PRESENTATION OF THE RESULTS OF THE ANNUAL PROFICIENCY TEST FOR THE SEROLOGICAL DIAGNOSIS OF EQUINE INFECTIOUS ANEMIA CONDUCTED IN ITALY BETWEEN 2006-2010.

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Introduction

Equine infection anemia (EIA) is a viral infection of equidae, caused by a virus (EIAV) of the Lentivirus genus, Retroviridae family. Since 2006, Italy has adopted a national surveillance plan based on the serological screening of all animals older than 6 months except for meat horses. According to the Italian Regulations, the official confirmatory test is the agar gel immunodiffusion (AGID), which detects antibodies against the p26, a highly conserved and primary immunogenic structural protein of the virus (1). The serological analysis of the samples, collected for the surveillance plan, can only be performed by the network of the national official laboratories, belonging to the Isitituti Zooprofilattici Sperimentali, mostly operating according to the ISO IEC 17025 and annually participating to proficiency tests.
In this context, since 2002, the national reference centre for equine infectious anemia (CRAIE) has conducted inter-laboratory agid proficiency tests (2) with an increase in the number of participating laboratories.
The aim of this paper is to present and discuss the results of the proficiency tests conducted in Italy during the years 2006-2010 for the diagnosis of EIA using the AGID test.

Materials & methods

Over the period 2006-2010, 65 laboratories participated to the annual proficiency test, performing both methods prescribed for AGID for AIE: "the Coggins test" which uses two layers of agar (1.5% and 0.7% respectively and wells of 7mm in diameter and 3 mm apart) and the OIE prescribed by the OIE (3). The panel distributed to each laboratory was each year made up of 10 sera, 3 negatives and 7 positives, with different level of antibodies against the P26 antigen (high to weak positives). Only in 2008, the panel was constituted of 2 negative and 8 positive sera. Before dispatching the samples to each laboratory, they were subjected to homogeneity and stability tests for their reactivity (2), so as to correctly evaluate reproducibility and repeatability parameters. Statistical analysis was carried out using K Cohen statistic, which was calculated for each laboratory and for all laboratories gathered together (4).

Results

Of the 65 participating laboratories, 27 constantly had a K value equal to 1. The results of the different laboratories per year are reported in table 1.

Table 1: K value results per year.

		Results of the 65 laboratories per year				
		2006	2007	2008	2009	2010
		N. of laboratories				
K Value	0.21-0.40	8	1	2	1	0
	0.42-0.60	5	0	0	0	1
	0.61-0.80	7	2	0	2	1
	0.81-1	45	62	63	62	63

On the other hand when considering the samples not correctly identified (K < 1) in both methods for all laboratories, those mostly misclassified were weak positive, 7.6%, against the 1.4% of the strong positive sera.
When taking into account the concordance of samples for the two different methods, the percentages of error for different AGID methods were respectively 5.2 for the Coggins test and 3.8 for the OIE method.
The K analysis conducted to evaluate the concordance among the different laboratories for each year is reported in table 2. The results using the two different AGID methods are not significantly different.

Table 2: K value results for all raters *per year using both AGID methods.*

K value results* among the raters *per year					
YEAR	**2006**	**2007**	**2008**	**2009**	**2010**
AGID Coggins	0.78	0.94	1	0.94	0.95
AGID OIE	0.77	0.98	1	0.97	0.98

Discussion & conclusions

The constant increase of participating laboratories to the proficiency tests during the years is an important element for evaluating the efficiency of the diagnostic system at national level for detecting EIA infection. These results stress the importance of performing such trials to achieve efficient diagnostic standards for each official laboratory as well as for the national diagnostic network.
Furthermore, the increase in the number of laboratories during the years, having a K value > 0.8, demonstrates an improvement of the diagnostic performances, as also the achievement of good quality standards, with very satisfactory reproducibility and repeatability parameters for both AGID tests, even if better for the AGID OIE. It is important to underline that some difficulties occurred in the interpretation of positive samples with lower levels of antibody against the p26 antigen (weak positive sera). This represents a problem especially because this error during routine performance is expected to be higher in consideration of an expected higher attention in reading a proficiency test. This may constitute a potential bias of the results obtained, generating the calculation of a better proficiency than the effective.
In this respect it our opinion that a more sensible technique such as the ELISA should be introduced to improve the efficacy and efficiency of the surveillance system. The introduction of such a screening test, with its characteristic of high sensitivity, rapidity and objectiveness is surely a more useful instrument for the laboratories performing EIA diagnosis, especially within an official surveillance and control programme. Another important issue emerging from our results is that the laboratories having lower level of concordance were those testing fewer samples in their diagnostic routine. This further remarks the importance of the experience in the execution and interpretation of a test such as the AGID.

References

1. Ordinanza 8 agosto 2010 Piano di sorveglianza nazionale per l'anemia infettiva degli equidi. G.U. Serie Generale n. 219 del 18 settembre 2010
2. Guidelines OIE 1998. Guidelines of the Office International des Epizooties for laboratory quality evaluation, for international reference standards for antibody assays and for laboratory proficiency testing. Rev. Sci. Tech. Off. Int. Epiz., 17 (2), 600-609
3. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010, Chapter 2 . 5 . 6 .Equine infectious anaemia
4. Langton S.D., Chevennement R., Nagelkerke N., Lombard B., 2002. Analysing collaborative trials for qualitative microbiological methods: accordance and concordance. International Journal of Food Microbiology 79: pp. 175-181
5. Charles J. Issel, R. Frank Cook (1993)REVIEW ARTICLE A review of techniques for the serologic diagnosis of equine infectious anemia J Vet Diagn Invest 5: 137-141