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Comparison of "Pathotypes" Among Chlamydial (Psittacosis) Strains Recovered From Diseased Birds and Mammals[1]

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ABSTRACT

Studies of the pathogenicity for laboratory animals of organisms of the genus *Chlamydia* (psittacosis-LGV-trachoma group) recovered from diseased birds and mammals were extended to include three strains isolated from cows which had a history of encephalomyelitis or abortion. Each of the strains was titrated or passaged in chicken embryos, mice, guinea pigs, pigeons, turkeys, sparrows, parakeets and sheep. In terms of pathogenicity for these animals, one of the bovine abortion strains was identical to a previously studied pigeon strain in that it produced severe systemic disease indistinguishable from psittacosis in mice, pigeons, sparrows, parakeets and turkeys, while not affecting sheep or guinea pigs. The second abortion strain produced lesions in mice and chicken embryos only. The encephalomyelitis strain caused death and severe lesions in guinea pigs and dwarfing of chicken embryos but otherwise failed to affect the other test species. The "pathotype" determination may reveal significant epizootiologic relationships and may be useful in identifying the source of chlamydiae to domestic animals or in recognizing mutants with new pathogenic capabilities.

INTRODUCTION

The approach to this work was to compare strain of *Chlamydia*[2] originally isolated from diseased wild and domestic animals for their pathogenicity for selected laboratory animals in the hope that the resultant pathogenicity patterns might reveal which "pathotypes" (=defined pathogenicity spectrum) were potential disease agents for domestic animals. Also, comparisons of pathotypes might illumine relationships between strains which previously were thought to cause only one disease syndrome in certain animal species. Knowledge of these relationships would assist in the identification of reservoir hosts which might serve as sources of chlamydial agents to domestic animals.

Little evidence, if any, has been reported to ascertain the original source or reservoir of the chlamydial agents to affected animals in past epizootics of encephalomyelitis, abortion, arthritis, or

[1] Oral presentation at the 67th Annual Meeting of the American Society for Microbiology, New York, N.Y., May 1, 1967.

[2] The taxonomy Subcommittee on the Chlamydiaceae of the American Society for Microbiology has approved a proposal[11] to include all organisms of the psittacosis-LGV-trachoma group in the genus *Chlamydia*.

pneumonia in domestic cattle and sheep in the United States after the causative chlamydiae were isolated and identified. This is not true in the case of man, for one of the major reservoirs of chlamydiae for humans since the 1890's has been infected psittacine birds. Other wild and domestic birds have also been proven to be sources of the disease agent for man. In chlamydial epornitics in domestic turkeys, investigations have revealed that pigeons,[1] sparrows,[9] or nest mites[3] in the immediate vicinity of the diseased turkeys were also infected. Ticks and other arachnids have been implicated as mechanical carriers of chlamydiae in areas where epizootic bovine abortion occurs.[7] In no epizootics in domestic animals, however, has the natural reservoir been identified. This aspect of chlamydial epizootiology deserves further investigation.

The pathogenicity spectra of chlamydial strains from a variety of human and animal sources have been charted for diagnostic purposes previously by Meyer and Eddie[8]. The spectra developed through their investigations were based on observations of laboratory animals inoculated by several routes with large numbers of organisms. Their information was useful for selecting the appropriate animal species and route for isolating and propagating various chlamydial strains. The work reported here was concerned with chlamydial strains that presumably have an opportunity to infect wild reservoir hosts and domestic animals, all of which intermingle in the same farm environment. Thus, our test animals included wild and domestic birds and a domestic mammal, as well as the usual laboratory animals.

The immediate objectives were (1) to determine the pathogenicity patterns of three chlamydial strains recovered from cattle which had signs of encephalomyelitis or had aborted, and (2) to compare the results of these tests with the patterns previously established[12] for four chlamydial strains recovered from a diseased turkey, a pigeon, an aborted ovine fetus and a lamb with infectious polyarthritis.

MATERIALS AND METHODS

Except for variances noted below, the technical details connected with the procurement, preliminary testing, management, infection, and clinical, pathologic and serologic observations on the test animals are found in a previous report[12]. However, a brief recounting of essential facts and methods is desirable.

