EVALUATION OF THE ELISA FOR DIAGNOSIS OF MYCOPLASMAL PNEUMONIA OF SWINE (MPS): I. CROSS-REACTIVITY BETWEEN *M. HYOPNEUMONIAE* AND THE OTHER PORCINE MYCOPLASMAS TO REFERENCE ANTISERA

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An accurate serodiagnostic test is needed for control of mycoplasmal pneumonia of swine (MPS). The ELISA is a sensitive and specific serologic test and has shown promise for serodiagnosis of diverse diseases,[1] including MPS.[2,3] For example, the pattern of titers of caesarean-derived, colostrum-deprived (CD:CD) swine infected experimentally with MPS detected by the ELISA compared well with those detected by the complement fixation and indirect hemagglutination tests but ELISA titers persisted much longer.[4] However, questions about the specificity of the ELISA have arisen. Bruggmann *et al.*[2] concluded that the ELISA was not specific for antibodies to *Mycoplasma hyopneumoniae* (HyoP). Preliminary results from this laboratory indicated cross-reactivity to HyoP antigen of sera from swine immunized monospecifically with *M. flocculare* (Floc), as well as less reactivity with *M. hyorhinis* (HyoR) and *M. hyosynoviae* (HyoS) antisera.[3] Nicolet *et al.*[5] have subsequently suggested that purification of the antigen used for the ELISA might improve specificity of the test. The initial promise of the ELISA suggested more extensive examination of the antigenic cross-reactivity of the porcine mycoplasmas.

Procedures. Type strains of HyoP, Floc, HyoR and HyoS were used for the preparation of antigen for immunization of CD:CD swine for the preparation of monospecific reference antisera. Two swine were used for all but one (HyoS) of the type species. Antigen for the ELISA was prepared by 10 cycles each of freezing and thawing and sonic disruption and, finally, by SDS extraction.[4]

The Gilford PR-50, Semi-Automated Processor-Analyzer System was used for all determinations. Data were summarized and statistics determined with a calculator whose linear regression function was used to extrapolate titers to an endpoint of 0.2 O.D., using data points over the range of 0.1 to 1.0 O.D. This value was selected since 0.2 O.D. was ∿3 S.D. greater than the mean of 2000 serum control values, i.e. all reagents except antigen. Titers were calculated as the log base 2, i.e. ($\log_{10}$ reciprocal titer x 3.32). Thus, titers of 100, 200, --- 1600, etc. were expressed as $\log_2$ titers rounded to 6.64, 7.64, --- 10.64, etc. Differences in titers were calculated as the $\Delta\log_2$ titer, rounded to the nearest integer, representing the number of $\log_2$, i.e. 2-fold, differences between two titers.

Results. Quality control procedures were applied to insure that reagent controls were within control limits (i.e. ±2 S.D.) of the mean. Standard homologous (anti-HyoP) and heterologous (anti-Floc) serum tests were included in each ELISA. Reference positive and negative serum test values fell within 1 $\log_2$ dilution of the mean, i.e. 10.10±1 and 4.00±1, respectively.

Titers of the homologous and heterologous sera tested against HyoP antigen-coated cuvettes were:

		Days, Post-Inoculation			
		27	64	75	104(F)
		Reference Reciprocal and ($\log_2$) Titer			
Ser	Anti-				
42	HyoP	1495 (10.55)	1010 (9.98)	5361 (12.39)	5181 (12.34)
		Reciprocal and ($\Delta\log_2$ Reference) Titer			
43	HyoP	1079 (<1)	660 (<1)	8445 (+1)	9623 (+1)
45	Floc	1091 (<1)	905 (<1)	1895 (-2)	1046 (-2)
44	Floc	102 (-4)	73 (-4)	335 (-4)	274 (-4)
40	HyoR	274 (-3)	204 (-2)	310 (-4)	104 (-6)
41	HyoR	201 (-3)	66 (-4)	55 (-7)	84 (-6)
46	HyoS	126 (-4)	104 (-3)	207 (-5)	70 (-6)

Titers of the anti-HyoP sera did not differ significantly over the period of the response and 42F was selected as the homologous reference standard. Cross-reactivity of the other sera varied at different times during the response but attention was focused on the final (F) antisera. Titers as little as 2 $\log_2$ units (i.e. 4-fold) less than the homologous reference were noted for one anti-Floc serum (45F). Cross-reactive titers of other swine were generally lower. However, if even the lowest of these titers was typical of those of naturally-infected swine, a titer as much as 6 to 7 units less than that of the reference standard would be equivalent to a titer of 40 to 80 and thus be a possible point of confusion in titer interpretation.

Since comparable (i.e. ∿2 $\log_2$ units) differences in reactivity were observed for titers of homologous and heterologous sera tested on HyoP and Floc antigen coatings, other experiments were performed to determine the degree of cross-reactivity indicated by the relative degree of binding. Cuvettes were coated with equivalent (0.1 µg) amounts of protein for all four mycoplasmal antigens. The reciprocal, $\log_2$ and $\Delta\log_2$ reference titers for sera titrated on HyoP and Floc antigen-coated cuvettes were:

	HyoP Coat			Floc Coat	
Serum	Anti-		Serum	Anti-	
42F	HyoP	1072 (10.06)	45F	Floc	4202 (12.03)
	Reciprocal and ($\Delta\log_2$ Reference) Titer				
43F	HyoP	886 (<1)	44F	Floc	1485 (-2)
45F	Floc	265 (-2)	42F	HyoP	790 (-2)
44F	Floc	254 (-2)	43F	HyoP	594 (-3)
40F	HyoR	110 (-3)	40F	HyoR	223 (-4)
41F	HyoR	22 (-5)	41F	HyoR	53 (-6)
46F	HyoS	100 (-3)	46F	HyoS	255 (-4)

These data indicated a high reciprocal cross-reactivity between HyoP and Floc antisera, with only a 4-fold difference between titers with cuvettes coated with HyoP or Floc. Titers of heterologous sera on HyoR or HyoS antigen-coated cuvettes, shown below, were 5 $\log_2$, or more, units lower than for the homologous sera.

	HyoR Coat			HyoS Coat	
Serum	Anti-		Serum	Anti-	
40F	HyoR	78799 (16.26)	46F	HyoS	5575 (12.44)
	Reciprocal and ($\Delta\log_2$ Reference) Titer				
41F	HyoR	47608 (<1)	40F	HyoR	203 (-5)
42F	HyoP	821 (-7)	41F	HyoR	112 (-6)
43F	HyoP	291 (-8)	42F	HyoP	49 (-7)
44F	Floc	314 (-8)	43F	HyoP	103 (-6)
45F	Floc	322 (-8)	44F	Floc	186 (-5)
46F	HyoS	413 (-8)	45F	Floc	314 (-4)

These results indicate that cross-reactivity among the porcine mycoplasmas, especially HyoP and Floc, explains the apparent non-specificity of the ELISA which has been of concern in attempts to develop a practical serodiagnostic test for MPS.

Selected References. [1]Van Weemen,B.K. *et al.* Chim.Acta 1977, *81*:1. [2]Bruggmann,S. *et al.* Vet.Rec. 1977, *101*: 109. [3]Armstrong,C.H. *et al.* Proc.,Am.Ass.Vet.Lab.Diag. 1978, *21*:377. [4]Armstrong,C.H. *et al.* Proc ,IPVS 1980, p.224. [5]Nicolet,J. *et al.* Proc., IPVS 1980, p.225.