PATHOGENESIS AND INTERACTION OF SELECTED MICROORGANISMS CAUSING RESPIRATORY DISEASE IN THE CHICKEN

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CHAPTER I

INTRODUCTION

The poultry industry is of great economic importance in some areas of the United States, especially in Alabama, Arkansas, California, Delaware, Georgia, Maryland, North Carolina, Texas and Virginia where the large broiler operations are located. Several thousand chickens are reared together on the floor and a respiratory disease agent can spread rapidly almost before the raiser is aware that there is a problem.

Research is continually being conducted on poultry diseases. The etiologic agent(s), its pathogenesis and its interaction with other agents are being determined. Once this information is known, methods of disease control and prevention can be implemented. Considerable knowledge about the pathogenesis (multiplication and migration) and the effect of mycoplasma infection, alone and in combination with other disease agents, in poultry has been accumulated in recent years. In contrast, little experimentation has been done with the paramyxovirus strain Yucaipa (PMY) isolated from chickens a few years ago. Studying the pathogenesis of this paramyxovirus by itself and in combination with mycoplasma should contribute useful information

in understanding how it affects poultry before any serious outbreak occurs. After isolation of the virus in 1960, Bankowski et al. (6) stated that it did not appear to be wide spread, but it might be capable or instrumental in triggering or aggrevating otherwise silent infections into full scale disease outbreaks.

PMY was isolated from a chicken flock in California with a respiratory infection tentatively identified as infectious laryngotracheitis. This virus is very similar to Newcastle disease virus (6). A survey made of chicken serum obtained from 169 flocks with respiratory infection found antibodies to PMY in only 3 of the flocks (6).

Mycoplasma has been isolated from chickens affected by practically all kinds of respiratory infections. Chu (10) states that there is some evidence that there may be more than one type of mycoplasma in poultry, some types being able to cause disease by themselves while others are capable of acting as secondary invaders. Other mycoplasma e.g. Mycoplasma species (strain Tu), are not considered to cause disease at any time even in combination with viruses or bacteria (15).

Mycoplasma can cause a chronic infection in chickens and can be transmitted through the egg. The organism can be recovered several months after infection, as seen by the isolation of mycoplasma from the caseous material from the oviduct of mature hens which were inoculated at one day of age via the umbilicus with Mycoplasma gallisepticum

(Mg) (21). Respiratory distress may be the first sign of mycoplasma infection in a flock or there may be no signs of infection. The Mg infection in the trachea is not uniform, rather only limited areas are infected (14). Tu by itself does not produce clinical signs or gross pathologic lesions. Its multiplication in the tracheal exudate is not altered by the presence of Mg unless a viral infection is superimposed (15).

Similar results have been reported on the effect of antibotics on mycoplasma infected chickens. Barnes et al. (9) found that one parenteral injection of tylosin (5 mg/lb) controlled experimental infection in chickens as determined by weight gains, serology, occurrence of lesions, and the recovery of mycoplasma. Experiments by Olesiuk et al. (27) with tylosin tartrate, chlorotetracycline, and erythromycin showed tylosin to be highly effective in treating mycoplasmosis, thus improving weight gains and reducing or preventing the incidence of clinical signs of infection.

The multiplication of mycoplasma in the trachea is greatly enhanced when a virus such as Newcastle disease or infectious bronchitis damages the intact layer of mucus and tracheal mucosa. The degree of enhancement is directly proportional to the amount of damage to the tracheal system with no enhancement seen when the trachea is in a state of repair (13). In addition the incidence of airsacculitis greatly increases with a resulting increase in mortality and condemnation on processing (20).

CHAPTER II

REVIEW OF LITERATURE

Due to the lack of marked clinical signs in mycoplasma (PPLO) infections, the importance of this infection may be underestimated. Many factors, physical and chemical, as well as other infective agents can change a mycoplasma infection from a mild disease to a severe one.