The test animals and ages were: mice, 4 weeks of age; guinea pigs, 6 weeks of age; pigeons, 6-8 weeks of age; English sparrows, 3-12 months of age; parakeets, 3-6 months of age; Broad Breasted Bronze turkeys, 6 weeks of age; Dorset lambs, 2 months of age; and White Leghorn chicken embryos, 7 days of incubation. All of the animals except chicken embryos, mice and guinea pigs were housed in plexiglas cages. Each cage had its own separate filtered air intake and exhaust and sewage disposal systems which prevented any cross contamination between groups of animals. Preliminary serologic, clinical and necropsy studies of all groups of animals assured procurement of healthy animals (except one case noted in Results) which were negative for previous chlamydial infection. After 2 to 3 days acclimation of the animals to their cages, they were inoculated on the same day with the appropriate suspension of chlamydiae. All of the animals were inoculated by the intraperitoneal route except pigeons (intracerebral route) and chicken embryos (yolk sac route). After inoculation, the animals were observed several times daily for two weeks, after which all survivors were bled for serology (except mice), killed and examined. Animals which died during the test were promptly necropsied. Records of clinical signs and gross lesions were made, and tissues from representative groups of animals (with and without lesions) were saved for chlamydial isolation attempts.

Chlamydiae were reisolated from tissues of affected test animals either by (a) inoculating mice intraperitoneally, looking for typical chlamydial lesions at necropsy two weeks later, and examining exudates for the presence of *Chlamydia* infected monocytes (Figure 1); or, (b) inoculating triturated tissues into chicken embryos and looking for typical gross lesions in the yolk sac and embryo when the embryo died. Identification of the newly isolated organisms was confirmed by preparation of an antigen from infected yolk sacs and titrating the antigen against chlamydial antiserum in a complement fixation test.

For purposes of inoculation, a single series of tenfold dilutions of a suspension of each

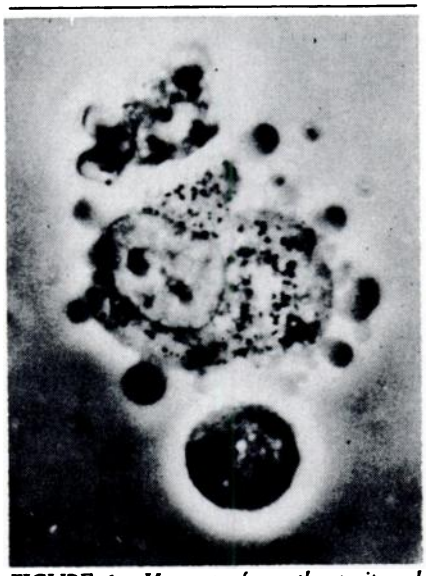

FIGURE 1. *Monocyte from the peritoneal exudate of mouse inoculated with Chlamydia. The cytoplasm of the cell is dotted with maturing chlamydial bodies. Phase contrast photomicrograph.* 4500X.

chlamydial strain was prepared, and certain volumes of each of selected dilutions were inoculated into groups of each animal species. The full series of dilutions from 10^{-1} to 10^{-8} were inoculated into chicken embryos, mice and guinea pigs to determine the lethal and infective 50 percent endpoints for each chlamydial suspension. The other test animals received a single amount of the 10^{-1} dilution of the suspension only. The volume of each inoculation dose is listed in Table 2.

The number of animals used per dilution of the original suspension of organisms was ten chicken embryos, ten mice, six guinea pigs, six pigeons, eight sparrows, six parakeets, six turkeys, and one lamb.

The direct complement fixation method for detection of chlamydial *group* antibodies was used for the pre- and post-inoculation serology of the guinea pigs, pigeons and lambs. The indirect complement fixation method as previously described[10] was used for sera from sparrows, parakeets and turkeys. Only animals clinically and serologically negative with respect to psittacosis were used so that the post-inoculation presence of circulating chlamydial antibodies in animals reflected a specific chlamydial antigenic stimulation. The chlamydial inocula were prepared by propagating each strain in the lungs of mice (after intranasal instillation); thus, harvested, triturated, Chlamydia-infected mouse lungs were used as inocula for pathogenicity tests. The purpose in using mouse-propagated chlamydial inocula instead of egg-propagated inocula was to avoid the development of antibodies to egg proteins in the test animals. Such antibodies would interfere with the complement fixation tests since the chlamydial antigen used in the serologic tests was prepared wtih egg-propagated chlamydiae.

