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Evaluation of novel diagnostic techniques for contagious bovine pleuropneumonia (CBPP) in Uganda

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Abstract

Field surveys were conducted in Mbarara, Bugiri and Kamuli districts suspected to have CBPP in order to get blood samples and nasal swabs from infected and non-infected cattle. Cattle were examined clinically for CBPP. Blood samples were taken from the animals for Complement Fixation Test (CFT) and competitive ELISA (cELISA). Nasal swabs or tracheal scrapings from the same suspected cattle were mixed with PBS buffer for extraction of *Mycoplasma mycoides* DNA. A total of 954 blood samples were collected for preparation of serum samples for CFT and cELISA, and 174 nasal swabs for DNA preparation for subsequent use in PCR. A total of 621 out of 907 serum samples from Mbarara district have been analysed by CFT and cELISA (Table1). Eleven of 907 serum samples were positive by CFT while 16 were positive by cELISA. The two tests had a concordance of 97.2%, which indicates good agreement in the results of the two tests evaluated.

Introduction

Contagious Bovine Pleuropneumonia is an infectious bacterial disease of cattle. It is caused by *Mycoplasma mycoides* subspecies *mycoides* (small colony type).
The disease is characterised by chronic pleuritis and pneumonia.
The disease had been checked in some countries of Africa through annual vaccination campaigns. Howerever, there is a resurgence of the disease in Uganda and has spread from its traditional focus of Karamoja and parts of northern Uganda to western, central and southern districts. It is now endemic in most districts of Uganda (Twinamasiko *et al* 1996; Annon, 1996). CBPP is one of the most important threats to cattle production in Uganda because infected animals are incurable and meat from such cattle cannot be exported to Mycoplasma-free countries. This disease has a high mortality rate resulting in significant economic losses to the farmers. CBPP is spread by inhalation of infective droplets from active or carrier cases of the disease. The focus of infection is provided by recovered carrier animals which remain potential sources of infective organisms for periods as long as 3 years.This study aims at evaluating and adapting new diagnostic techniques for CBPP and improvement of diagnostic skills of extension staff through practical training.

Materials and methods

Field surveys were conducted in Mbarara, Bugiri and Kamuli districts suspected to have CBPP in order to get blood samples and nasal swabs from infected and non-infected cattle. Cattle were examined clinically and blood samples were taken from the animals for Complement Fixation Test (CFT) and competitive ELISA (cELISA). Nasal swabs or tracheal scrapings from the same suspected cattle were mixed with PBS buffer for extraction of *Mycoplasma mycoides* DNA. A total of 954 blood samples were collected for preparation of serum samples for Complement fixation

Test (CFT) and competition ELISA (cELISA) and 174 nasal swabs for DNA preparation for subsequent use in PCR.

Complement fixation test (CFT)
Complement fixation test was carried out as described by the manufacturer of CFT CBPP Kit (CIRAD-EMVT, Sante animal, montpellier, France).

Competitive enzyme linked immunosorbent assay (cELISA)
Competition Enzyme Linked Immunosorbent Assay (cELISA) was performed as described by the manufacturer CIRAD-EMVT, Sante animal, montpellier, France.

Results and discussion

A total of 621 out of 907 serum samples have been analysed by complement fixation test (CFT) and competitive ELISA (cELISA)(Table1). Eleven of 907 serum samples were positive by CFT while 16 were positive by cELISA. The seroprevalences of CBPP as stimated by CFT and cELISA are 1.2% and 1.7% respectively. The low number of CBPP positive cattle may indicate low prevalence of the disease or cattle in the incubative and early stages may have given negative reactions with the currently used serological tests. However, the two tests (CFT and cELISA) had a concordance of 97.2%, which indicates good agreement in the results of the two tests evaluated. Because of the insiduous nature of CBPP, it may go undetected for months before cattle develop noticeable lesions, hence there is a need for a more sensitive test such as PCR-ELISA, which detects current infections. The newly developed PCR-ELISA is being evaluated for effective diagnosis of CBPP. The test is claimed to be highly sensitive and specific and would therefore help to identify carrier animals.

Expected outputs

- Validation of PCR test that amplify the DNA of selected pathogenic *Mycoplsama mycoides* of cattle shall be applied to assess CBPP prevalence and assist policy makers in focusing and implementing appropriate disease control strategies.
- Detection of CBPP foci of infection by diagnosing infected cattle that test negative by ELISA and CFT due to low sensitivities of these two tests, will help in disease eradication.
- Rural poor farmers would benefit from this since infected animals would be slaughtered to reduce the spread of the disease.
- Improved monitoring of control programme with a more sensitive diagnostic test will result in a large number of cattle identified as infected and slaughtered; and animals will be non infected correctly identified.

Table 1: Results of analysis of serum samples by CFT and cELISA tests

Location	Tests				Total
	CFT.		cELISA		
	+	-	+	-	
Mbarara	11 (139,219 270,547 611,636 654,658 709,713 715)	**896**	16 (327,542 543,547 549,554 560,597 599,600 604,614 625,627 640,733)	**891**	907

Table 2. Comparative analysis of CFT and cELISA tests in the diagnosis of CBPP

	cELISA		Total
CFT	+	-	
+	1	10	11
	15	881	896
Total	16	891	907

The concordance of the two tests = $\frac{(1+881) \times 100}{907}$ = 97.2%

Expected benefits

Laboratory: A more sensitive diagnostic test for CBPP is being evaluated for use in the lab.for screening samples. Both scientists and technicians will be trained in the use of these techniques.

Farmers: Infected animals will be slaughtered and thus reduce spread and mortality, resulting in an increase in farmers' income.

Country: The use of a more sensitive diagnostic techniques will improve monitoring of control and eradication programmes as infected cattle will be identified and slaughtered. Mycoplasma-free Uganda will enable export of meat from cattle to other countries, hence earn income.

Recommendation

It is recommended that periodic screening of cattle using CFT and ELISA before being transported to other areas be re-instituted and where there is a positive case in a herd, PCR technique be used to detect animals in incubative and early stages or carrier cases.

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