In his work on PPLO and respiratory diseases of poultry, Chu(10) isolated Mycoplasma species not only from chickens with chronic respiratory disease but from chickens affected by respiratory disease due to infectious laryngotracheitis, infectious bronchitis, and Newcastle disease virus (NDV), and from apparently normal chickens. He concluded that there was more than one species of mycoplasma involved in respiratory disease, with possibly a specific mycoplasma species responsible for producing uncomplicated chronic respiratory disease.

Chu(10) isolated two types of mycoplasma, morphologically different, from uncomplicated chronic respiratory disease. One was characterized by the production of medium sized macroscopic colonies with the characteristic raised center in 24-48 hours. Stained smears of these cultures showed mainly rings, globules, bipolar bodies, and a small

number of coccobacilliform bodies. The second type, containing a large number of coccobacilliform bodies, produced a colony, visible only microscopically, on artificial medium after 4-5 days incubation which often lacked the characteristic raised center. Upon intranasal inoculation into chickens, the second type produced a coryza while the first type did not cause respiratory signs.

Adler et al. (3) also isolated two types of mycoplasma from chickens. They were similar to the two types described by Chu. Adler showed these two types to be serologically different with the smaller colony being a pathogen and the larger colony type a nonpathogen.

The pathogenesis of mycoplasma in chickens has been studied by Corstvet and Sadler (14) using the fluorescent antibody technique. They found that Mycoplasma gallisepticum (Mg), after intratracheal inoculation, multiplied in both the trachea and air sacs. The multiplication in the trachea was not uniform as to the area of trachea infected or the amount of infection in any area. The amount of multiplication in the trachea or the tracheal area infected did not influence the production of infection in the air sacs. Their results indicated that before Mg could enter the log phase of growth it had to adjust to its surroundings and alter them in some way. Its pathogenic action may be due to physical interference with the physiologic processes of the cells since it must line the epithelial cell wall before its pathogenic action is

manifested. The irregular and inconsistant findings of tracheal and air sac infection and multiplication of Mg in these tissues was reflected in the irregularity and inconsistency of the serologic response.

Several investigators (21,22,23,29,31) have inoculated Mg into chickens by various routes and observed similar pathologic changes in the respiratory tract. After inoculation of Mg, clinical signs of infection were observed in some of the chickens. Clinical signs were seen in uninoculated penmates about a month after signs of infection were observed in inoculated chickens. Agglutinins to Mg were first detected in the serum a few days to two weeks after inoculation of the organism, depending on the route of inoculation (21,22). The Mg agglutinins were first observed in chickens inoculated intravenously or inoculated intravenously and in the air sacs and last in those inoculated in the foot pad. Contact chickens produced agglutinins to Mg about a week after the inoculated chickens (21,22). The Mg organisms could be reisolated from various tissues for varying lengths of time after inoculations, e.g. up to 206 days from the trachea of chickens inoculated in the foot pad (21,23,29).

By intranasal inoculation of Mg or inoculation into the infraorbital sinus, McMartin (23) found the infection was restricted almost entirely to the nasal passages. Mg was not isolated from the air sacs and was isolated from only one trachea. He was able to produce infection in the

air sacs only by direct inoculation of Mg into the air sacs of susceptible chickens. No air sac infection occurred in chickens which had been inoculated intranasally with Mg 21 days previously or chickens which had been in contact with Mg infected chickens even though Mg was inoculated into the air sacs (23,25).

Mycoplasma can be transmitted to the embryo through the egg resulting in chickens which are infected at the time of hatching. Several investigators (18,19,28) have examined the affect on egg transmission of early exposure of uninfected chickens to Mg. All the investigators found that when Mg was inoculated into chickens less than five weeks old, egg transmission of Mg was reduced or did not occur. Control chickens which became infected naturally by eight weeks of age transmitted Mg through the egg.

Corstvet et al. (15) observed that as a single infection Mycoplasma species (strain Tu) did not produce clinical signs or gross pathologic lesions. It did multiply in the tracheal exudate. This multiplication was not affected by the presence of Mg unless NDV was superimposed. The increased multiplication of Mg as a result of the NDV infection increased the duration and the amount of exudate produced thus increasing and prolonging the multiplication of Tu. No Tu specific agglutinins were detected in the sera of infected chickens.