Chlamydial Strains. The bovine abortion strains were obtained from two sources.. The first strain, labelled Wolfsen Cattle (WC), was received from Dr. B. Eddie of the University of California's Hooper Foundation in San Francisco. This strain was isolated in 1963 from the intestinal tissues of a cow with hemorrhagic enteritis. The organism was implicated in an epizootic of bovine abortions in California and also was implicated in a case of fatal pneumonitis in the owner of the affected cattle[2]. A 10th yolk sac passage culture of the organism was passaged in mice for use in the present pathogenicity tests.

The second bovine abortion strain, labelled "EBA" was obtained from Dr. D. G. McKercher of the University of California, Davis. This strain was isolated in 1959 by Dr. J. Storz from tissues of an aborted bovine fetus[16]. The pathogenicity of this strain for pregnant cows, for various tissue cultures, and for chicken embryos has been described[15], but its pathogenicity for other animals has not been reported. A 10th yolk sac culture propagated in mice was used as inocula for the present tests.

The bovine encephalomyelitis strain, labelled "McNutt" and "E58" was isolated in Iowa in 1940 by Dr. S. H. McNutt from the pleural exudate of one of a group of calves showing signs of encephalomyelitis[8]. The strain was passaged in chicken embryos and frozen; infected yolk sacs from these embryos were sent to the laboratory of Dr. Herald Cox of Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y., from which cultures were sent to Dr. John Enright of the University of California, Davis. Dr. Enright gave the author a 14th egg passage culture of the strain. A 16th egg passage culture propagated in the lungs of mice was used in the present tests.

RESULTS

Results of the inoculation of groups of various species of animals with three chlamydial strains isolated from diseased cattle are listed in Tables 1 and 2, and a schematic diagram of these results in comparison with those previously pub-

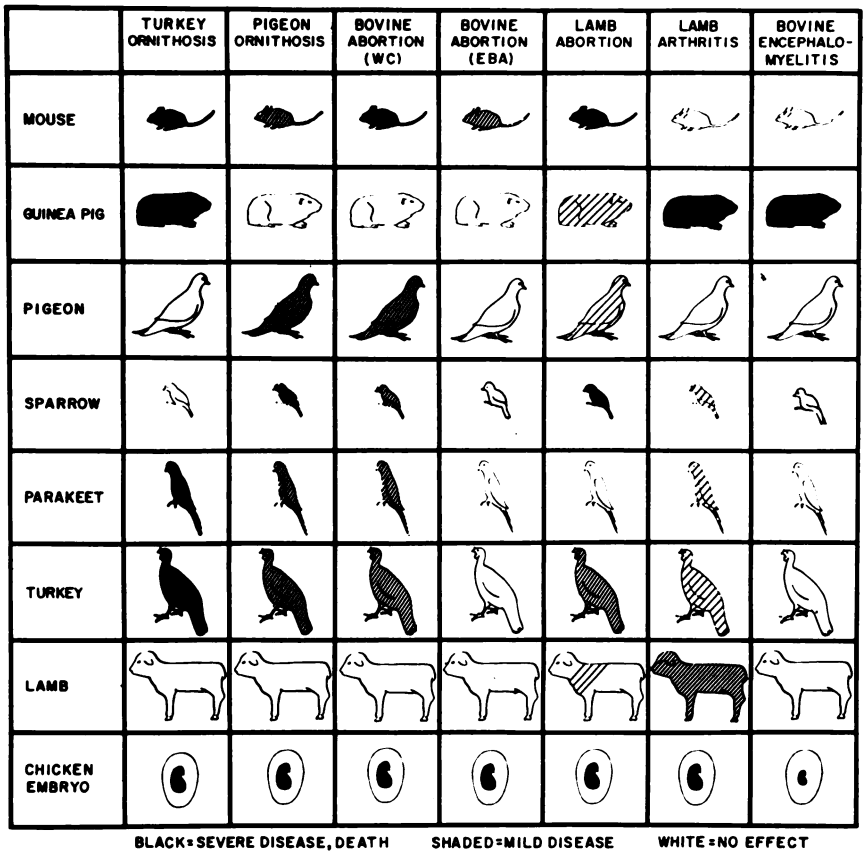

FIGURE 2. *Schematic chart representing the pathogenicity of various chlamydial strains for eight species of test animals.*

lished for four other chlamydial strains is presented in Figure 2.

