

## Evaluation of a Modified Complement Fixation Test and an Indirect Hemagglutination Test for the Serodiagnosis of Melioidosis in Pigs

ANNETTE D. THOMAS,<sup>1\*</sup> GEORGE A. SPINKS,<sup>1</sup> TERESA L. D'ARCY,<sup>1</sup> AND DENIS HOFFMANN<sup>2</sup>

*Oonoomba Veterinary Laboratory, Queensland Department of Primary Industries, P.O. Box 1085, Townsville, Queensland 4810,<sup>1</sup> and Australian Centre for International Agricultural Research, Canberra, Australian Capital Territory 2061,<sup>2</sup> Australia*

Received 12 February 1990/Accepted 22 May 1990

**A complement fixation test modified by the addition of porcine serum and an indirect hemagglutination test were used to detect antibodies to *Pseudomonas pseudomallei* in pigs. These tests together with cultural examinations were carried out with 250 pigs. The sensitivity and specificity values were 79.3 and 99.5% and 82.8 and 93.2% for the modified complement fixation and hemagglutination tests, respectively. When results from the combination of both tests were considered, the values were 86.2 and 92.8%, respectively.**

*Pseudomonas pseudomallei*, a soil saprophyte and opportunistic pathogen of animals, including humans, causes a chronic form of melioidosis in pigs in Queensland, Australia (11, 17), where the disease is not often recognized until slaughter of the animal. Melioidosis then becomes an economic problem, because it results in total carcass condemnation (8). When infection occurs by ingestion or wound entry, nodules are found mainly in the spleen (11, 17). Infection by inhalation also involves the lung (8, 15).

Serodiagnosis is important when determining prevalence and distribution of infection. The serum agglutination, hemagglutination, and complement fixation (CF) tests for porcine melioidosis are unreliable (6, 15), and there is little correlation between test and culture results (9, 13, 14). A recent trial (A. D. Thomas and G. A. Spinks, Conf. Workshop Ser. QC83011, Queensland Dept. Primary Industries, 1983, p. 27-35) suggested that a CF test modified by the addition of porcine serum (CFPS) and an indirect hemagglutination (IHA) test were the tests of choice, and this study was undertaken as a more comprehensive evaluation of these tests.

For the purposes of this paper, pigs from which *P. pseudomallei* was isolated are described as "culture positive" and pigs from which *P. pseudomallei* was not isolated are described as "culture negative."

**Pigs.** Serum samples were collected from 250 pigs (54 domestic and 196 feral) from areas throughout Queensland, Australia. All pigs were available for cultural examination.

**Bacteriology.** Methods for culture of clinical samples and identification of isolates have been described previously (18).

**Serology.** The CFPS test (Thomas and Spinks, Conf. Workshop Series, 1983) was a modification of the microtiter CF test for *Mycoplasma hyopneumoniae* (16). Briefly, guinea pig complement (GIBCO Laboratories, Grand Island, N.Y.) was reconstituted in Veronal buffer containing 1% normal unheated pig serum recently collected from 6- to 8-week-old pigs. The test had an overnight fixation step at 4°C. Doubling dilutions of sera commencing at 1:4 were used, and titers were graded 0 or 1 to 4, depending on the degree of hemolysis or buttoning at the bottom of the microtiter well. A (baseline) positive value was a serum dilution of  $\geq 1:8$ .

The IHA test of Alexander et al. (1) was used. Doubling dilutions of sera commencing at 1:10 were made, and titers were graded 0 or 1 to 4, depending on the amount of buttoning at the bottoms of the microtiter wells. A (baseline) positive value was a serum dilution of  $\geq 1:40$ .

**Antigens.** The antigens were prepared from a local porcine isolate of *P. pseudomallei* serotype 1. Antigens and sensitized erythrocytes were prepared by standard procedures (1). Preliminary trials had shown that antisera prepared in rabbits to antigens of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pleisomonas shigelloides*, *Escherichia coli*, and *Acinetobacter calcoaceticus* (all isolated from pigs) did not cross-react with the *P. pseudomallei* antigens used in this test (data not shown).