Several investigators (7,8,9,17,27,30,32,33,34) have examined the affects of antibiotics on mycoplasma

infections. The antibiotics used included streptomycin, erythromycin, tetracycline, oxy- and chlorotetracycline, and tylosin. Tylosin was found most effective, followed by tetracycline and oxytetracycline (32).

Ose et al. (30) injected embryos with Mg 2-3 days before hatching which resulted in a uniform infection in the hatched chickens. The chickens were vaccinated at one day of age with either infectious bronchitis (IB), Newcastle disease virus (NDV) strain B_1, or IB and NDV strain B_1 and in some cases again at 3 weeks of age. The vaccinations were used as a stress factor in an attempt to simulate field conditions. Varying amounts of tylosin were given in the feed for 5 days after hatching and again for 24 hours when the chickens were revaccinated. All treatment groups gained more weight than the infected controls. At 3 weeks of age no Mg antibody titers had developed in the groups receiving 400 or 800 grams of tylosin per ton of feed. The severity of lesions produced decreased as the tylosin concentration was increased. However, none of the levels of tylosin used eliminated the mycoplasma from the respiratory tract of the chicken as shown by recovery of Mg from the trachea and air sacs.

Olesuik et al. (27) compared tylosin, chlorotetracycline, and erythromycin for effectiveness in the control of Mg in experimental infection in 6 week old chickens. They found tylosin more effective than erythromycin as measured by increased weight gains and feed conversion and

more effective than chlorotetracycline for reducing the numbers of Mg that could be reisolated from the trachea at 14 and 21 days postinoculation. Tylosin administered in the drinking water at the rate of 5 gm/gal. for 5 days after Mg inoculation or injected subcutaneously, 12.5 or 25 mg/pound of body weight, at 5 or 7 days after Mg inoculation reduced the recovery of Mg from inoculated chickens. Tylosin may reduce the occurrence of Mg agglutinins following experimental Mg inoculation. As Ose et al. (30) found, high doses of tylosin administered for 5 or 10 days after Mg inoculation prevented development of a serologic response in a large percentage of inoculated chickens. However, it did not alter the serologic response in inoculated chickens which had attained detectable levels of Mg agglutinins.

Similar results with tylosin have been observed by Barnes (7,8,9). However, he found that a single dose of tylosin greater than 3 mg/pound, or 5 mg/pound of tylosin tartrate or lactate injected intraperitoneally was highly effective in controlling mycoplasma infections, based on the isolation of Mg from the trachea and air sacs, evaluation of the lesions produced, production of Mg antibodies, and improved weight gains. Both Olesuik and Barnes observed that the higher dosage levels reduced or prevented the development of clinical signs and gross pathologic lesions in chickens inoculated intratracheally with Mg.

All the antibiotics studied to date have been aimed at increasing weight gains and feed conversion, reducing the amount of Mg that can be recovered or preventing pathologic changes that occur in Mg infected chickens. No study has been conducted with the purpose of examining the affect of antibiotics on the pathogenesis of Mg or on Mg infections at the cellular level.

The influence of Newcastle disease and infectious bronchitis viruses on mycoplasma infection have been examined (2,11,13,14,15,24,26). Adler et al. (2) and McMartin (24) investigated the interaction of Mg and IB with similar results. Neither observed clinical signs in chickens inoculated with Mg for 28-31 days until IB was superimposed. After IB inoculation coughing, tracheal rales, nasal discharge, and coryza developed and these clinical signs were still evident 3 weeks later.

