Tanzania Journal of Science 48(4): 954-961, 2022
ISSN 0856-1761, e-ISSN 2507-7961

Effects of Long Acting Oxytetracycline on Contagious Bovine Pleuropneumonia Experimentally Infected Cattle

Beatrice Otina[1*], Philip Kitala[1], Lilly Bebora[2], Moses Olum[3], Alexander Kipronoh[4], Lizzie Chesang'[5], Kristin Stuke[5] and Hezron Wesonga[4]

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P. O. Box 29053-00625, Nairobi Kenya*

[2]Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, Kenya

[3]Department of Protozoology, KALRO-Veterinary Science Research Institute, Muguga, Kenya

[4]Department of Bacteriology, KALRO-Veterinary Science Research Institute, Muguga, Kenya

[5]GALVmed (Global Alliance for Livestock Veterinary Medicines)

[*]Corresponding author, Email:beatriceotina3@gmail.com

Co-authors' emails: pkitala@uonbi.ac.ke; lilybebora@yahoo.com; mosesolum@gmail.com; kkalexdoc@gmail.com; lizzie.Chesang@galvmed.org; kristin.stuke@galvmed.org; hezronwesonga@gmail.com

Received 6 Jul 2022, Revised 30 Dec 2022, Accepted 31 Dec 2022, Published Dec 2022

DOI: https://dx.doi.org/10.4314/tjs.v48i4.20

Abstract

Contagious bovine pleuropneumonia is an important disease of cattle. Many strategies employed for its eradication and control have had shortcomings. This study was conducted to determine the effects of long acting Oxytetracycline on its course. The study involved 30 indigenous zebu cattle sourced from an area free of the disease, infected by contact transmission and randomly allocated to Oxytetracycline or saline treatment groups. Clinical observations were recorded on the two groups concurrently. Cattle were tested for the disease using complement fixation test. The mean clinical scores of the groups for each observation was compared post treatment on GENSTAT using unpaired *t*-test for single sample in groups. Full post-mortem was conducted on the cattle and samples collected for *Mmm SC* isolation. The clinical scores were worse in the control treatment group; there was no fever in the Oxytetracycline-treated group post treatment. Lesions were observed in 93% of the control and 27% of the Oxytetracycline-treated group. In this study, as in others, Oxytetracycline was shown to lower the severity of the clinical signs of the disease. This is important at slaughter houses meat inspection where decision on whether to pass or condemn the animal is based on the clinical signs and post-mortem findings.

Keywords: Contagious bovine Pleuropneumonia, Oxytetracycline, Bovine respiratory distress, Trans- boundary diseases.

Introduction

Contagious bovine pleuropneumonia (CBPP) is a transboundary disease with high potential for rapid spreading. It causes great economic losses due to mortality, loss of weight, reduced fertility, reduced milk production, and reduced performance for draught animals, restriction of trade and indirect costs of controlling the disease. Introduction of the disease to a susceptible herd leads to devastating losses in terms of morbidity and mortality. The indirect costs include costs of vaccination, loss of cattle and money in compensation at test and slaughter

(Tambi et al. 2006, Thomson 2005). Such an endemic and transboundary disease has devastating effects on the poor communities in the developing countries. It hinders pathways of alleviation of poverty through livestock by reducing productivity as treatments increase the costs of production (Perry et al. 2002). Within a country, the disease leads to restrictions of animal movements due to confinements due to outbreaks. The disease is one of the most serious trans-boundary diseases of cattle in Africa due to export restrictions imposed on infected countries by the disease-free countries like United State of America, countries of the European Union and China.