The "WC" bovine abortion strain produced lesions typical of psittacosis in mice, pigeons, sparrows, parakeets, and turkeys, and no lesions in guinea pigs and lambs. The severe lesions caused by this strain in turkeys, for example, are seen in Figure 3. As shown in Figure 2, the "WC" strain had the same pathogenicity spectrum for the test animals as did the chlamydial strain isolated from pigeons. In fact, the gross lesions produced by either strain in a given test animal were indistinguishable.

In contrast, the "EBA" strain was of very mild virulence; for it produced visible lesions only in mice and in chicken embryos.

The "McNutt" encephalomyelitis strain caused a severe, fatal infection of guinea pigs, paralleling that caused by either the ovine arthritis or turkey ornithosis strain. The "McNutt" strain was unique among all the strains, however, in that it caused consistently a dwarfing of embryos (Fig. 4) and a late embryonic death pattern in chicken eggs inoculated with diluted suspensions of organisms. This strain failed to produce any visible effect on any of the other test species, except in the instance noticed below.

TABLE 1. *Results of titration of suspensions of three chlamydial strains in chicken embryos, mice, and guinea pigs.*

Host	Endpoint	Number 50 Percent Endpoint Units per Ml[1] Bovine Abortion Strains "WC"	"EBA"	Bovine Encephalomyelitis Strain "McNutt"
Chicken embryo	Death	80,000,000	250,000	12,500
Mouse	Death	630,000	0	0
	Lesions	63,000,000	10,000	0
Guinea pig	Death	0	0	20
	Lesions	0	0	100,000

[1] A single suspension of each chlamydial strain was titrated in all of the animal species. Lethal and infective endpoints were determined by the method of Reed and Muench[13].

TABLE 2. *Gross Lesions and serology of animals inoculated with three chlamydial strains isolated from diseased cattle.*

Host	Strain	Route	Dose (ml)	Gross Lesions and Signs	Positive Serology[2]
Chicken embryo	WC	YS	0.1	Severe vascular congestion, early death.	Not done.
	EBA	YS	0.1	Vascular congestion, late death.	Not done.
	McN.	YS	0.1	Dwarfed embryo; vascular congestion, very late death.	Not done.
Mouse	WC	IP	0.5	Severe fibrinous peritonitis,[1] splenomegaly, early death.	Not done.
	EBA	IP	0.5	Splenomegaly, survival.	Not done.
	McN.	IP	0.5	None.	Not done.
Guinea pig	WC	IP	0.5	None.	2 of 6
	EBA	IP	0.5	None.	2 of 6
	McN.	IP	0.5	Severe fibrinous peritonitis, perihepatitis, splenomegaly, death.	6 of 6
Pigeon	WC	IC	0.1	Splenomegaly and airsacculitis.	5 of 6
	EBA	IC	0.1	None.	5 of 6
	McN.	IC	0.1	None.	5 of 5
Sparrow	WC	IP	0.2	Severe peritonitis, airsacculitis, splenomegaly, survival.	8 of 8
	EBA	IP	0.2	None.	0 of 8
	McN.	IP	0.2	None.	0 of 8
Parakeet	WC	IP	0.2	Severe peritonitis, airsacculitis, splenomegaly, hepatomegaly, death.	2 of 5
	EBA	IP	0.2	None.	0 of 5
	McN.	IP	0.2	None.	0 of 5
Turkey	WC	IP	1.0	Severe peritonitis, airsacculitis, perihepatitis, pericarditis, survival.	4 of 4
	EBA	IP	1.0	None.	1 of 6
	McN.	IP	1.0	None.	2 of 6
Lamb	WC	IP	1.0	None.	1 of 1
	EBA	IP	1.0	None.	1 of 1
	McN.	IP	1.0	None.	1 of 1

[1] The evidence for the presence of peritonitis, airsacculitis, etc. was based on phase contrast microscopy of exudates from the peritoneal cavity, airsac membranes or organ surfaces. These exudates invariably contained large numbers of mononuclear cells which often had intracytoplasmic chlamydiae (Fig. 1).

[2] Column indicates number of serologically positive animals of total number inoculated.