Corstvet working with several other investigators studied the effect of NDV on pathogenic and nonpathogenic species of mycoplasma. They found that the damage to the intact layer of mucus and tracheal muscosa resulting from the virus infection enhanced the multiplication of Mg in the trachea. The degree of enhancement was a reflection of the damage in the tracheal tissue. No increase in Mg multiplication was seen when the trachea was in a state of repair or when the respiratory tract was immune to the virus. The pathogenesis of the NDV infection was not influenced by prior or subsequent Mg infection. The Mg

was seen to multiply intracellularly more often in enhanced infections than in nonenhanced infections. Fluorescent antibody staining showed the Mg infection in the trachea was irregular in degree and distribution and of low incidence in the air sacs while the NDV uniformly infected the trachea and air sacs.

Serologic studies showed that Mg agglutinin production was directly related to the degree of multiplication of the organism in the trachea. The NDV hemagglutination inhibition (HI) antibody response was greater than average when the NDV Roakin strain was inoculated 5 days prior to the Mg inoculation (13). Corstvet et al. (14) also observed that Mg multiplied in the respiratory tract in the presence of Mg agglutinins but that the NDV multiplication ceased suddenly after humoral antibody was detected by the HI test. In experiments using Mg and Tu with NDV, the presence of the nonpathogenic mycoplasma did not detectably alter the pathogenesis and pathogenic action of Mg and NDV.

There is little information about the pathogenicity and the interaction with Mg of less virulent viruses such as chicken embryo lethal orphan (CELO), NDV strain B_1, and paramyxovirus strain Yucaipa (PMY) in the respiratory tract of the chicken. Monreal (26) investigated the influence of CELO virus on experimental Mg infection. Clinical signs were not observed in hens (15 months old) inoculated intratracheally or intrasinoidally with Mg or

Mg with CELO but air sac lesions were seen on post mortem examination. In young chickens (3 and 10 weeks old) clinical signs were not observed and no air sac lesions were found at post mortem. The formation of antibody to Mg was weak in a few of the Mg inoculated chickens but strongly evident in the Mg-CELO inoculated chickens.

Upon intratracheal inoculation, NDV strain B_1 produced a slight exudative tracheitis in some of the chickens and no exudative airsacculitis. No clinical signs or gross lesion were seen in chickens 10 to 11 days after NDV-B_1 inoculation even when Mg was inoculated 5 days after the virus, indicating that the multiplication of Mg was not enhanced.

Bankowski and Corstvet (5) observed no sign of disease in chickens inoculated intramuscularly or intrabursally with PMY when they were 37 days old. Very mild, moist rales and abnormal chirps, unlike those heard with infectious bronchitis, were exhibited by chickens inoculated intratracheally. Serum samples taken 25 days after inoculation had no or low PMY antibody titers. Nine-day-old chickens showed no signs of infection when inoculated intramuscularly or intratracheally.

PMY was isolated in 1960 by Bankowski and Corstvet (5) from the tracheas of 3 week old chickens with a respiratory disease tentatively diagnosed as infectious laryngotracheitis. PMY hemagglutinates erythrocytes while the infectious laryngotracheitis virus does not. Results

of the biological and physical properties of PMY (size, stability, pathogenicity for susceptible chickens and embryos, hemagglutination of erythrocytes, and the production of HI antibody) showed that it was not related to the viruses of infectious bronchitis, infectious laryngotracheitis, fowl pox, Fahey-Crawley, chicken embryo lethal orphan, or quail bronchitis. Neutralization tests done with other viruses which hemagglutinate erythrocytes showed that PMY was not serologically related to parainfluenza types 1, 2, and 3. Thus PMY appeared to be antigenically unrelated to known poultry pathogens.

Antibodies to PMY were detected in the sera of 3 of 169 flocks of chickens with respiratory infection in 1960 (6). In 1968 PMY antibodies were detected in the serum of turkeys with respiratory disease and reproductive failures throughout the United States (4).

While PMY is recognized as causing infection or complicating other infections in the turkey, no survey for the presence of antibodies to PMY in the chicken has been published or any report of its virulence or pathogenicity for the chicken since the Bankowski et al. report in late 1960. Thus there is no way of knowing its importance as a disease causing (complicating) agent or how wide spread it is in the chicken population in the United States.