Many efforts are employed in attempts to control and eradicate CBPP. Restricting cattle movement was deployed successfully in Europe. Achieving this in the current African settings would make the control strategies more expensive than the costs of the disease itself (Chandapiwa 2011). It has also been noted that the European strains could have been less virulent than the African; Afadé strain (Abdo et al. 1998), hence the later may take more effort in resources to control. Pastoral livelihood practices including transhumance, communal grazing, congregation at marketplaces, watering points and night kraal, have increased the spread of the disease in Africa. Another strategy that has worked elsewhere is test and slaughter. This has led to loss of livestock and threatened the livelihood of affected communities (Thiaucourt et al. 2004). Also, without compensation the strategy may not be socially acceptable. The strategy applied in Africa currently is vaccination. This strategy faces a host of challenges ranging from the quality of vaccines produced, costs of repeated campaigns, handling of the vaccines, inadequate infrastructure and of lack of funding. The importance of sero-surveillance to monitor outbreaks and contain infections is recognized (Ter Laak 1992, Rurangirwa 1995).

Failure of vaccination strategy to control the spread of the CBPP has led to reconsideration of alternative strategies including use of antibiotics. Although antibiotics are not fully accepted as for treatment of CBPP (FAO 2006), their use has been widespread especially in pastoral communities (FAO 2003). A possible cure may be found from three classes of antibiotics that are effective against *mycoplasmas*: *Tetracyclines, Fluoroquinolones and Macrolides.*

Treatment of CBPP using antibiotics has been discouraged based on presumptions that lack scientific basis. Several studies have been done on use of antibiotics for the treatment of the diseases. Differences in virulence of Mmm SC strains has been documented (Abdo et al. 1998, Vilei et al. 2000) and most of the results were not conclusive (Windsor and Masiga 1976, Niang et al. 2010, Muuka et al. 2017), calling for more research on use and effects to various strains. This study, an experimental trial that enabled good follow up, used multiple diagnosis approach, facilitated early detection of cases, used a strain of high virulence, used a single dose formulation, included isolation of the treated and control groups, sought to address the concerns that use of antibiotics, in particular Oxytetracycline, has no beneficial effects on the course of the disease in CBPP infected cattle.

Materials and Methods

Infection of cattle

One hundred and forty-six male castrated cattle aged between 24 and 42 months, confirmed to be negative for CBPP antibodies, using Complement fixation test (CFT), were sourced from Kakamega, a CBPP-free zone of Kenya. They were put under quarantine within KALRO Muguga for a month,vaccinated against Lumpy Skin Disease, Foot and Mouth disease and anthrax. Of the 146 recruited cattle, 60 were randomly selected and challenged by endo-tracheal intubation with pathogenic *Mmm* SC cultures, Afadé strain donated to the International Livestock Research Institute (ILRI) in Kenya by CIRAD (French Agricultural Research Centre for International Development). When 8/60 (13.3%) of the inoculated cattle had indicative signs of CBPP (cough, depression, respiratory distress and/or pyrexia), 60 of the

remaining 86 healthy cattle were co-mingled with the inoculated animals, sharing the same airspace in a 1:1 ratio to facilitate contact transmission. All the cattle were housed continuously indoors for one week to ensure close contact. Thereafter, they were housed indoors only at night.

At the time when 12 of the 60 (20.0%) cattle in-contact with the intubates had shown elevated temperatures above 39.5 °C (an indication of infection), the group (n = 60) was randomly allocated into four treatment groups of 15 cattle each. Of the four groups, two were allocated to either Oxytetracycline or placebo (saline) treatment (Muuka et al. 2019). The treated cattle were put in different pens, where they were observed and bled in the course of the study period. Cattle that were allocated to Oxytetracycline treatment were injected with a single dose of 20% Oxytetracycline on the gluteal muscle at a dose rate of 6 mg/kg body weight. Cattle that were allocated to the placebo treatment were injected with single dose saline subcutaneously on the neck area at a dose rate of 6 mg/kg body weight. Data of the Oxytetracycline and placebo (saline) treatment groups are presented in this paper.

Animal health monitoring

General health observations were made daily and recorded. In addition, rectal temperatures were monitored and recorded daily, equally for the two groups. Individual animal clinical manifestations were observed by a veterinarian and recorded. To ensure that bias was reduced the veterinarian who administered the treatment was not involved in any other part of the study. In addition, those making observations were not aware which treatment groups the cattle belonged to. Also, those handling and processing the samples were not aware which group received what treatment. Parameters recorded in clinical observations included general body condition, appetite, respiratory distress, nasal discharge, cough, and rectal temperatures. The signs were categorized as absent (score 0), mild (score 1), moderate (score 2), or severe (score 3). All veterinarians and animals' attendants were blinded to the treatment allocations.