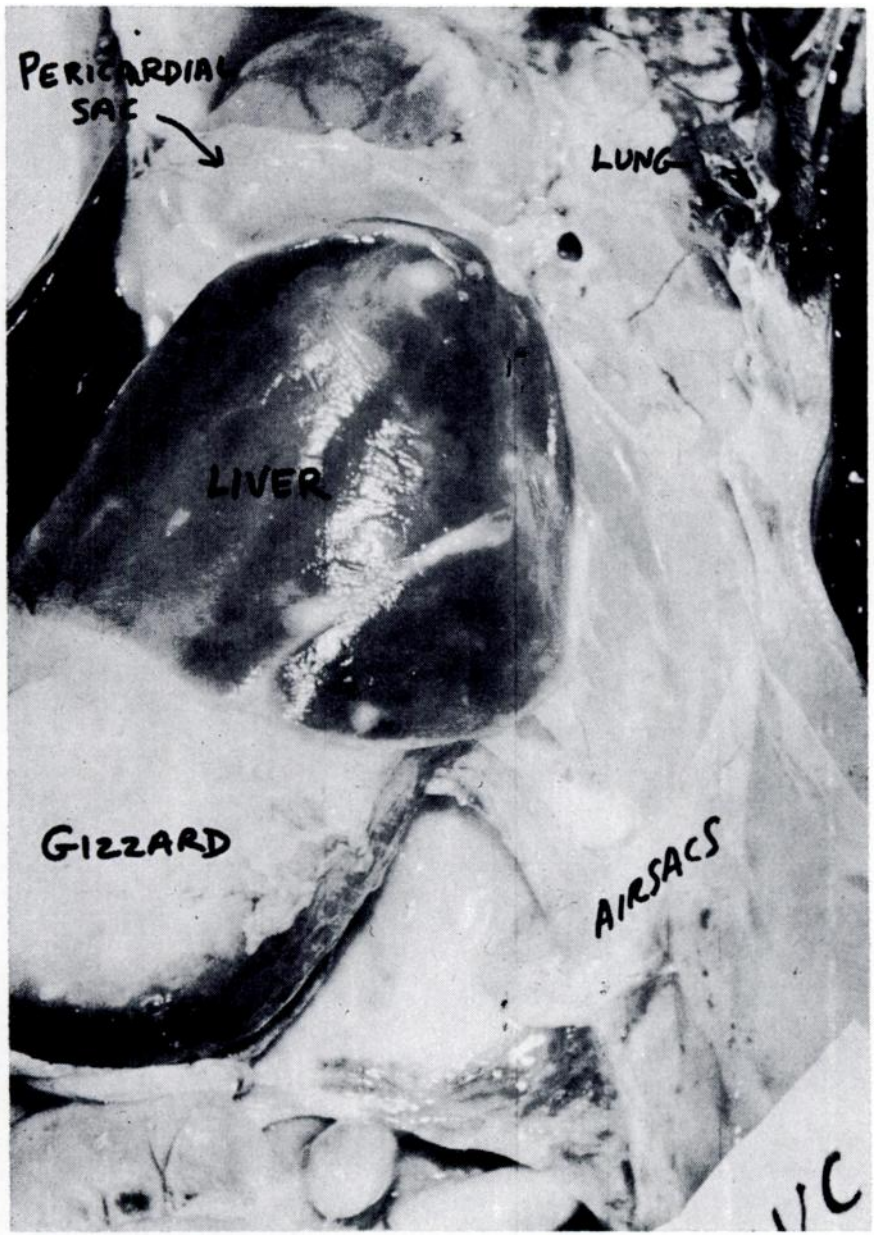

FIGURE 3. *Diseased viscera of a turkey inoculated with the "WC" bovine abortion strain of* Chlamydia. *Note fibrinous film covering liver and the thickened, exudate covered thoracic and abdominal air sacs.*

FIGURE 4. *The embryo on the left shows dwarfing with hemorrhage and congestion of the yolk sac capillary beds caused by growth of the "McNutt" bovine encephalomyelitis strain. The infected embryo was inoculated via the yolk sac at 6 days of incubation and died at 20 days of incubation. The normal embryo on the right was killed at 20 days of incubation.*