**Test evaluations.** Sensitivity and specificity values were calculated by the method of Fletcher et al. (5). The criterion for infected animals was culture-positive tissue. Observed percentage agreement and kappa statistics were determined by the method of Martin et al. (12). Kappa is a quotient used to measure the agreement between tests by adjusting for agreement due to chance. A value of +1 represents perfect agreement, and a value of  $-\infty$  represents perfect disagreement. Kappa values of 0 indicate no agreement beyond chance, values from 0.4 to 0.6 indicate moderate agreement, values from 0.6 to 0.8 indicate good agreement, and values above 0.8 indicate very strong agreement (2, 12).

Table 1 shows the relationship between culture results and serological status for the 250 pigs. Of the 221 culture-negative pigs, 16 had sterile lesions indicative of melioidosis in regression. Of these 16 pigs, 15 had CFPS-negative, IHA-positive titers, and 9 of these IHA titers were  $\geq 1:160$ .

Sensitivity and specificity values for the two tests are

TABLE 1. Serological results for and culture status of 250 field pigs by using the CFPS and IHA tests for *P. pseudomallei*

Result with:		No. of pigs	
CFPS	IHA	Culture positive (n = 29)	Culture negative (n = 221)
+	+	22	0
+	-	1	1
-	+	2	15
-	-	4	205

\* Corresponding author.

TABLE 2. Sensitivities and specificities of the CFPS and IHA tests for porcine melioidosis

Test	Sensitivity (%; n = 29 <sup>a</sup> )	Specificity (%; n = 221 <sup>a</sup> )
CFPS	79.3 (60-92) <sup>b</sup>	99.5 (99-100)
IHA	82.8 (64-93)	93.2 (90-97)
CFPS and IHA in parallel	86.2 (73-99)	92.8 (89-96)

<sup>a</sup> n, Number of porcine serum samples used per test.

<sup>b</sup> 95% confidence interval.

shown in Table 2. The values given are from examinations of each test alone as well as examinations of both in parallel (either test positive).

The percentage of agreement between the two tests is shown in Table 3, as is the kappa value of agreement.

Our results have shown that the CFPS and IHA tests are useful for the serodiagnosis of porcine melioidosis. The tests may be used in parallel, or alternatively, the more sensitive IHA test could be used as a screening method followed by the more specific CFPS technique as a confirmatory test. Laws (10) found little correlation between serological titers and culture results with a CF test on pig sera. The increased sensitivity of the CFPS test was due to the addition of normal unheated porcine serum to the guinea pig complement. This added the porcine C1<sub>q</sub> component of the complement system necessary for specific fixation to the antigen-antibody complex, since guinea pig C1<sub>q</sub> is not effective in porcine CF reactions (3, 4, 16).

Johnson (7) and Laws (9, 10) (in studies with humans and sheep and goats, respectively) reported that CF antibodies did not persist for very long after melioidosis infection had been resolved and that persistence of a CF titer indicated persistence of infection. Six false-negative CFPS reactions were recorded in the present study. These reactions were noted for pigs with chronic melioidosis highlighted by a few well-encapsulated nodules in single organs. Lowered sensitivity values for the CF test for goats with latent chronic infections have been reported (19).

Observed agreement between the two tests was high (92.4%), and the kappa quotient (0.66) showed good agreement. Some disagreement is expected, since the two tests are measuring different antigen-antibody reactions. The IHA test was less specific than the CFPS test (Table 2). This was due to the 15 false-positive reactions in the IHA test, as compared with 1 in the CFPS test. The 15 pigs with false-positive IHA reactions had sterile lesions indicative of melioidosis in regression, a common occurrence in pigs (11, 15, 18); therefore, these pigs could have recovered from infection with *P. pseudomallei*. However, by definition (5), specificity is calculated by using infected animals with culture-positive tissues. Other factors that could contribute to the lowered specificity of the IHA test are infections with cross-reacting organisms such as *Salmonella* sp. or other pseudomonads (1, 4, 17) and the presence of natural agglu-

tinins in endemic regions due to frequent exposure to *P. pseudomallei* (6, 13, 15).

The CF and IHA tests have been recommended for the serodiagnosis of human (1) and caprine (19) melioidosis. An enzyme-linked immunosorbent assay is being tested in our laboratory, but until a more sensitive and specific test or combination of tests is evaluated, the CFPS and IHA tests, run in parallel, are recommended for the serodiagnosis of porcine melioidosis.