Sample collection and handling

For detection by complement fixation test (CFT), cattle were bled every two weeks. To do this, cattle were restrained in a crush. About 5-10 mL of blood was collected from the jugular vein into a vacutainer tube, without anticoagulant. The collected blood was left to stand in a slanting position at +4 to 8 °C in a refrigerator overnight. It was then removed, left at room temperature for about an hour and centrifuged at 1500 rpm for 10 minutes. The supernatant was separated by pipetting into 2.5 mL cryovial aliquots. The resulting serum samples were stored in cryovials, placed in marked polythene bags, at ≤-35 °C until ready for testing.

Complement fixation test

The complement fixation test (CFT) was carried out as described by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (standard chapter 2.04.08). On each dilution plate, containing 90 microliters of buffer in the wells, ten microliters of test serum were pipetted to each well. The mixture was agitated by inspiration and ejection using the microliter pipettes onto wells on a working plate containing 25 µL of buffer. Twenty-five microlitres of the mixture was pipetted to the well. To this 25 µL of antigen was added and incubated for 30 minutes at 37 °C while shaking continuous and moderately. Twenty-five microliters of haemolytic system were added and incubated for 30 minutes at 37 °C with moderate shaking. Complement was then added, and the plate left for at least 4 hours and read. Where there was no complement fixation (haemolysis) the serum was read as nonreactive and scored at 0. Where there was complement fixation (no haemolysis), the serum was recorded as reactive with a score of 1 to 4. Serum of scores 2 and above was considered positive.

Necropsy

A full necropsy was performed on study animals of the clinical trial that died in course of study, euthanized during the study or at end of clinical trial, from days 92–103 after treatment. The intubates that were still alive by the time of treatment exited the study but did not leave the research facility. Euthanasia was done by captive bolt and exsanguination. Lung tissue lesions were scored by a pathologist and were classified into acute, sub-acute, chronic and in remission. The sizes of the lesion were also recorded. Tissues were collected for culture and characterization of *Mmm* Sc.

Data collection and analysis

The data collected for this study included clinical observations (up to 31 days post treatment), serology results, post-mortem findings, culture and isolation. Clinical observations were made daily. Information for individual animals was captured separately on data capture forms against animal identification. Animal handlers took rectal temperatures of each animal daily and recorded in data capture forms. Post-mortem findings were captured on pre-formulated data capture forms and were filled concurrent to the post-mortem. The serological tests samples were laid in a rack and a layout sheet drawn. The layouts were used to capture CFT results. Laboratory readings for CFT were captured in sample layout sheets and transferred to an Excel sheet. Culture and isolation results were recorded in an isolation book according to KALRO standard operating procedures (SOPs) then entered in Excel. The serological, clinical observation and isolation data were captured as categorical data.

Data on clinical observations for the two groups were imported from the Excel file, summarised over time, and compared using a two-sample unpaired *t*-test with unequal variances in GENSTAT. In GENSTAT, comparison of pathological score and rectal temperature for individual days was also computed with the same t-test. Counts on serological results were computed directly from Excel.

Ethical approval

Institute Animal Care and Use Committee (IACUC) approval, as per animal welfare regulations, was granted to the project before commencing the study (KALRO-VSRI/IACUC010/07102016).

Results

Rectal temperatures and clinical observations

Eight to forty-nine days prior to treatment, 5/15 (33.3%) of cattle in the control group (to be inoculated with placebo) and 4/15 (26.7%) in the group to be treated with Oxytetracycline had fever of 39.5 ℃ and above. The difference in the mean temperatures in the two groups was not statistically different ($p = 0.54$). Six of the 15 (40.0%) cattle in the control group had rectal temperature of ≥ 39.5 ℃ between days 1 to 31 days post treatment. Only 1 of these 6 cattle had fever prior to treatment. It had rectal temperature of 40.5 ℃ on one day pre-treatment. On the other hand, none of the cattle treated with Oxytetracycline developed fever post treatment. The rectal temperatures mean between the two groups post treatment were different (< 0.05) between days 7 and 15 post treatment for the days shown below (Table 1).

