decrease in milk production and protrusion of the tongue. The affected animals lagged behind the herd and looked for shade. One cow, which later was culled for PME, had a lowered and extended head, arched back, and oedematous swelling of the throat region. The total number of animals affected by CBPP from May 2011 to December 2011 was 235, giving a morbidity rate of 59.6% (235/394; excluding the 36 unweaned calves). The total number of animals which died as a result of CBPP was 19, resulting in a mortality rate of 4.8% (excluding the 36 unweaned calves). The outbreak involved all age groups and the same was true for the sex and breed of animals (Horro – the local zebu – and their crosses with Jersey and Holstein–Friesian). However, the disease was more severe in crossbreeds than in the local zebu animals.

The highest peak of cases was observed in June (Fig. 1) and the outbreak declined over the next six months. Cases were treated with oxytetracycline 10%, oxytetracycline 20% LA, penicillin–streptomycin, and tylosin 20% (Table I). Twenty-nine relapsed cases were observed during the eight-month follow-up period (Table I); the relapse rate (carrier state) was 12.3% (29/235). Animals treated with oxytetracycline 20% LA were more likely to become sick again (relapse) compared to those given other antibiotics. The average time interval between initial infection and relapse was found to be 2.44 ± 1.31 months (±SD; standard deviation). The average time interval to relapse for those treated with oxytetracycline 20% was longer (4.6 ± 0.2 months) than with oxytetracycline 10% (2.48 ± 1.35 months), although the difference was not statistically significant. However, the duration before relapse in those given penicillin–streptomycin (1.68 ± 0.28 months) was significantly shorter (p = 0.0124).

Post-mortem findings

In the two cows on which PME was conducted, consolidation of the lungs was found, with a typical marbled appearance on the cut surface. A large quantity of straw-coloured pleural fluid was observed

in the chest cavity of one cow. No fluid was seen in the second case, but there was a firm attachment of the apical lobe of the left lung to the chest wall, which may have been due to the chronicity of the case.

Bacteriological examination

The two cows that underwent PME were found to be positive for *Mycoplasma* growth; typical *Mycoplasma* colonies show raised central growth with a peripheral expansion on the agar surface. Rapid growth was observed in one sample (from the first cow that underwent PME): growth was seen after four days of culture. The second sample showed *Mycoplasma* growth after one week.

Competitive enzyme-linked immunosorbent assay

A total of 355 serum samples were tested in the first round, of which 255 (71.8%) were positive for CBPP antibody (Table II). In the second round, 120 (100 from cattle that were negative in the first round and 20 others not tested in the first round) serum samples were collected and 107 were found to be positive (89.2%) (Table II). The occurrence of CBPP was higher (79.9%) in females than in males (51.5%), and the difference was significant (Pearson chi^2 [1 degree of freedom] = 28.88; $p = 0.000$). Overall, CBPP had spread to almost the entire herd, involving 362 out of the 374 adult animals tested (96.8%).

Discussion

In this outbreak, early detection and intervention to control the spread of CBPP were essential. The disease was not known in the area previously, and the first case was tentatively diagnosed as simple pneumonia. Later, CBPP was suspected after the infection had involved many animals, loss of milk production had occurred and recovered animals fell sick again. The first clinical case was observed on 5 May 2011. By the time the screening test for CBPP was completed, the disease had infected almost the whole herd. Within 214 days (seven months and four days) of the outbreak, the disease was found to have spread to 362 out of 374 (96.8%) animals tested. In susceptible herds morbidity can reach 100% (4). Sick and apparently

healthy animals were kept in adjacent pens, but close enough that they could lick each other. A distance of at least 6 m should be maintained between diseased and healthy animals (6). The viability of the organism in the environment is poor and close contact is important for transmission to occur. In non-outbreak situations, a disease prevalence of 10.3% in Ethiopia (7) and 9.7% in Kenya (8) has been reported. In the present outbreak, treatment with oxytetracycline, penicillin–streptomycin and tylosin was given to clinical cases, but the antibiotics were ineffective in reducing the spread because the prevalence increased during the course of the outbreak. However, relapses were not seen with tylosin-treated animals; this may have been a true cure or be due to the short period of observation for treatment with tylosin, which was started in the final phase of the outbreak. However, in a controlled experimental study another antibiotic, danofloxacin, was found to reduce the spread of CBPP to healthy in-contact cattle (9). One of the issues in the use of antibiotics to treat CBPP is the possibility of creating carrier animals, which are thought to be a source of fresh infection in a herd. Among recovered animals, 25% may become carriers, with chronic lung lesions in the form of sequestra of variable size (4). In an experimental study in Mali (10), long-acting oxytetracycline treatment did not have a significant negative effect on the formation of sequestra in CBPP-infected animals. In that study, about 10 of the 13 infected but untreated animals developed sequestra; however, among the nine infected and treated animals only one animal presented a small sequestrum.

Regarding the occurrence of a carrier state in the outbreak reported here, out of 235 clinical cases, 29 (12.3%) were relapsed cases and these animals were a source of infection for the herd and the surrounding livestock because there was no complete isolation and no vaccination. In this outbreak, female cattle were significantly more likely to be affected, and a similar finding was reported in Kenya (8). The role of age as a confounding factor in this outbreak was not assessed. In the Kenyan study, when age was evaluated within sex, increasing seroprevalence was associated with increasing age for both male and female cattle.

Concerning the introduction of the disease to the farm in this study, two potential sources were suspected. The first was animals that had been purchased and mixed with the herd without quarantine. The second, but less likely, possibility was mixing of farm animals with the animals of the surrounding community, which may have introduced the disease, because the research farm is located within rural farm land.

