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SEROLOGIC EVIDENCE OF NATURAL AND EXPERIMENTAL TRANSFERS OF Chlamydia psittaci BETWEEN WILD AND DOMESTIC ANIMALS

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Organisms of the genus Chlamydia present challenging problems in epizootiology because of their wide distribution in nature and the variety of diseases they cause. Their presence has been detected in over 120 species of birds and numerous species of wild and domestic mammals. They have even been isolated from a variety of arachnids³ — although these hosts as ectoparasites were associated at one time or another with infected hosts. In avian or mammalian hosts, Chlamydia psittaci causes generalized septicemia, pneumonitis, polyarthritis, pre-natal infection resulting in abortion, encephalomyelitis, conjunctivitis, or enteritis depending upon which tissue is attacked. Mortality is generally low and restricted to the weak, the young, or those with concurrent disease. In severe epizootics, however, virulent strains of C. psittaci may cause death rates of 20% in humans, 30% in birds, 5% in lambs, and 50% in some wild mammals. Abortion losses may reach 10% of the unborn in infected cows and ewes. Economic losses caused by these diseases in domestic hosts approximate 10 million dollars annually in the U.S.

Our mission at the NADL ultimately is to devise methods for controlling animal diseases, and one aspect of control to is to identify and eliminate the sources of the disease agents to domestic animals, or at least to break the chain of transmission. With respect to the chlamydioses, these sources have been very difficult to identify, for when an investigator studies an epizootic in domestic animals, he must determine whether the disease agent came from any of a variety of hosts which excreted it into the environment of the susceptible animals, or whether the agent was already present in the affected herd or flock. Ectoparasite or venereal transmission cycles are also possible.

The first possibility — that is, that the source of disease agent was an outside group of infected mammals or birds — is what has interested us most, and we should like to mention some serologic investigations that relate to this aspect of transmission. In these investigations, we have used serology as a useful indicator of infection when clinical observations or disease agent recoveries are lacking. Regarding exogenous sources of chlamydiae, birds are probably the most common and most widely distributed reservoirs of chlamydiae. The parrot (Psittacidae) and pigeon (Columbidae) families are the most common carriers of chlamydial organisms, and members of the latter family are the most likely suspects for contamination of the environment of domestic animals. Most farms in the U.S., for example, have a continuous natural complement of feral pigeons intermingling with the domestic populations. Surveys have shown that pigeons all over the world are infected with chlamydial organisms. Of 16,500 pigeons tested by 50 investigators in 24 countries, 4,400 or 27% of the birds were seropositive for chlamydiosis, and 20% of 3,200 birds examined for the presence of chlamydiae were carriers.² In the authors' experience only two serologically negative groups of pigeons have been found among 50 different groups comprising approximately 1,000 birds in California and Iowa.

Listed in Table 1 are some reports based on natural epizootics with suggestive serologic evidence of interspecies transfers of chlamydiae. The first two reports in this table involved diseased turkeys in California. In the first report, pigeons were suspected as being the source of the disease agent since the turkey flock had been a closed, healthy breeding flock for 14 years prior to the uninvited arrival of pigeons on the turkey ranch.

Case	Clinical problem host	Incidental or source host?	State, year, and reference
1	1000 diseased TURKEYS 6 seropositive/6 tested 3 carriers/3 tested	15 healthy PIGEONS 14 seropositive/15 tested 4 carriers/15 tested	California, 1954 (Bankowski & Page, 1959)
2	2100 diseased TURKEYS 70 seropositive/75 tested 4 infected/4 tested	5 healthy SPARROWS Pooled tissues pos. for chlamydiae	California, 1956 (Page & Bankowski, 1959)
3	2100 LAMBS, 50 lame with polyarthritis 10 seropositive/10 tested 2 infected/5 tested	500 feeder CATTLE, apparently healthy, 34 seropos./38 tested	Iowa, 1966 (Page & Cutlip, 1968)
4	200 COWS, 10 abortions, 30 neonatal deaths	37 healthy PIGEONS 27 seropos./37 tested 0 isolations/7 tested	Oregon, 1964 (Page and Erickson, unpublished data)
	140 EWES, 30 neonatal deaths	3 healthy MAGPIES 0 seropos./3 tested	
		140 EWES 27 seropos./32 tested	
		20 CHICKENS 4 seropos./12 tested	