Table1: Summary of mean rectal temperatures post-treatment

Days post treatment	Control group (mean °C)	SEM	Oxytetracycline group (mean °C)	SEM	*t*-statistics	*p* –value
7	37.8	0.16	37.1	0.24	2.26	0.032
8	38.4	0.22	37.6	0.09	3.02	0.007
9	38.3	0.25	37.4	0.13	3.09	0.005
12	38.1	0.21	37.3	0.09	3.79	0.001
14	37.9	0.29	37.0	0.11	2.85	0.011
15	38.0	0.25	36.9	0.11	3.86	0.001

The means are of the rectal temperatures in degree Celsius (°C) taken in the period post treatment. The control group received the saline treatment. The treatment group received Oxytetracycline treatment.

After contact challenge with mycoplasma-intubated cattle, and before treatment, cattle that were to be treated with Oxytetracycline developed signs that were consistent with CBPP infections including depression (11 out of 15, 73.3%), coughing (13 out of 15, 86.7%) and respiratory distress (7 out of 15, 46.7%). During the same period, cattle that were to be assigned as control developed similar signs including depression (10 out of 15, 66.7%), coughing (12 out of 15, 8.0%) and respiratory distress (10 out of 15, 66.7%).

After treatment of the cattle in the two groups, the clinical signs were monitored for 31 days post treatment as displayed in Table 2. Of the 7 clinical signs considered all were more severe in the control group than in the Oxytetracycline treated group, only appetite, nasal discharge and diarrhoea were not statistically significant (Table 2). The greatest difference between the two groups was on respiratory distress with the score much higher in the control group.

Table 2: Summary of statistical analysis of post treatment mean clinical observations

Parameter	Control mean	SEM	Treatment mean	SEM	*t*-statistic	*p* -value
Appetite	0.263	0.051	0.091	0.075	1.89	0.069
Cough	0.551	0.124	0.108	0.085	2.95	0.006
Depression	0.341	0.040	0.133	0.083	2.25	0.036
Diarrhoea	0.054	0.036	0.002	0.002	1.42	0.177
Nasal discharge	0.635	0.064	0.516	0.059	1.36	0.184
Respiratory distress	0.640	0.065	0.182	0.091	4.1	<0.001
General body condition	1.341	0.149	0.910	0.118	2.28	0.031

The control group received the saline treatment, while the treatment group received Oxytetracycline treatment post challenge. Challenge was by contact transmission from intubated cattle. The data were summarized for each animal as an average score over a 31-day period post treatment.

Serological detection of contagious bovine pleuropneumonia

The CFT titres ranged from 1/20 to 1/640. In the Oxytetracycline treated group, 6/15 (40.0%) cattle tested positive on CFT, after treatment with titres ranging from 1/20 to 1/80. In the control treatment group, 13/15 cattle tested positive on CFT, after treatment with titres ranging from 1/20 to 1/640.

Necropsy findings

The CBBP infected cattle in both experimental groups (6 from control group and 1 from the Oxytetracycline group) were euthanized during the study. Those that survived were euthanized at end of the study. Post-mortem changes were documented in all. Some 14/15 (93.3%) of the cattle in the control group had CBPP lesions. By

comparison, only 1/15 (6.67%) of cattle in the Oxytetracycline group had CBPP lesions in the lungs. The main lesions observed were hepatinisation, fibrous tags, adhesions and sequestration. The sequesters ranged in size from 5 cm to more than 20 cm. One sample from the Oxytetracycline group yielded mycoplasma. Eight samples from 5 cattle in the control group yielded the organisms.

