EVALUATION OF THE ELISA FOR DIAGNOSIS OF MYCOPLASMAL PNEUMONIA OF SWINE (MPS): II. ANTIGENIC RELATIONSHIPS BETWEEN *M. HYOPNEUMONIAE* AND *M. FLOCCULARE* OF REFERENCE ANTISERA AND SERA OF MPS-AFFECTED SWINE

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Previous experiments[1] indicated extensive cross-reactivity among porcine mycoplasmas; particularly between *Mycoplasma hyopneumoniae* (HyoP), the etiologic agent of MPS, and *M. flocculare* (Floc), a widely distributed porcine mycoplasma whose role, if any, in MPS is not established; when antisera from monospecifically immunized swine were tested by the ELISA. The present study was concerned with extension of these studies with sera of swine naturally-infected with MPS.

Procedures. The ELISA, quality controls and methods for comparative binding studies of cross-reactivity have been described.[1] HyoP and Floc reference antisera and sera from swine naturally-infected with MPS were studied by specific antigen inhibition, comparative binding and absorption tests for comparison of cross-reactivity between HyoP and Floc antigens.

Results. The results of duplicate determinations of the effects of reciprocal inhibition of reference antisera reacted on cuvettes coated with either homologous or heterologous antigen are shown as the reciprocal and reference $\log_2$ titers for each serum without inhibition (0) or as the $\log_2$ titer reduction after inhibition with either soluble HyoP (ΔH) or Floc (ΔF) antigen. The results were:

HyoP Coat				Floc Coat			
Serum	Anti-	Ag Inh		Serum	Anti-	Ag Inh	
42F	HyoP	0	862 {9.75}	45F	Floc	0	2566 {11.33}
		ΔH	27 (-5)			ΔH	1949 (<1)
		ΔF	195 (-2)			ΔF	41 (-6)
		Reciprocal and (ΔLog$_2$ Reference) Titer					
43F	HyoP	0	526 {9.04}	44F	Floc	0	618 {9.27}
		ΔH	5 (-7)			ΔH	329 (-1)
		ΔF	125 (-2)			ΔF	5 (-7)
45F	Floc	0	227 {7.82}	42F	HyoP	0	618 {9.27}
		ΔH	3 (-6)			ΔH	31 (-4)
		ΔF	19 (-4)			ΔF	24 (-5)
44F	Floc	0	162 {7.34}	43F	HyoP	0	510 {8.99}
		ΔH	10 (-4)			ΔH	<1 (-9)
		ΔF	5 (-5)			ΔF	24 (-4)

Thus, inhibition of homologous and heterologous reference antisera with HyoP antigen abolished their titers (endpoints <30) on HyoP antigen-coated cuvettes. Inhibition with Floc antigen also abolished titers (<20) of the anti-Floc sera but reduced the titers of the anti-HyoP sera by only ∿2 $\log_2$ units. The residual titers of 195 and 125, respectively, for 42F and 43F, presumably reflected HyoP-specific antibody.

The reciprocal experiment using Floc antigen-coating indicated inhibition of the reactivity of the anti-Floc sera by Floc antigen (titers <41) but only ∿1 $\log_2$ decrease after inhibition with HyoP antigen. Inhibition with both antigens abolished the titers of both anti-HyoP reference sera (titers <31).

Subsequently, effects of HyoP and Floc antigen inhibition on the ELISA titers of sera from swine affected with MPS were studied using HyoP antigen-coated cuvettes. Swine which had high ELISA titers were selected for cultural examination and gross and microscopic necropsy examinations. Eight sera with the highest ELISA titers from a total of 27 swine culturally positive for either HyoP or Floc or both were studied. The results were as follows:

Ag Inh	Ser	Cult	Titer	Ser	Cult	Titer	Ser	Cult	Titer
0	42F	NA	2532 {11.30}	45F	NA	391 {8.61}	--	---	----
ΔH			84(-5)			32(-5)			----
ΔF			2130(≤1)			15(-4)			----
0	C63	HyoP	611 {9.25}	W64	HyoP	1087 {10.08}	W98	HyoP	2791 {11.44}
ΔH			314(-1)			41(-5)			127(-5)
ΔF			717(≤1)			763(<1)			2640(<1)
0	B83	Floc	1014 {9.98}	Z16	Floc	528 {9.04}	D49	Floc	<50 {<5.64}
ΔH			184(-3)			32(-4)			----
ΔF			479(-1)			227(-1)			----
0	B57	HyoP & Floc	303 {8.24}	B46	HyoP & Floc	<50 {<5.64}	--	---	----
ΔH			34(-3)			----			----
ΔF			188(-1)			----			----

Thus, inhibition with HyoP but not Floc reduced the titers of two of three sera from swine from which HyoP had been isolated. All three had gross and microscopic lesions. HyoP but not Floc also inhibited the titers of two sera from swine (B83,Z16) from which Floc was isolated and of one swine (B57) from which both organisms were isolated. One swine (B83) from which only Floc was isolated had gross and microscopic lesions. With the exception of one swine (C63) from which HyoP had been isolated, these results indicated that inhibition with HyoP was suggestive of a role for HyoP infection in the genesis of the ELISA titer. The incomplete correlation of the gross and microscopic lesions and the isolation of HyoP or Floc was not surprising since serologic reactivity to HyoP detected by the ELISA may persist for up to 12 months after experimental infection of CD:CD swine with *M. hyopneumoniae*,[2] whereas the persistence of lesions and the ability to isolate the etiologic agent are recognized to be more transient.

Comparative binding[1] of the same antisera to HyoP or Floc antigen-coated cuvettes was also investigated. Sera of three swine from which HyoP had been isolated had titers 2 or more $\log_2$ units lower on Floc-coated cuvettes. The three swine from which only Floc was isolated, or the one swine from which both organisms were isolated, all had titers on the Floc antigen-coat which were not different than their titers on HyoP antigen-coat.

Since assessment of antigen binding capacity was not completely successful in indicating the basis of serologic reactivity, further studies were conducted to determine if absorption with intact mycoplasmal cells would remove cross-reactive antibodies more effectively.

Reductions of titers were comparable to those observed for the inhibition experiment (above), i.e. effective abolition of reactivity to HyoP antigen when the test anti-HyoP serum was reacted on HyoP antigen-coat. Inhibition with heterologous antigen had little effect on reactivity on HyoP antigen-coated cuvettes.

Therefore, both inhibition and absorption tests have promise as aids in establishing the origin of serologic reactivity to HyoP and Floc antigens and thus may aid development of a practical serodiagnostic test for MPS.

Selected References. [1]Sands,L.L. *et al.* Proc., IPVS 1982. [2]Armstrong,C.H. *et al.* Proc., IPVS 1980, p.224.