TABLE 1. Natural chlamydiosis involving two or more species on the same premises

Acute, lethal chlamydiosis appeared in the turkeys one year following the arrival of the pigeons. At necropsy, no gross lesions were observed in the pigeons, although their cloacal contents contained millions of chlamydiae.¹

In the second report, sparrows with no gross lesions but carrying chlamydiae nested in the only tree on a turkey breeding premises where the turkeys became severely diseased.⁵

In the third report, feeder lambs imported into Iowa from several western states developed chlamydial polyarthritis within two months after arrival. At the same time, 90% of clinically healthy calves maintained in a lot contiguous to that of the sheep, were seropositive. Probably the lambs were infected prior to their importation into Iowa, but the high incidence of seropositiveness in the calves is evidence that they were also infected. Did the calves become infected from the lambs? A strain of *C. psittaci*, isolated from lame sheep, was infectious and lethal for calves, but it was not possible to examine the calves on the farm further so the question cannot be answered.

An interesting unpublished case investigated by the authors occurred in Oregon, where 200 cows on a ranch near Baker, Oregon, had experienced 40 abortions and neonatal calf losses in March of 1964 (Ranch A in Table 2). Of 43 cows tested at that time, 86% were seropositive for chlamydial antibodies. An effort was then made to trace potential sources of the disease agent by serologic surveys of domestic and wild animals in the immediate environment of the cattle. Of 32 ewes on this ranch which had a high incidence of neonatal lamb loss, 84% were seropositive; of 12 chickens tested, 33% were positive; of 37 pigeons, 73% were positive, and of 3 magpies, none was positive. Except for the magpies, the incidences of seropositiveness in all of the species on this ranch were much higher than normal for corresponding animals on other farms as detailed in Table 2. Except for one herd, the incidence of positive chlamydial serology in cattle on five neighboring ranches was equal to or less than the general incidence among cattle in the U.S. which is around 20%. Of a group of 19 pigeons on neighboring Ranch B, none was seropositive. This finding is in contrast to a 73% incidence of seropositiveness in pigeons on Ranch A. Similarly, 5 pheasants taken on neighboring ranches in October 1964 were seronegative, although by March 1965, 2 other pheasants were seropositive.

An attempt was made to determine the persistence of circulating antibody in the cattle on Ranch A to see if there was any further antigenic stimulation through chlamydial reinfection. While 86% of the cows tested were seropositive in May of 1964, 70% of those tested in October were positive, and by February 1966 the date of the next bleeding, only 45%of those tested were positive. All of this indicated a definite downtrend in the titers and reflected a declining exposure to chlamydial antigens. The results of tests on serums of 26 individual cows — representing 3 con-

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TABLE 2. Serology	of chlamydial infections in wild and domestic	animals on
	ranches near Baker, Oregon, in 1964	

Ranc cod		Number animals	r Iı GCattle		of positive Chickens		ial serolo; Pheas.	gy* Magpies
A	Bovines 10 abortions 30 deaths of newborn. Mar. 64	200 cows 140 ewes 20	39/43 (89%) May 64 62/87 (71%)	27/32 (84%) Oct. 64	4/12 (33%) Oct. 64	27/37 (73%) Oct. 64		0/3 Oct. 64
	Ovines 30 deaths of newborn from 140 ewes	Chicken	^s Oct. 64 39/87 (45%) Feb. 66					
В	Bovines 100 deaths of newborn: with enteritis Mar. 64; 1 abortion, Mar. 65.	1000 cows	3/23 (13%) Oct. 64			0/19 (0%)	0/4 Oct. 64 1/5 Mar. 65	
С	Bovines 150 deaths of newborn with enteritis; Mar. 64	900	9/40 (22%) Oct. 64		_		0/1 Oct. 64 1/1 Mar. 65	0/6 (0%)
D	Bovines Some abortions; 140 infertiles	435	4/26		_		_	
E	Bovines 3 abortions, Mar. 65	400	13/27 (48%) Oct. 64		_		_	
F	<i>Bovines</i> 29 abortions (Mar-Oct. 64)	240	1/11 (9%) Oct. 64				_	
G	Ovines "numerous" abortions, Mar. 65			4/4** Mar. 65			_	

*Number serologically positive animals/number tested. CF test was used with "EBA" strain of *Chlamydia psittaci* as antigen for bovine serums.

