Cloacal swabs: an alternative to PCR monitoring and sequencing of infectious bursal disease vaccination

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INTRODUCTION

Infectious bursal disease (Gumboro disease) is one of the most serious diseases in poultry farming, because of the economic consequences and the high levels of mortality that arise with this disease.
The Avibirnavirus RNA of infectious bursal disease is usually identified by PCR in the bursa of Fabricius. To avoid sacrificing animals and to make transporting samples easier, cloacal swabs are under consideration as an alternative for PCR screening for infectious bursal disease virus. The studies presented here were carried out following vaccination with the 228E strain. As a preliminary step, the presence of viral RNA in the cloaca was confirmed, and the ideal time to take a sample was determined. Following this, the appropriateness of the analysis was validated in 10 groups of broilers.

MATERIALS AND METHODS

Samples

For the preliminary step of the study, cloacal swabs and samples of the bursa of Fabricius were collected from 10 birds in a batch of 25,000 broilers which had been vaccinated with the 228 E strain via the drinking water. These samples were taken every two days following vaccination just until slaughter. For the second stage, cloacal swabs were collected from 10 chickens, 10 to 16 days post-vaccination, from 10 batches of 25,000 broilers that had been vaccinated with the 228E strain via the drinking water.

Sample method for the cloacal swabs

For each bird, a dry swab was rubbed against the cloacal mucosa, and then deep-frozen until required.

PCR method

Viral RNA was extracted from the bursa of Fabricius and cloacal swabs of vaccinated birds. Briefly, 200 mg of bursa was crushed in 1 ml of sterile physiological water and each swab was suspended in 1 ml of sterile physiological water. Viral RNA was then extracted from 140µl of bursa homogenate or swab suspension using QIAamp RNA Mini Kit (Qiagen).
VP1 and VP2 target sequences were amplified using specific primers, according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrian Animals (OIE 2008), and One Step RT PCR kit (Qiagen).
Sequencing of amplified products was done using BigDye® Terminator kit (Applied Biosystems) with M13 specific primers and analysed using capillary ABI PRISM 3130xl (Applied Biosystems). Alignments of nucleotidic sequences were done using BioEdit software (Hall, 1999).

RESULTS

Table 1

The preliminary study demonstrated that viral RNA can be identified from cloacal swabs and in sufficient quantities to carry out sequencing of genes encoding VP2 viral proteins. Moreover, the authors identified a sampling 'window' between 10 and 16 days after vaccination.

Table 2

Second stage results:
8 out of 10 batches gave positive results by PCR, and VP2 (viral protein 2) sequencing, confirming that it was indeed the RNA of the vaccination strain administered.

Age	Samples	Identification	VP2 result
V+4	Pooled swabs 1	1 to 5	-
	Pooled swabs 2	6 to 10	-
	Bursas	6 to 10	-
V+8	Pooled swabs 1	21 to 25	-
	Pooled swabs 2	26 to 30	-
	Bursas	26 to 30	-
V+10	Pooled swabs 1	31 to 35	+
	Pooled swabs 2	36 to 40	-
	Bursas	36 to 40	-
V+12	Pooled swabs 1	41 to 45	+
	Pooled swabs 2	46 to 50	+
	Bursas	46 to 50	+
V+14	Pooled swabs 1	51 to 55	-
	Pooled swabs 2	56 to 60	+
	Bursas	56 to 60	+
V+16	Pooled swabs 1	61 to 65	-
	Pooled swabs 2	66 to 70	+
	Bursas	66 to 70	+

TABLE 1

Batch	Age	PCR VP2	Sequencing
PR	28	2/2 positive	100% 228E
A	32	2/2 positive	100% 228E
B1	28	1/2 positive	99.8% 228E
CC	27	negative	
AD	32	2/2 positive	100% 228E
GD	31	negative	
R3	28	2/2 positive	100% 228E
R2	27	2/2 positive	100% 228E
MR	32	2/2 positive	100% 228E
B2	28	2/2 positive	99.8% 228E

TABLE 2

DISCUSSION

Window of detection: the study by G. A. Abdel Alim and Y. M. Saif (Avian Diseases 45: 646-654, 2001) , showed that after vaccination, the detection of the virus or of its RNA in the bursa of Fabricius may be delayed in commercial broilers compared to specific pathogen-free (SPF) birds. This is due to the presence of maternal antibodies, relevant when only a low dose of virus is administered. Detection was possible only 14 days post-infection in commercial broilers, compared to 7 days in SPF birds. Our results, obtained from broilers following administration of a vaccine strain in the drinking water, are compatible with those of the referenced study, the cloacal excretion being closely tied with the presence of virus in the bursa of Fabricius.

If viral RNA was found in the bursa, it was always also found in the cloacal swab of the same animal. Two pools of 5 cloacal swabs seemed to suggest a good sampling method to confirm a vaccine response.

Testing 10 batches from the same organisation on the one hand allowed validation of the results, and on the other hand, underlined the value of such testing. After further investigation, one of the negative batches came from a unit where the birds had not been vaccinated, and the second came from a unit which was subjected to an audit.

CONCLUSION

Cloacal swab-based sampling improves animal welfare by preventing the need to sacrifice healthy animals and reduces the constraints imposed by sending samples to the laboratory, such as the need to refrigerate tissue samples.
In addition to the laboratory work-up already performed routinely, serology and histology,
using the Nobivet® Gumbo+ commercial PCR on cloacal samples is therefore a simple means of monitoring Infectious bursal disease vaccination.
These studies equally demonstrate the potential value for monitoring other vaccine strains or for epidemiological tracking of the strains circulating within a production unit.

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