EVALUATION OF DIAGNOSTIC TESTS FOR THE EARLY DETECTION OF JOHNE'S DISEASE IN A DAIRY HERD IN URUGUAY

Suanes A [1], Nunez A [1], Piaggio J [1], de Freitas J [1], Zaffaroni R [1], Gil A [1].

E-mail: alesuanes@hotmail.com
[1:] Universidad de la República- Facultad de Vetrinaria
A. Lasplaces 1620 CP 11600 Montevideo- Uruguay

Key words: *Mycobacterium avium paratuberculosis*, Johne's disease, cattle, Interferon-gamma, ELISA, Baciloscopy, Uruguay

Introduction

Johne's disease is chronic and progressive enteric pathology in cattle, produced by bacteria: *Mycobacterium avium paratuberculosis* (Map). The young animals are the most susceptible, but they shown no symptoms until adult hood. Animals with subclinical disease can spread the microorganism and cause important economic losses. *M. avium* subsp. *Paratuberculosis* can infect monogastric species (1, 3), and although this is still highly controversial, *M. avium* subsp. *Paratuberculosis* has been implicated as a causal or exacerbating agent in human Crohn's disease (2, 5). The principal transmission occur through oral-fecal contamination. Several diagnostic techniques have been developed trying to detect the etiology agent but their results are very variable, depending on the sensitivity and specificity of each test. The latest reported data in Uruguay at the year 2003 indicate that the seroprevalence is 14,68% and the seropositives herds are 85% (6). The objective of this study is the comparison of intradermical skin (IS), Interferon gamma (IFN), ELISA, and direct baciloscopy (DB) tests for the early detection of Map in different categories of animals.

Materials and Methods

A total of 200 cattle all born in the same farm were sampled. The animals were stratified in 5 groups: group 1: 4 to 6 months, ,group 2: 6 to 8 months, group 3: 8 to 10 months, group 4:10 to 12 month and group 5: older than 1 year. At each sampling time, heparin stabilized blood (15 U/ml), non- stabilized blood and fecal samples were collected from each animal. Moreover, each animal was inoculated with tuberculin antigen (M. bovis and M. avium). The tuberculin test was interpreted 72 ± 6 hs after cattle were injected. The Interferon gamma test was performed as previously described (4). Briefly, 1,5ml whole blood samples were incubated (18 h/37°C) with each of the following antigen preparations in separate wells: PBS (nil antigen), 100 µl PPB (M. bovis) and 100 µl PPA (M.avium). Plasma was harvested and

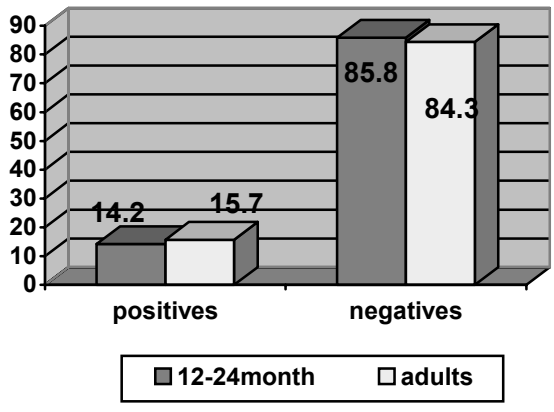
analyzed for IFN–γ contents by a commercially available assay (BOVIGAM ™, CSL Ltd., Parkville Australia) according to the manufacturer's instructions. Test results were interpreted using the optical density OD difference PPA-PBS as the test response value. Blood samples of 10 ml were collected from the selected animals. Sera were stored at -20°C until needed. Antibodies to *M. paratuberculosis* were detected with an ELISA commercial kit (Svanova Lab Kit, Sweden). Optical density was obtained by an ELISA reader (Multiscan II, Labsistem) at 450 nm. Absence or presence of antibody was determined by the rate between sample OD and positive control OD. Positive sample resulted on data greater or equal to 0,53. A new plastic sleeve was used for collection of fecal samples from each animal. Fecal sample were processed by baciloscopy method (Ziehl- Neelsen). Agreement was estimated by the Kappa index at p = 0,05 level of significance.