**Titers were very high ranging from 1:128 to 1:1024.

secutive bleedings of each animal — were then compared (Table 3). All 26 were seropositive in May 1964, but by October 1964, 12 animals had become seronegative, and by February 1966, 17 of the 26 were seronegative. So over a period of 21 months, the titers of 25 of the 26 cows

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Number	Tite	Titer		
cows	May 1964	October 1964	February 1966	trend
1	32	16	8	Downward
1	32	8	8	21/26
1	16	16	8	(81%)
1	16	16	4	. ,
2	16	8	4	
1	16	8	Neg	
2	16	Neg	Neg	
2	8	8	Neg	
2	8	Neg	Neg	
8	4	Neg	Neg	
1	16	32	16	Slight rise
1	16	32	4	and fall
2	4	8	Neg	4/26 (15%)
1	4	4	8	Slight rise 1/26 (4%)
26	26 Pos./26	14 Pos./26	9 Pos./26	
	(100%)	(54%)	(34%)	

TABLE 3. Chlamydial serology of individual cattle from Ranch A, Baker, Oregon

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had dropped. One had a slight rise. The decline in titers reflected a lack of continued antigenic stimulation by chlamydiae, a fact which correlated with the subsequent clinical experience in the herd, for no further abortions occurred. One can conclude from the serology here that the chlamydial epizootic was a single event and did not result in a large number of cows remaining as infected carriers.

We also attempted to transfer chlamydiae between various species of hosts experimentally, (Table 4). In the first test, 5 infected pigeons were caged above 10 normal, young turkeys in a coop. All of the birds were bled for serology at biweekly intervals. By the 6th week, all of the turkeys became seropositive. At the eighth week, the pigeons were removed, the quarters were cleaned, and 5 normal turkeys were added to the group. All 5 became seropositive within 4 weeks. An additional 4 normal turkeys were added later and 2 of these 4 birds became seropositive within 4 weeks. Although the birds remained clinically healthy, these results indicated that chlamydiae can be transmitted by natural means from pigeons to turkeys and then passaged again by natural means through successive groups of turkeys.⁴

In other tests conducted by the senior author, caged sheep and calves were exposed for periods up to six weeks to a continuous stream of exhaust air from cages of experimentally infected birds. One lamb exposed to pigeon cage air in this manner developed a chlamydial antibody titer within 6 weeks. It remained clinically healthy and was essentially normal at necropsy, but chlamydiae were not isolated from its tissues. Two other

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TABLE 4. Serology of experimental transfers of chlamydiae between different hosts

Case	Infected primary host	Route or vector	Secondary host	Reference
1	PIGEON	Airborne	TURKEYS (10 seropos./10 exp.)	Page (1960)
			TURKEYS (5 seropos./5 exp.)	
			TURKEYS (2 seropos./4 exp.)	
2	PIGEON	Airborne	LAMB (1 seropos./1 exp.)	Page (unpub.) 1965
3	SPARROW	Airborne	LAMB (0 seropos./2 exp.)	Page (unpub.) 1965
4	PIGEON	Airborne	CALF (0 seropos./2 exp.)	Page (unpub.) 1965
5	SHEEP	Cage contact	TURKEYS 15 seropos./20 exp. 11 infected/11 examined.	Pierce et al. 1964

lambs exposed for 6 weeks to a continuous stream of exhaust air from a cage of sparrows infected with C. *psittaci* originally isolated from sparrows failed to develop positive serology, clinical signs or lesions from the experience.

In two other tests, 2 calves exposed to overhead caged pigeons infected with C. *psittaci* originally isolated from a cow with enteritis failed to develop chlamydial antibodies, or clinical signs.

In the fifth case listed in Table 4, Pierce and his colleagues⁷ at Texas A & M, found that virulent chlamydiae transferred readily from experimentally infected ewes to turkeys kept in the same confines. Fifteen of 20 exposed turkeys became infected, and 11 had severe lesions of chlamydiosis.

In summary, we have briefly reviewed some investigations of potential interspecies transfers of chlamydial organisms and presented serologic evidence of natural and experimental infections of the agents among wild and domestic animals.

These observations indicate that transfers of chlamydiae between different host species are possible, and that the likelihood is great that they actually occur in nature.

It cannot be concluded from the evidence presented that transfers of chlamydiae between wild and domestic animals play a significant role in disease problems in domestic animals. However such transfers of organisms would cause occasional, subclinical infections and provide periodic antigenic stimulation of farm animals. This in turn would account for a steady "normal" incidence of seropositiveness among these domestic animal populations.

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