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DETECTION OF HOBI-LIKE INFECTED BUFFALOS WITH A BLOCKING BVDp80 ELISA KIT

Paula Melisa Favaro[1]; S. Gascon[2]*; M. Badosa[2]; X. Rebordosa[2]; Maria Jose Dus Santos[3]; Andrea Pecora[3]
[1]Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Buenos Aires, Argentina. /
[2]HIPRA, Amer (Girona), Spain. / [3]Virology Institute, CICVyA, INTA Castelar, Buenos Aires, Argentina.
Corresponding author (sandra.gascon@hipra.com)
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INTRODUCTION

HoBi-like viruses are a group of emerging pestiviruses affecting cattle and buffalo. Reliable diagnostic tests are necessary to control and/or eradicate the disease they cause. Seroneutralization (SNT) and ELISAs are the most common techniques for antibody detection.

There are some antigenic differences between HoBi-like viruses and BVDV-1 and BVDV-2 strains. The performance of BVDp80 ELISA kits detecting antibodies against this virus is not well known. For this reason, the aim of this study is to evaluate the detectability of a blocking BVD ELISA kit against sera from HoBi-like infected buffalos.

MATERIALS AND METHODS

The study was conducted using 9 sera from HoBi-like naturally infected buffalos. These samples, provided by INTA (Instituto Nacional de Tecnología Agropecuaria, Argentina), had been previously analyzed by SNT and represented a sequence of SNT titer values from 2^3 to 2^{10} (see Table 1; Andrea Pecora *et al.*, 2017).

Samples were analyzed using a blocking BVD p80 ELISA kit (CIVTEST® BOVIS BVD/BD P80) according to the manufacturer's instructions. The kit expresses results as an Inhibition Percentage (%IN) value, and differentiates between low positive values (%IN 50-80) and high positive values (%IN >80). Samples were evaluated using both protocols of the kit: (i) long protocol; sample incubation o/n at +4ºC and (ii) short protocol; sample incubation 1 hour at +37ºC.

RESULTS AND DISCUSSION

Quantitative and qualitative ELISA results for each protocol and sample are shown in Table 1. The kit was able to detect as positives those samples with SNT titers equal or superior to 2^5 using the long protocol. Buffalo 2 sample, with a 2^5 SNT titer, was the only sample detected as "low positive". The other sample with SNT titer of 2^5 (Buffalo 6) together with the rest of samples with SNT titers from 2^6 to 2^{10} were interpreted as "high positives".

	SNT Titers HoBi-like viruses	Log_2 SNT Titer HoBi-like viruses	SHORT PROTOCOL		LONG PROTOCOL	
			%IN	Interpretation ELISA kit	%IN	Interpretation ELISA kit
BUFFALO 27	8	3.0	-1,26	NEG	-2.74	NEG
BUFFALO 28	8	3.0	4.01	NEG	-0.39	NEG
BUFFALO 21	16	4.0	16.73	NEG	23.39	NEG
BUFFALO 2	32	5.0	46.82	NEG	71.59	LOW POS
BUFFALO 6	32	5.0	74.08	LOW POS	91.6	HIGH POS
BUFFALO 10	64	6.0	74.63	LOW POS	88.89	HIGH POS
BUFFALO 3	256	8.0	61.59	LOW POS	88.47	HIGH POS
BUFFALO 4	1024	10.0	85.39	HIGH POS	94.25	HIGH POS
BUFFALO 11	1024	10.0	94.42	HIGH POS	94.3	HIGH POS

Table 1. Sample descriptions, SNT titer and results from the short and long protocol are shown.

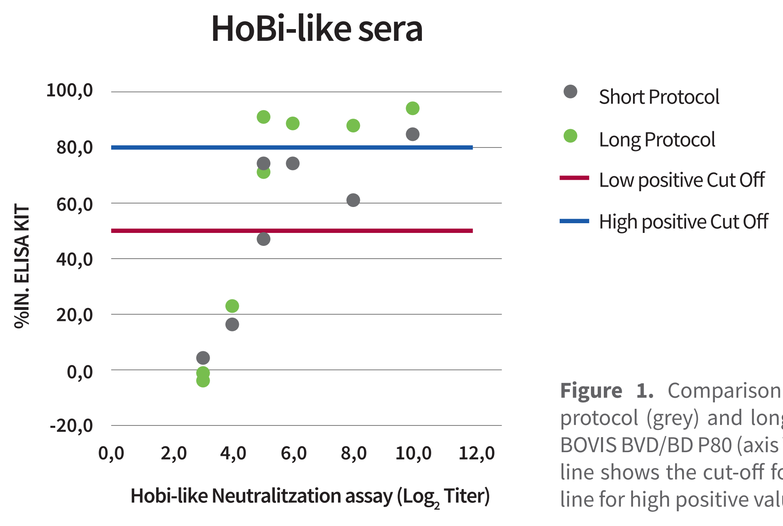

Figure 1. Comparison between results from short protocol (grey) and long protocol (green) of CIVTEST® BOVIS BVD/BD P80 (axis Y) and SNT titer (axis X). The red line shows the cut-off for low positive values and blue line for high positive values.

By using the short protocol the kit detected as positive only one of two samples with SNT titer of 2^5 (Buffalo 6 classified as "low positive"). Samples with SNT titers from 2^6 to 2^8 were also interpreted as "low positive" and only the samples with SNT titer of 2^{10} were interpreted as "high positive".

Comparison between SNT and ELISA (%IN) by both protocols is shown in Figure 1. Neither of the protocols detects samples with a SNT titer lower or equal than 2^4.

CONCLUSION AND DISCUSSION

CIVTEST® BOVIS BVD/BD P80 was able to detect HoBi-like infected buffalo. The kit showed greater detectability by using the long protocol than the short one, as it was able to detect all samples with SNT titers equal or superior than 2^5. In these conditions, the kit shows the best qualitative performance, indicating that it is suitable to detect HoBi-like infected buffalos.

On the other side, the short protocol, despite showing less sensitivity, allows a better semi-quantitative analysis of the samples, since it is able to differentiate between "low positives" (samples with a SNT titer between 2^5 and 2^8) and high positives (samples with SNT titer > 2^8). So, depending on the type of information we need, we could use one protocol or the other; we would use the long protocol as a screening system, and the short protocol to obtain quantitative information on a positive sample.

This work helps to determine the suitability of commercial kits to detect antibodies against this new pestivirus species. Above all, we would like to highlight the usefulness of these tools in regions where Hobi-Like virus already circulates.

REFERENCES

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