Discussion

This study has shown that Oxytetracycline treatment of CBPP infected cattle modifies the effects of the disease by reducing the severity of the disease as determined by the clinical signs and post-mortem changes. There were statistically significant differences between the treated group and the control group on most of the clinical signs and post-mortem changes, e.g. respiratory distress, loss of appetite, depression, and loss of body condition. All these signs were more severe in the control group than in the treatment group. Similarly, post-mortem changes in the lungs (sequestration, lung adhesions and, hepatinisation) were more severe in the control group relative to the treatment group. The results of this study are consistent with those of other researchers who reported that Oxytetracycline treatment can indeed improve the course of CBBP (Windsor and Masinga 1976, Niang et al. 2010, Muindi 2014). In the Zambian study on antimicrobials by Muuka et al. (2019) where the Caprivi strain was used, similar results were reported where Oxytetracycline treatment was shown to give 77.5% protection against CBPP infections.

The use of antibiotics in treatment of CBPP infected cattle may have some practical implications especially in pastoral areas where CBPP occurs in an endemic status. At slaughter CBPP infected cows if severely emaciated, the whole carcass is condemned (Meat Control Act of Kenya cap 356). However, if they are not severely emaciated, it is only the lungs that are condemned and the carcass may pass meat inspection and then be graded. If CBPP infected cattle are treated with Oxytetracycline when sick from the disease, pastoralists would be able to sell cattle that would otherwise be condemned in total at slaughter houses meat inspection due poor quality of the carcasses. The benefits of using Oxytetracycline regarding carcass values are demonstrated in several studies. There was a significant difference in the post-mortem lesion with 76.9% and 11.1% of the untreated and treated presenting a sequestrum according to a study (Niang et al. 2010). In a study by Muuka et al. (2017) at post-mortem 16.6% of lesions were from an Oxytetracycline treated group, while 83.4% were from the untreated group. Fibrotic lesions were equally observed in both groups (45.8% and 52.2%, respectively) and this was considered a sign of healing. These findings support the current study in demonstrating benefits of using Oxytetracycline in treating CBPP as contributing to reduced lesions and hence improved carcass score at meat inspection. The twin study in Zambia that was done with this study also supported the benefit of treatment in post-mortem changes and hence carcass value (Muuka et al. 2019). In this study, only 1 sample from the Oxytetracycline group yielded *Mmm* SC compared to 8 from the control group. These results are supported by other similar studies that showed that *Mmm* SC organism are not transmitted to susceptible animals exposed to experimentally infected cattle and treated with long acting Oxytetracycline (Windsor and Masiga 1976, Niang et al. 2010, Muuka et al. 2019). However, antibiotics should be used with caution and not indiscriminately or incorrectly by employing the services of veterinarians. The correct dosages, routes and withdrawal periods should be strictly adhered to.

The results from this study and indeed from other similar studies should, however be interpreted with caution. This study was conducted under confined laboratory conditions and not in the animals' real-life environment. There are difficulties in interpreting results from such studies (Dohoo et al. 2010). There is therefore a need to conduct large scale studies in the form of clinical trials that are conducted in the

animals' natural environment. These studies would serve to confirm or otherwise the results of the laboratory studies.

Conclusion

The results of this study showed that 20% Oxytetracycline treatment of CBPP infected cattle lowers the severity of the clinical signs as well as reduces pathological lesions associated with the disease. The improvement of the body condition of the infected cattle by the Oxytetracycline treatment has practical implication saving cattle from condemnation at slaughter house meat inspection. However, the results of this study need to be confirmed by large scale clinical trials.

Competing interests

The authors declare that they have no competing interests.

Funding and acknowledgements

The study is based on research funded in part by the Bill & Melinda Gates Foundation and aid from the UK Government through GALVmed (grant number OPP1009497). GALVmed facilitated review support during the write-up of the manuscript. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation or the UK Government. The funders (Bill & Melinda Gates Foundation and the UK Government) had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We would like to appreciate Jane Poole for statistical support while writing the manuscript.

