COMPARATIVE SENSITIVITY BETWEEN PCR AND AGPT METHODS IN DIAGNOSING INFECTIOUS BRONCHITIS CASES

SIR, Infectious bronchitis virus (IBV) is an important virus causing upper respiratory tract disease in commercial chickens around the world. Other than causing respiratory syndrome, IBV has also been known to cause reproductive disease and nephrosis-nephritis syndrome.

Prior to the development of polymerase chain reaction (PCR) technique, conventional methods have been used routinely to diagnose IB cases. The latter technique includes virus isolation in the embryonated eggs, and agar gel precipitation test (AGPT). The drawbacks of the conventional method are that it is consuming and laborious. With the advent in nucleotide diagnosis, diagnosing of IB cases is rapid and specific.

A study was conducted to determine the sensitivity of reverse transcriptase PCR (RT-PCR) in diagnosing IB cases from post-mortem (PM) samples; and to compare this method with a conventional AGPT. For the PCR method, a published universal oligonucleotide was used that would generate complimentary deoxyribonucleic (cDNA) with all IBV isolates including vaccine strain. Samples negative with PCR are subjected to nested PCR to further amplify the first PCR product and thus increase the sensitivity of detection. Oligonucleotide primers used were UTR1 and UTR2+ while for nested PCR, UTR3- and UTR4+ (Williams *et al.* 1993) were used. These set primers are derived from the untranslated region of the genome, which is the conserve region of the avian coronavirus.

Seven cases of chicken suspected of IB were necropsied and their trachea, lung and kidney were harvested. Their organs were pooled, processed and the virus suspension was inoculated into embryonated chicken egg according to standard procedure. After 72 hours, the choriollantoic membrane (CAM) and the allantoic fluid were harvested. The CAM were minced and subjected to AGPT using IBV polyclonal antibodies whilst the allantoic fluid were subjected to RNA extraction for RT-PCR (Adzhar *et al.*, 1996).

Out of seven cases of IB, only one case tested positive with AGPT, despite passaging the negative samples three times. Sample which was AGPT positive was also positive for PCR. However, out of 6 cases negative with PCR, 5 cases were positive when nested PCR was performed and yield expected product of 174 bp (Table 1). Nested PCR has proven to be a sensitive, specific and rapid test. The sensitivity and rapidity nature of this technique has enable it to be used as a tool for surveillance monitoring and characterising IBV isolates into vaccine strains, nephropathogenic (Aziz *et al.*, 1996) or a new IB variant.

Table 1. Summary on IBV detection in 7 samples suspected IB cases using AGPT, RT-PCR and Nested PCR methods

Sample no.	AGPT	RT-PCR	Nested PCR
1	-	-	+
2	-	-	+
3	-	-	+
4	-	-	-
5	-	-	+
6	-	-	+
7	+	+	Not done

Legend; +, positive; -, negative

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