Although both the "EBA" and "WC" strains were avirulent for *healthy* turkeys (Table 2), it was fortuitously observed that the same strains were surprisingly virulent for turkeys concurrently infected with fowl pox virus. In separate tests not recorded in table 2, the "EBA" and "McNutt" strains were inoculated into poults which had unknowingly been vaccinated with a suspension of live fowl pox virus just prior to procurement of the birds for experimental purposes. Other members of the source flock of turkeys from which the experimental birds were purchased had shown signs of a natural fowl pox infection, and the owner promptly vaccinated all of his birds without mentioning the fact to procurement officers. One day prior to the intraperitoneal inoculation of the turkeys with chlamydiae, the birds showed skin lesions typical of fowl pox around their heads and at the site of vaccination in the wing web. It was decided to infect the birds with chlamydiae anyway. The dually infected birds were held for two weeks, killed and examined. It was immediately apparent that the turkeys had developed lesions of severe ornithosis: thickened airsacs covered with inflammatory exudate, enlarged spleens, perihepatitis and pericarditis (Figure 5). Control birds which had not been inoculated with chlamydiae developed pox lesions also, but had no other gross lesions of the airsacs, viscera and other organs. Subsequently, the pox virus was isolated from skin lesions of the latter birds and was introduced in pure culture intradermally and intraperitoneally into normal turkeys. The virus produced typical skin lesions in these birds and failed to cause any visible changes in airsac membranes. The "EBA" and "McNutt" chlamydial strains were also inoculated intraperitoneally into another set of normal turkeys and both failed to produce any sign of airsacculitis or gross lesions similar to those caused by the "WC" strain or other pigeon or turkey strains of *Chlamydia*.

Titration of the various strains in laboratory animals emphasized the greater sensitivity of guinea pigs over chicken

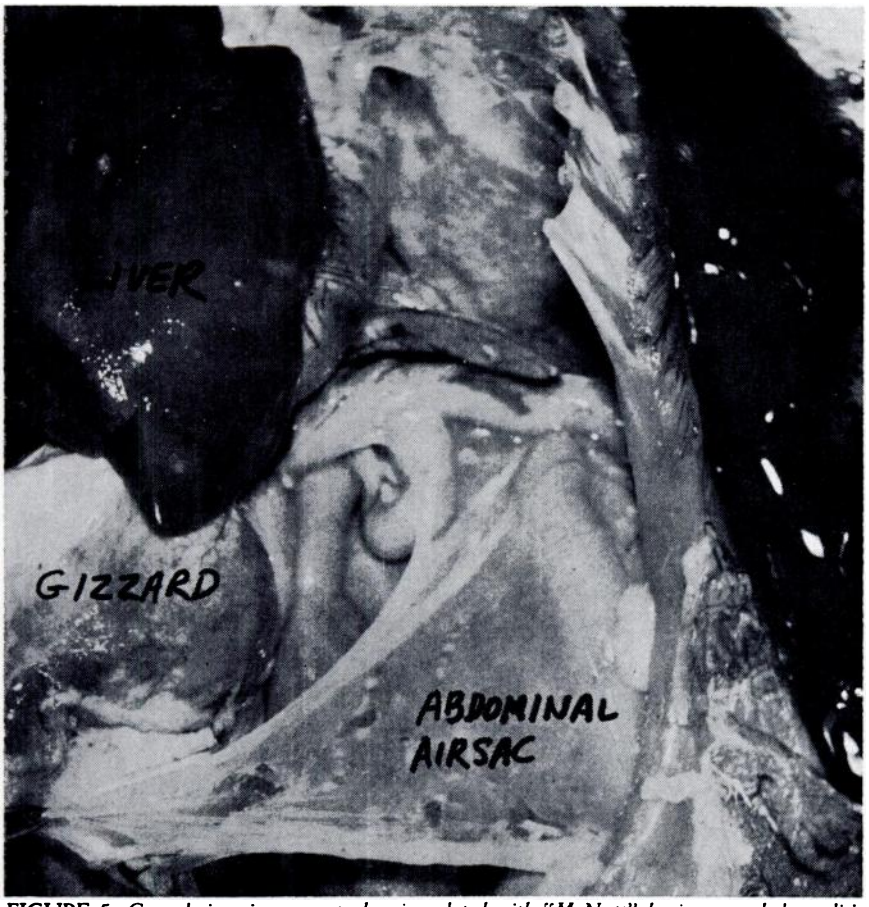

FIGURE 5. *Gross lesions in young turkey inoculated with "McNutt" bovine encephalomyelitis strain of Chlamydia following inadvertent vaccination for fowl pox. The combined infection produced severe airsacculitis and serositis in turkeys whereas fowl pox virus alone or the "McNutt" strain alone did not produce airsacculitis in other turkeys.*