References

Abdo EM, Nicolet J, Miserez R, Gonçalves R, Regalla J, Griot C, Bensaide A, Krampe M and Frey J 1998 Humoral and bronchial immune responses in cattle experimentally infected with *Mycoplasma mycoides* subsp. *mycoides* small colony type. *Vet. Microbiol.* 59(2-3): 109-122.

Chandapiwa M 2011 Contagious bovine pleuropneumonia in Botswana experience with control, eradication, prevention and surveillance. *Vet. Ital.* 47(4): 397-405.

Dohoo I, Wayne M and Stryhn H 2010 Veterinary Epidemiologic Research, 2nd edn. VER Inc., Charlettetown, PEI.

FAO 2003 Consultative group on contagious bovine pleuropneumonia, third meeting, Rome 12 to 14, November 2003: https://www.fao.org/3/y5510e/y5510e.pdf

FAO 2006 Consultative Group Meeting on CBPP in Africa Rome, 6-8 November 2006. https://www.fao.org/3/ah672e/ah672e.pdf.

Muindi PW 2014 *A qualitative assessment of the gendered effects of contagious bovine pleuroneumonia (CBPP) outbreaks and control among the Somali Pastoralists of Ijara Sub-County, Garissa County, Northeastern Kenya*. Master Thesis, University of Nairobi.

Muuka GM, Songolo A, Kabilika S, Sikwese H, Bowa B and Kabunda O 2017 Observations of oxytetracycline treatment effects in a contagious bovine pleuropneumonia naturally infected herd in Zambia. *J. Vet. Sci. Anim. Health* 9(6): 110-115.

Muuka G, Otina B, Wesonga H, Bowa B, Gicheru N, Stuke K, Jane Poole E, Salt J and Colston A 2019 Evaluation of new generation macrolides for the treatment and metaphylaxis of contagious pleuropneumonia (CBPP) in cattle experimentally infected with Mycoplasma mycoides subspecies mycoides. *BMC Vet. Res.* 15: 451.

Niang M, Sery A, Doucouré M, Koné M, N'Diaye M, Amanfu W and 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. Health* 2(4): 35-45.

Perry BD, Randolph TF, McDermott JJ, Sones KR and Thornton PK 2002 Investing in animal health research to alleviate poverty *Nairobi: ILRI*. https://hdl.handle.net/10568/2308.

Rurangirwa FR 1995 Uses of serology for the diagnosis of contagious bovine

pleuropneumonia. *Revue scientifique et technique* International Office of Epizootics 14(3): 603-609.

Tambi NE, Maina WO and Ndi C 2006 An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. *Rev. Sci. Techn. Office Int. Epizooties* 25(3): 99-110.

Ter Laak EA 1992 Contagious bovine pleuropneumonia a review. *Veterinary Quarterly* 15: 104-110.

Thiaucourt F, Van der Lugt J and Provost A 2004 Contagious bovine pleuropneumonia. In: Coetzer JAW and Tustin RC (Eds) *Infectious Diseases of Livestock* 3(2), Cape Town: Oxford University Press.

Thomson GR 2005 Contagious bovine pleuropneumonia and poverty: a strategy for addressing the effects of the disease in sub-Saharan Africa. Research report. Department for International Development (DFID) Animal Health Programme Centre for Tropical Veterinary Medicine University of Edinburgh, United Kingdom.

Vilei EM, Abdo EM, Nicolet J, Botelho A, Gonçalves R, and Frey J 2000 Genomic and antigenic differences between the European and African/Australian clusters of *Mycoplasma mycoides subsp. mycoides* SC. *Microbiology (Reading, England* 146 (2): 477–486.

Windsor RS and Masiga WN 1976 The effect of post vaccinal treatment with the antibiotic Tylosin on immunity produced by T strain of *Mycoplasma mycoides sub species mycoides*. *J. Comp. Pathol.* 86(2): 173-181.

Windsor RS and Masiga WN 1977 Investigations into the role of carrier animals in the spread of contagious bovine pleuropneumonia. *Res. Vet. Sci.* 23(2): 224–229.