



## Molecular Detection of Hemoplasma Species (*Mycoplasma* spp.) in Cattle in Cebu, Philippines

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### ABSTRACT

Epidemiological reports on vector-borne diseases affecting cattle in the Philippines have been limited. The present study aimed to detect the presence of the Hemoplasma pathogen (*Mycoplasma* spp.) in dispersal cattle in selected plainlands and marginal uplands in Cebu, Philippines, using peripheral blood smear examination (PBSE) and polymerase chain reaction (PCR) methods. A total of 14 cattle were tested. Using PBSE, no inclusion bodies were observed. However, using PCR, 4 out of 14 cattle showed positive results. Results indicate the high sensitivity of the PCR methods in detecting *Mycoplasma* spp. than that of the PBSE. The present study adds new information on the biodiversity of vector-borne pathogens in cattle in Cebu, Philippines, and is the first report of detection in the country.

Vector-borne diseases continue to threaten the health and productivity of livestock animals worldwide. In the Philippines, *Anaplasma*, *Babesia* and *Trypanosoma* species (Ybañez *et al.*, 2013b; Ybañez *et al.*, 2013c) have been reported in cattle, but there has been no official report yet on the presence of hemoplasmas or hemotropic mycoplasmas (*Mycoplasma* spp.), which are believed to be tick-borne pathogens (Messick, 2004) with wide geographic distribution (Scott and Woldehiwet, 1993). These are bacteria that can cause infectious anemia in various mammalian species (Smith *et al.*, 1990; Messick, 2004; Peters *et al.*, 2008). They were previously known as Haemobartonella and Eperythro-

zoon species, but were reclassified to the genus *Mycoplasma* on the basis of their 16S rRNA gene sequences and morphologic similarities (Neimark *et al.*, 2001). In cattle, 2 distinct species have been identified, namely, *Mycoplasma wenyonii* (Mw: formerly, *Eperythrozoon wenyonii*) (Nishizawa *et al.*, 2010), and a provisional species “Candidatus *Mycoplasma haemobos*” (CMh: synonym, “Candidatus *M. haemobovis*”) (Tagawa *et al.*, 2008). The present study aimed to detect the presence of the Hemoplasma pathogen in dispersal cattle in selected plainlands and marginal uplands in Cebu, Philippines, using peripheral blood smear examination (PBSE) and polymerase chain reaction (PCR) methods.

DNA samples obtained from 14 cattle from selected plainland and marginal uplands in 2012 in

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Cebu, Philippines were used. These included samples which were used in a previous study (Ybañez *et al.*, 2013c). The cattle were part of the dispersal program of the National Dairy Authority of the Philippines to the remote and/or mountain barangays. DNA was sourced from the blood of each cattle collected from the jugular vein using 5 ml sterile syringes and EDTA-coated vacutainer tubes. At the time of blood collection, cattle sources showed varying degrees of emaciation and tick infestation. DNA was extracted using QIAmp DNA blood Mini kit (Qiagen, Hilden, Germany), eluted with 200 µl of the buffer provided by the kit, and stored at -30°C until further use.

PCR amplification was performed using a primer pair F<sub>2</sub> (5'-ACGAAAGTCTGATGGAGCAATA-3') and R<sub>2</sub> (5-ACGCCCAATAAATCCGRATAAT-3) (Jensen *et al.*, 2001) in a 25 µl reaction mixture containing 5 µl of each DNA template. The used primers can amplify the 16S rRNA genes of most Hemoplasma species, including *M. haemofelis*, *M. haemocanis*, 'Candidatus *M. haemominutum*', 'Candidatus *M. haemopurvum*', *M. wenyonii* and 'Candidatus *M. haemobos*' (Jensen *et al.*, 2001; Tagawa *et al.*, 2008). The negative and positive controls used were double distilled water (DDW) and DNA prepared from the blood of a *Mycoplasma* spp.-infected cattle from Japan (Tagawa *et al.*, 2012). The amplification products were visualized under UV light using 1.5 % agarose gel in Tris-borate-EDTA (TBE) buffer after migration for 30 minutes and

staining with ethidium bromide. Upon visualization, amplicons with longer fragments (approximately 190 bp) and shorter fragments (approximately 170 bp) indicate *M. wenyonii* and 'Candidatus *M. haemobos*', respectively (Tagawa *et al.*, 2008).

Out of 14 cattle, 4 showed positive results using the PCR method for *M. wenyonii*. Figure 1 shows the gel electrophoresis result of an infected cattle. However, using PBSE, none of the samples revealed stained bodies, suggesting Hemoplasma infection (Table 1). Although the diagnosis of Hemoplasma infection has been traditionally performed by microscopic examination of the bacterial pathogen on the surface of the erythrocyte or in the plasma, the sensitivity and specificity of this method are low (McAuliffe *et al.*, 2006; Tagawa *et al.*, 2012). Moreover, PBSE requires proficiency in identifying bacterial pathogens and is prone to errors because artifacts can be construed as inclusion bodies or pathogens (Ybañez *et al.*, 2013a). The results of the study corroborated with previous studies showing the high sensitivity of the PCR methods in detecting the pathogen compared to that of the PBSE (McAuliffe *et al.*, 2006; Tagawa *et al.*, 2012). Recent studies have also favored the use of PCR as an aid in diagnosing bovine hemoplasmosis (Messick, 2004; Tagawa *et al.*, 2008; Tagawa *et al.*, 2010; Giroto *et al.*, 2012; Tagawa *et al.*, 2012).