In conclusion, CBPP affected almost the whole cattle population of Bako Agricultural Research Centre farm, where there was no history of exposure or vaccination. In countries such as Ethiopia, where culling with compensation is unlikely to occur, early detection and isolation of sick animals seem to be more important in controlling the disease than treatment, although it may be difficult to generalise from this outbreak alone. However, for this particular centre, which is engaged in distribution of heifers, it is essential to have a CBPP-free herd to avoid being a source for the dissemination of disease. Therefore, among other measures, selection of animals with high breeding value, which should be kept quarantined for three years, and culling of all remaining cattle were recommended. Re-stocking must be accompanied by testing for CBPP, and annual vaccination must be practised. The role of treatment in curing clinical cases, its role in the spread of the disease, and the use of other control options, including vaccination and movement control, need further study.

Acknowledgments

The authors would like to thank the late Dino Awol who was heavily involved in sample collection but fell sick afterwards and never returned to his job, which he loved more than anything else. They are also indebted to staff at the research centre, Huluageresh Adamu, Letbrhan Yimesgen and Belachew Durra, for the laboratory testing and all logistics.

References

1. World Organisation for Animal Health (OIE) (2014). – Resolution No. 17: Recognition of the contagious bovine pleuropneumonia status of Member Countries. *In* Final Report of the 82nd General Session, 25–30 May, Paris. OIE, Paris, 152. Available at: www.oie.int/fileadmin/Home/eng/About_us/docs/pdf/A_FR_2014_public.pdf (accessed on 11 March 2016).

2. Tuli G. (2010). – Contagious pleuropneumonia. *In* Status of major animal diseases in Ethiopia (G. Abie, T. Eguale, E. Yimer & H. Lemecha, eds). Ministry of Agriculture, Ethiopia, 28–42.

3. Lesnoff M., Diedhiou M., Laval G., Bonnet P., Workalemahu A. & Kifle D. (2002). – Demographic parameters of a domestic cattle herd in a contagious-bovine-pleuropneumonia infected area of Ethiopian Highlands. *Rev. Elev. Méd. Vét. Pays Trop.*, **55** (2), 139–147. Available at: http://remvt.cirad.fr/cd/derniers_num/2002/EMVT02_139_147.pdf (accessed on 11 March 2016).

4. Baker J.C. (1998). – Respiratory diseases of cattle. *In* The Merk Veterinary Manual (S.E. Aiello, ed.), 8th Ed. Merck and Co., Kenilworth, New Jersey, USA, 1078–1079.

5. Quinn P.J., Carter M.E., Markey B.K. & Carter G.R. (1999). – Clinical veterinary microbiology, 2nd Ed. Mosby International, London, 377–344.

6. Radostits O.M., Gay C.C., Hinchcliff K.W. & Constable P.D. (2007). – Veterinary medicine: a textbook of diseases of cattle, sheep, goats, pigs and horses, 10th Ed. Saunders-Elsevier, Edinburgh, 1133–1137.

7. Mekonnen G. (2004). – Serological, clinical and participatory epidemiological survey of contagious bovine pleuropneumonia in Somali region, Ethiopia. MSc thesis, Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia. Available at:

http://etd.aau.edu.et/bitstream/123456789/5278/1/7.%20Gedlu%20Mekonnen.pdf (accessed on 17 February 2016).

8. Mtui-Malamsha N.J. (2009). – Contagious bovine pleuropneumonia (CBPP) in the Maasai ecosystem of south-western Kenya: evaluation of seroprevalence, risk factors and vaccine safety and efficacy. PhD thesis, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh. Available at: https://core.ac.uk/download/files/39/279871.pdf (accessed on 17 February 2016).

9. Huebschle O.J., Ayling R.D., Godinho K., Lukhele O., Tjipura-Zaire G., Rowan T.G. & Nicholas R.A. (2006). – Danofloxacin (Advocin) reduces the spread of contagious bovine pleuropneumonia to healthy in-contact cattle. *Res. Vet. Sci*., **81** (3), 304–309. doi:10.1016/j.rvsc.2006.02.005.

10. Niang M., Sery A., Doucouré M., Koné M., N'Diaye M., Amanfu W. & Thiaucourt F. (2010). – Experimental studies on the effect of long-acting oxytetracycline treatment in the development of sequestra in contagious bovine pleuropneumonia-infected cattle. *J. Vet. Med. Anim. Hlth*, **2** (4), 35–45. Available at: http://agritrop.cirad.fr/558021/1/document_558021.pdf (accessed on 11 March 2016).

Table I

The association of relapse with the type of drug

Name of drug	Number of animals treated			% (relapses)
	At 1st sickness	At 2nd sickness (relapse)	At 3rd sickness (relapse)	
Oxytetracycline 10%	121	19	1[(a)]	15.7
Oxytetracycline 20%	27	7	–	25.9
Tylosin 20%	30	0	–	0
Penicillin–streptomycin	57	3	–	5.3
Total	235	29	1	12.3

a) relapsed twice (the same animal)

Table II

Prevalence of contagious bovine pleuropneumonia by sex and age

	First round test (*n* = 355)				Second round test (*n* = 120)			
	Positive	Negative	Total	%	Positive	Negative	Total	%
Female	203	51	254	79.9	65	7	72	90.3
Male	52	49	101	51.5	42	6	48	87.5
Age (years)								
0.5–2	43	30	73	58.9	36	6	42	85.7
>2–4	98	43	141	69.5	45	4	49	91.8
>4	58	26	84	69.0	26	3	29	89.7
Unknown	56	1	57	1.8	0	0	0	0

Fig. 1
Trend of cases in the 2011 contagious bovine pleuropneumonia outbreak at Bako Agriculture Research Farm