A NOVEL *MYCOPLASMA HYOPNEUMONIAE* ELISA, SPECIALLY DESIGNED FOR HIGH SPECIFICITY

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Introduction

Mycoplasma hyopneumoniae (M.hyo) diagnostics are considered to be a dilemma[1]. For serology there is no true Gold Standard available and until now serological assays lacked specificity. When testing M.hyo free herds, a low specificity will lead to too many false positive results, which cannot be confirmed with Gold Standard testing. On this basis there is a strong need for a highly specific assay with good sensitivity. This paper describes the first set of results with a high specificity M.hyo ELISA.

Materials and Methods

Sera sensitivity panel

This panel contains 108 samples from experimental infected animals. Animals were challenged at day 42 and sampled at day 63 of the study.

Sera specificity panel

In total, 1695 samples from SPF herds from five different studies of different geographical regions were tested. Samples were taken from animals of different ages, different breeds and different farm management systems.

ELISA

The BioChek *Mycoplasma hyopneumoniae* Antibody Test Kits was used according to the manufacturer's instructions.

Statistical analysis

For test evaluation, WinEpiscope 2.0 was used to calculate Sensitivity and Specificity with a 95% confidence level.

Results and Discussion

The results on all sera are given in Table 1. In table 2, a summary of the statistical test evaluation is given.

Table 1. Results on *Mycoplasma hyopneumoniae* antibody positive and negative samples

	M. hyopneumoniae positive and negative samples (n=1803)	
	Sample True Positive	Sample True Negative
ELISA positive	92	13
ELISA negative	16	1682

To serologically confirm the M.hyo free status of herds, it is imperative that the test has high specificity *combined* with good sensitivity to be sure that recent outbreaks are not missed. In the different studies described, the overall specificity of the ELISA is high (>99%), while at 21 days post infection the sensitivity is 85.2%. This is considerably sufficient in monitoring programs and therefore, based on the overall results, the ELISA is suitable for this purpose. Within the different specificity studies, different values for specificity were found, an overview of which is given in Table 3.

Table 2. Results of the statistical test evaluation

		95% Confidence level	
	Calculated (%)	Lower Limit (%)	Upper Limit (%)
Sensitivity	85.2	78.5	91.6
Specificity	99.2	98.8	99.6

Table 3. Results on *Mycoplasma* negative samples, 5 different studies

Total number of negative samples	Number of tested negative	Number of tested positive	Specificity	Remarks
993	986	7	99.3	
272	270	2	99.3	
70	70	0	100	
346	342	4	98.8	
14	14	0	100	*M. flocculare* positive samples

From Table 3 it is obvious that there could be regional differences, but still the ELISA shows high specificity, especially in confirmed *M. flocculare* positive sera where there is no cross reaction observed

Conclusion

The evaluated ELISA demonstrates high specificity and very good sensitivity which makes this ELISA suitable for monitoring M.hyo free herds.

Literature cited

1. Buddle, J.R, O'Hara, A.J., 2005. Aus.Vet. J., vol. 83, no.3, 134-139