COMPARISON OF DIRECT AND INDIRECT FLUORESCENT ANTIBODY TECHNIQUES FOR DETECTION OF MYCOPLASMA HYOPNEUMONIAE IN SWINE LUNGS

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Specific diagnosis of mycoplasmal pneumonia of swine (MPS) has long been hampered by the slow and fastidious growth of M. hyopneumoniae as well as by the frequent overgrowth of the organism by other mycoplasmas and bacteria. Rapid diagnosis has been achieved by use of direct (L'Ecuyer and Boulanger, 1970; Meyling, 1971 and Amanfu et al., 1980) and indirect (Gois et al., 1975) fluorescent antibody techniques. The relative usefulness of these techniques in comparison to culture and other diagnostic procedures has not been thoroughly evaluated. In the work reported here we evaluated the usefulness of DFA and IFA for detection of M. hyopneumoniae in swine lungs.

Methods: Pigs utilized in this study were described in a companion report (Piffer and Ross, 1982). All pigs were exposed by contact to other swine infected with M. hyopneumoniae and examined at necropsy 41-43 days (Experiment A) or 49-50 days (Experiment B) after contact-exposure began.

Tissues were collected, embedded in OCT, cut with a cryostat and the DFA procedure was done as described by Amanfu et al. (1980). The IFA procedure was similar to that described by Cherry et al. (1960) but with washing and timing as described by Rosendal and Black (1972). Commercial goat anti IgG conjugated with fluorescein and antiserum to M. hyopneumoniae prepared in rabbits (Ross and Karmon, 1970) were used in the IFA test. Appropriate dilutions of conjugate and hyperimmune serum were determined by block titration. The effect of an azo-dye counterstain (Potgieter and Ross, 1972) was also evaluated. Isolation of M. hyopneumoniae was achieved in a manner similar to that of Friis (1975). Macroscopic and microscopic evaluations for lesions typical of MPS were done according to standard methods.

Results: Distribution and intensity of staining of M. hyopneumoniae antigen was similar in both the DFA and IFA tests. The antigen appeared as a granular yellow green layer on the surface of the bronchial and the bronchiolar epithelium. DFA with counterstain, IFA with counterstain and IFA without counterstain were equally efficient for detection of mycoplasmal antigen (Table 1). Furthermore, no differences in intensity were observed among the techniques ($p > 0.05$).

Table 2 summarizes results obtained in comparisons between isolation of M. hyopneumoniae, DFA, macroscopic evaluation of lungs and microscopic evaluation. Although microscopic evaluation and culture tended to be positive more often, differences in efficiency of detection were not significant ($p > 0.05$).

Table 1.

Immunofluorescence procedure	No. of FA-pos. lobes[a]	No. of pos. lobes in graded categories of fluorescence[b]			
		1	2	3	4
Experiment A (18 pigs)					
DFA with counterstain	15	2	3	9	1
IFA without counterstain	15	3	4	4	4
IFA with counterstain	14	1	7	5	1
Experiment B (12 pigs)					
DFA with counterstain	15	2	0	9	4
IFA without counterstain	16	0	4	8	4
IFA with counterstain	16	2	5	6	3

[a]Both cardiac lobes from each pig examined.
[b]Intensity of fluorescence was graded 1 = least intense to 4 = most intense.

Table 2.

No. of lobes eval.	No. of lobes culture-pos. for M. hyop.	No. of lobes DFA-pos.	No. of lobes with macro. lesions	No. of lobes with micro. lesions
24	16	12	14	20

Discussion: Since no differences were observed between the immunofluorescence techniques utilized, it appears that IFA is more suitable than DFA for less-equipped laboratories because the fluorescein tagged globulin can be bought commercially. In addition, the antiserum can be used at a higher dilution than the direct conjugate. The azo-dye counterstain reduced nonspecific fluorescence and provided a better contrast with no evidence of reduced sensitivity.

Results obtained by means of FA test corresponded closely to those obtained by culture procedure and macroscopic and microscopic evaluation for MPS lesions. These results are in agreement with those obtained by L'Ecuyer and Boulanger (1970), and Amanfu et al. (1980). In spite of this fact, a few lobes that were culture-positive were negative by FA while in one case the reverse occurred.

Selected References: Amanfu, W., et al. Proc. 6th Congr. IPVS, Copenhagen 1980, 223; Cherry, W. B., et al. US Govt. Print. Office, Washington DC 1960; Friis, N. F. Nord. Vet. Med. 1975, 27:337; Gois, M., et al. Zbl. Vet. Med. B. 1975, 22:205; L'Ecuyer, C. and Boulanger, P. Can. J. Comp. Med., 1970, 34: 38; Meyling, A. Acta Vet. Scand. 1971, 12: 137; Piffer, I. A. and Ross, R. F. Proc. 7th Congr. IPVS, Mexico City 1982; Potgieter, L. N. D. and Ross, R. F. Am. J. Vet. Res. 1972, 33:91; Rosendal, S. and Black, F. T. Acta Path. Microbiol. Scand. B. 1972, 80: 615; Ross, R. F. and Karmon, J. A. J. Bact. 1970, 103:707.