embryos in determining the number of infectious particles in a suspension of certain chlamydial strains. When a suspension of either the encephalomyelitis strain or the ovine arthritis strain was titrated simultaneously in guinea pigs and chicken embryos and the infective and lethal 50 percent endpoints were calculated for each titration two weeks after inoculation, the ID_{50} endpoint in guinea pigs was considerably higher than the LD_{50} endpoint in chicken embryos. As shown in Table 1, the number of embryo LD_{50} units determined for the suspension of the "McNutt" strain was found to be 12,500 per ml while the guinea pig ID_{50} units was 100,000 per ml. Similarly, in a previous report[12], the number of embryo LD_{50} units in a suspension of ovine arthritis chlamydiae was 3,000,000 while the guinea pig ID_{50} units for the same suspension was 250,000,000. This makes the guinea pig the host of choice for isolation of chlamydiae from encephalitic cattle or arthritic lambs.

Complement fixation tests on the ser-

ums from the various infected animals substantiated that chlamydial organisms were indeed introduced into the test animals where they produced an antigenic stimulus (Table 2). The "WC" strain stimulated an antibody response in every species tested. The "EBA" and "McNutt" strains caused an antigenic stimulus in all species except sparrows and parakeets.

Organisms of the "WC" strain were readily reisolated in mice from tissues of all the affected species inoculated with this strain. The "EBA" and "McNutt" strains were reisolated in chicken embryos from all affected species.

DISCUSSION

In the author's view, the observations reported here have some bearing on the epizootiology of chlamydiosis (this term being used to describe disease in any species caused by a chlamydial organism). The fact that the "WC" and "EBA" bovine abortion strains differed widely in their pathogenicity spectra indicates (a) that no single chlamydial strain with a given pathotype is the sole cause of chlamydial abortion in cattle, and (b) suggests that the two strains originated from different chlamydial reservoir hosts. This is because a chlamydial strain, such as "EBA", with a limited pathogenicity range, would tend to be restricted to a few natural hosts (including bovines), whereas strains with a broad pathogenicity pattern, such as "WC", would tend to be causing varying disease syndromes in a variety of natural hosts. This broad pattern is characteristic of chlamydiae isolated from wild avian hosts. Supporting this theory is the fact that the "WC" strain was not only implicated as a cause of bovine abortion but also as the cause of fatal pneumonitis in the owner of the affected cattle[2].

The identity in pathogenicity spectra shown for the "WC" and previously studied pigeon ornithosis strain suggests that both strains had a common reservoir host. Probably the host is a pigeon species since other chlamydial isolants from pigeons have similar pathogenicity spectra.

There is an interesting parallel observation reported by Schachter[14]. He isolated chlamydiae from inguinal lymph node exudate of a man showing lesions typical of lymphogranuloma venereum. These organisms could not be distinguished by pathogenicity pattern or by tests for glycogen production or sulfadiazine sensitivity from chlamydiae normally recovered from infected psittacine birds.

Schachter's observations and those reported here point to the inadvisability of assigning any one chlamydial pathotype to a specific natural disease.

It should also be noted that serologic similarity between the specific cell wall antigens of two strains of chlamydiae does not necessarily reflect an identity of pathogenic capabilities for the two strains. Fraser and Berman[4] have shown that an ovine arthritis strain and the "McNutt" encephalomyelitis strain have cell wall antigens that specifically cross react in complement fixation tests. Yet, in our present tests, the two strains differed in their pathogenic effects on two of the eight test species. The arthritis strain invariably caused arthritis in lambs while the "McNutt" strain did not. The "McNutt" strain caused dwarfing of chicken embryos while the arthritis strain did not.

Thus it appears that although pathotyping or serotyping may be useful for epizootiologic purposes, those techniques do not allow the chlamydiae to fall into natural groups for taxonomic purposes. In regard to this, all of the strains represented in Figure 2 were tested for glycogen production and sensitivity to sodium sulfadiazine and found to be negative. Therefore all of these strains belong in the second of Gordon and Quan's[5] two natural groups of chlamydiae.

It seems likely that there is a large reservoir of chlamydial agents, perhaps with common morphologic or chemical characteristics[5] but with varying pathogenic or serologic characteristics, that intermingle among many species of the animal world.

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