We thank the Department of Primary Industry officers who collected the samples from abattoirs and during feral pig surveys.

#### LITERATURE CITED

- Alexander, A. D., D. L. Huxsoll, A. R. Warner, Jr., V. Shepler, and A. Dorsey. 1970. Serological diagnosis of human melioidosis with indirect hemagglutination and complement fixation tests. *Appl. Microbiol.* 20:825-833.
- Dohoo, I. R., P. F. Wright, G. M. Ruckerbauer, B. S. Samagh, F. J. Robertson, and L. B. Forbes. 1986. A comparison of five serological tests for bovine brucellosis. *Can. J. Vet. Res.* 50:485-493.
- Eskildsen, M. 1975. Complement fixation with porcine serum antibodies. *Acta Pathol. Microbiol. Scand. Sect. C* 83:315-324.
- Etheridge, J. R., and L. C. Lloyd. 1980. A complement fixation test for enzootic pneumonia of pigs using a complement dilution method. *Aust. Vet. J.* 56:101-105.
- Fletcher, R. H., S. W. Fletcher, and E. H. Wagner. 1982. *Clinical epidemiology—the essentials*, p. 46-47. The Williams & Wilkins Co., Baltimore.
- Fournier, J., and L. Chambon. 1958. *La mélioiidose: maladie d'actualité et la bacille de Whitmore*. Éditions Médicales Flammarion, Paris.
- Johnson, D. W. 1967. Melioidosis: report of four cases from Torres Strait. *Med. J. Aust.* 2:587-588.
- Ketterer, P. J., W. R. Webster, J. Shield, R. J. Arthur, P. J. Blackall, and A. D. Thomas. 1986. Melioidosis in intensive piggeries in south eastern Queensland. *Aust. Vet. J.* 63:146-149.
- Laws, L. 1967. Melioidosis in animals in north Queensland. V. Serological methods of diagnosis in sheep. *Queensl. J. Agric. Sci.* 24:207-221.
- Laws, L. 1967. Melioidosis in animals in north Queensland. VI. Serological methods of diagnosis in goats, pigs, cattle, horses and man. *Queensl. J. Agric. Sci.* 24:223-234.
- Laws, L., and W. T. K. Hall. 1963. Melioidosis in animals in north Queensland. I. Incidence and pathology, with special references to central nervous system lesions. *Queensl. J. Agric. Sci.* 20:499-513.
- Martin, S. W., A. H. Meek, and P. Willeberg. 1987. *Veterinary epidemiology: principles and methods*, p. 73-75. Iowa State University Press, Ames.
- Nguyen-Ba-Luong. 1956. A propos d'une épizootie porcine de mélioiidose dans une province méridionale du Vietnam. *Bull. Soc. Pathol. Exot.* 49:25-31.
- Nguyen-Ba-Luong. 1961. La mélioiidose porcine au Viet-Nam. *Off. Int. Epizoot.* 56:964-976.
- Omar, A. R., C. K. Kheong, and T. Mahendranathen. 1962. Observations on porcine melioidosis in Malaya. *Br. Vet. J.* 118:421-429.
- Slavik, M. F., and W. P. Switzer. 1972. Development of a microtitration complement fixation test for diagnosis of mycoplasma swine pneumonia. *Iowa State J. Res.* 47:117-128.
- Thomas, A. D. 1981. Prevalence of melioidosis in animals in northern Queensland. *Aust. Vet. J.* 57:146-147.
- Thomas, A. D., J. C. Forbes-Faulkner, T. L. D'Arcy, J. H. Norton, and D. Hoffmann. 1990. Experimental infection of normal and immunosuppressed pigs with *Pseudomonas pseudomallei*. *Aust. Vet. J.* 67:43-46.
- Thomas, A. D., G. A. Spinks, T. L. D'Arcy, J. H. Norton, and K. F. Trueman. 1988. Evaluation of four serological tests for the diagnosis of caprine melioidosis. *Aust. Vet. J.* 65:261-264.

TABLE 3. Agreement between the CFPS and IHA tests for melioidosis by using 250 porcine serum samples

IHA test result	No. of CFPS test results <sup>a</sup>	
	Positive	Negative
Positive	22	17
Negative	2	209

<sup>a</sup> Observed percentage of agreement, 92.4%; kappa value, 0.66.