Results and Discussion

The comparative study between the IS and IFN those not present a good agreement. The comparison of ELISA with the IS e IFN present a bad agreement (table 2). If we compared with DB, the agreement is low but statistically significant (table 3). The seroprevalence found in the animals of group 5 was 14,2% witch agree with adult population in other farms in Uruguay (Table 1 and Graphic 1). The study by category for the IS indicated that animals younger than 8 months did not react to the test, while the reaction was grated in animals older than 1 year (table 5). The INF did not present any differences among the various categories(table 6). With respect to DB, starting at 8 month the result found are similar in the categories analyzed(table 7). Serological study though presenting low sensitivity was the one which better detected animals without symptoms. There are varied diagnostic methods now a day, but each one revels different limitations which suggests that not all can be applied in the different stages of the disease.

Table 1. Serum-prevalence for category

Categories	Negatives	Positives
12 a 24 month	78	13
Adults	75	14



Graphic 1: Serum-prevalence of MAP for category

tuberculina
interferon | Negative Positive | Total

Negative	105	26	131
Positive	3	0	3
Total	108	26	134

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
78.36%	79.23%	-0.0418	0.0487	-0.86	0.8050

Table2: interferon tuberculina

tuberculin	baciloscopy Negativo	Positivo	Total
Negativo	9	3	12
Positivo	5	2	7
Total	14	5	19

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
57.89%	56.23%	0.0380	0.2227	0.17	0.4323

. table 3: tuberculina - Baciloscopy

interferon	Baciloscopy Negative	Positive	Total
Negative	78	26	104
Positive	2	0	2
Total	80	26	106

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
73.58%	74.51%	-0.0363	0.0446	-0.81	0.7922

. table 4 interferon Baciloscopy

category	tuberculina Negative	Positive	Total
4 a 6 month	32	0	32
6 a 8 month	31	0	31
8 a 10 month	18	2	20
10 a 12 month	15	10	25
12 a 24 month	53	36	89
Total	149	48	197

Table 5: tuberculin by category

Acknowledgements
The authors thanks the cattle herd owners. This study was supported financially by CSIC program.

interferon

category	Negative	Positive	Total
4 a 6 month	31	1	32
6 a 8 month	30	1	31
8 a 10 month	20	0	20
10 a 12 month	25	0	25
12 a 24 month	26	1	27
More than 2 years	85	2	87
Total	217	5	222

Table 6: interferon by category

	Baciloscopy		
category	Negative	Positive	Total
6 a 8 month	0	1	1
8 a 10 month	7	2	9
10 a 12 month	7	2	
More than 2 years	68	21	89
Total	82	26	108

Table7: Baciloscopy by category

References

1. Beard, P. M., M. J. Daniels, D. Henderson, A. Pirie, K. Rudge, D. Buxton, S. Rhind, A. Greig, M. R. Hutchings, I. McKendrick, K. Stevenson, and J. M. Sharp. 2001. Paratuberculosis infection of nonruminant wildlife in Scotland. J. Clin. Microbiol. 39:1517–1521.
2. Chamberlin, W., D. Y. Graham, K. Hulten, H. M. El-Zimaity, M. R. Schwartz, S. Naser, I. Shafran, and F. A. El-Zaatari. 2001. Mycobacterium avium subsp. paratuberculosis as one cause of Crohn's disease. Aliment. Pharmacol. Ther. 15:337–346.
3. Chiodini, R. J., and C. D. Buergelt. 1993. Susceptibility of Balb/c, C57/B6 and C57/B10 mice to infection with Mycobacterium paratuberculosis. J. Comp. Pathol. 109:309–319.
4.Jungersen, G., Huda, A., Hansen, J. J. and Lind, P.(2002). Interpretation of the interferon-gamma testfor diagnosis of subclinical paratuberculosis in cattle. Clinical and Diagnostic Laboratory Immunology, 9,453–460.
5. Naser, S. A., I. Shafran, D. Schwartz, F. El-Zaatari, and J. Biggerstaff. 2002. In situ identification of mycobacteria in Crohn's disease patient tissue using confocal scanning laser microscopy. Mol. Cell Probes 16:41–48.
6.Piaggio J, Nuñez A, Gil A. Johone´s disease serological prevalence in Uruguayan dairy cows. 10º Simposium International de Epidemiologic y Economic Veterinarian, Chile 2003.