It was noted that all the dispersal cattle had ticks and were pastured. Pastured cattle are known to have higher risk of exposure to blood-sucking

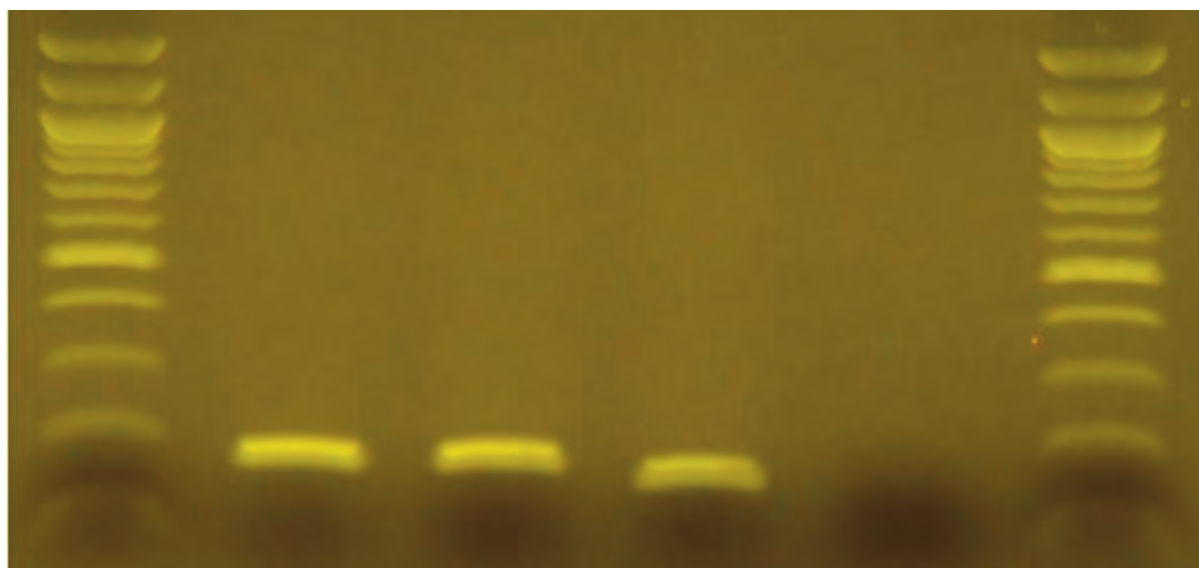


Fig. 1. Gel electrophoresis result of an amplified PCR product from a Hemoplasma-infected cattle from Cebu, Philippines. L-100 bp ladder, Lane 1 – Infected cattle, Lane 2- Positive control (*Mycoplasma wenyonii*), Lane 3 –Positive control ('Candidatus *M. haemobos*'), Lane 4 – Negative control (Distilled water).

Table 1. Results of Hemoplasma Testing using Polymerase Chain Reaction (PCR) and peripheral blood smear examination (PBSE) in Cattle in Cebu, Philippines.

Cattle	Location		Hemoplasma Detection	
	Area	Classification	PCR	PBSE
1	Tayud, Consolacion, Cebu	Plainland	-	-
2	Tayud, Consolacion, Cebu	Plainland	-	-
3	Binaliw, Danao, Cebu	Marginal Upland	-	-
4	Tabok, Danao, Cebu	Marginal Upland	-	-
5	Guba, Cebu City	Marginal Upland	-	-
6	Guba, Cebu City	Marginal Upland	-	-
7	Guba, Cebu City	Marginal Upland	-	-
8	Lusaran, Cebu City	Marginal Upland	-	-
9	Lusaran, Cebu City	Marginal Upland	-	-
10	Lusaran, Cebu City	Marginal Upland	-	-
11	Lusaran, Cebu City	Marginal Upland	-	-
12	Lusaran, Cebu City	Marginal Upland	-	-
13	Lusaran, Cebu City	Marginal Upland	-	-
14	Lusaran, Cebu City	Marginal Upland	-	-

arthropods that are capable of transmitting the Hemoplasma pathogen (Messick, 2004). Because Hemoplasma infection can be chronic and/or subclinical, or cause severe anemia, transient fever, lymphadenopathy, anorexia, weight loss and decreased milk production (Smith *et al.*, 1990; Messick, 2004; Genova *et al.*, 2011), its presence in the cattle population of Cebu can have an effect on their health and productivity. A study by Tagawa *et al.* (2013) also implied that calf birth weight may also be lower if infected with the pathogen. All these can impact the local farmer beneficiaries of the dispersal cattle program in the plainland and marginal uplands, who are usually underprivileged and at the low income bracket of the community.

Although complete blood count (CBC) was not performed in the study, the PCR detection of the pathogen was deemed enough to diagnose Hemoplasma infection. In a related study, a difference on the productivity performance between infected and non-infected cattle were seen, although no significant differences were observed on their blood values (Tagawa *et al.*, 2010). Therefore, relying on CBC alone can be unreliable in diagnosing bovine hemoplasmosis. On the other hand, the direct causal relationship of the emaciation observed on the infected cattle could not be clearly established as this clinical sign is non-specific, and may be

caused by other pathogens as well. Unfortunately, testing for other pathogens like bovine leukemia virus, *Trypanosoma* spp., *Theileria* spp. and *Babesia* spp. were not performed. These may also contribute to the emaciated condition of the dispersal cattle.

The present study provides new information on the biodiversity of vector-borne pathogens in cattle in Cebu, Philippines, and prompts for further studies to determine the prevalence of the pathogen and assess the impact it has on the local livestock industry. It is also the first report of detection in the country. Nonetheless, tick control must be made a part of the local animal health program as the pathogen is most likely to be transmitted via ticks. With the increasing recognition for the need of more advanced methods to detect diseases in the livestock industry, the Department of Agriculture, academe or local government health units should work hand in hand to set-up a diagnostic center that is accessible locally, and is capable of performing the more reliable molecular diagnostics compared to traditional methods.

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