CHAPTER III

MATERIALS AND METHODS

Experimental Chickens - Day old cockerals, Mycoplasma gallisepticum strain S_6 free, were obtained from Arbor Acres, Springdale, Arkansas. They were raised in isolation throughout the experiment. At 21 days of age, groups of 50 each were moved to four pens in a building that housed no other chickens and to four experimental houses located near by. The chickens were kept on cane pulp litter on the floor. A standard medicated ration containing 20% protein, 0.025% Nitrophenide, and 5 gm/ton procaine penicillin was fed throughout the experiment. The chickens were observed daily and a record made of any respiratory distress in the group as a whole and of any sick or dead chickens.

Inoculum - Mycoplasma gallisepticum (Richey strain) in yolk was passaged one time in chicken embryo yolk sacs. One-half (0.5) ml of the harvested yolk material was inoculated into the abdominal air sacs of 15-week-old chickens then passaged again in chicken embryo yolk sacs. The harvested yolk material was tested for purity by culture methods. The number of Mycoplasma gallisepticum (Mg) organisms per ml was determined by the drop technique.

Six 0.01 ml samples of 10 fold dilutions of yolk in broth were placed on agar and the number of colonies counted after incubation. The number of organisms was determined to be 1.6×10^5/ml. Mycoplasma species (strain Tu) was passaged one time in chicken embryo yolk sacs. The number of organisms was determined by the drop technique to be 2.2×10^5 per ml. One-half (0.5) ml of a 1:5 dilution of Mg or Tu infected yolk in sterile tryptose broth was inoculated intratracheally with a blunt curved needle on a 5.0 ml syringe. An equal volume mixture of the two mycoplasma organisms, diluted 1:5 with sterile broth was given to those chickens which were inoculated with both organisms.

Paramyxovirus strain Yucaipa (PMY) was passaged one time in the chicken embryo allantoic sac. The fluids were inoculated intratracheally into 15-week-old chickens. Four days later the tracheas were removed, divided into sections (upper, middle, lower), ground in sterile saline, and the fluid from each section inoculated into chicken embryos via the allantoic sac. The allantoic fluids were harvested and titrated by the hemagglutination (HA) method to determine which had the highest titer. The infected fluid, HA titer 160, was diluted 1:5 in sterile broth and inoculated intratracheally with a blunt curved needle on a 5.0 ml syringe.

Serologic Techniques - One-fourth of the chickens in each group were bled prior to challenge. These serum

samples were tested for Mg and Tu agglutinins and Newcastle disease virus and PMY HI antibodies. One lot of each mycoplasma strain antigen was used for the plate agglutination test performed according to Adler (1). The beta HI determination was performed for the detection of PMY and NDV antibodies.

Bacteriologic Procedure - The upper beak was removed posteriorly from the nares and a sterile cotton swab inserted into the sinus cavity. Samples were obtained from the trachea by insertion of a sterile cotton swab in the upper half of the trachea after the portions of tissue to be sectioned were removed. Samples from the air sacs were taken by insertion of a sterile cotton swab through the wall and swabbing the inner surface of the air sac; the air sacs were swabbed before the portion of tissue to be sectioned had been excised. The material on each swab was immediately streaked on mycoplasma agar medium, described by Corstvet and Sadler (11), in petri dishes, incubated in a moist atmosphere at 37° C and examined at 8 days postinoculation. Colony imprints were made from suspect mycoplasma colonies and identified with the fluorescent antibody technique.

Necropsy Procedure - Each chicken was examined and a blood sample taken from the wing vein before it was killed by electrocution. The sections of trachea were taken (just below the larynx, middle, and just above the tracheal bifurcation) and three air sacs (a portion of

the left anterior thoracic, the right anterior thoracic, and the abdominal). The tissues were collected with sterile instruments and each of the air sacs sampled was wrapped around one of the sections of trachea being sampled. This was done to expedite sectioning and to obtain a layering of air sac so that the pathogens could be seen within the architecture of the air sac. The tissues were frozen on dry ice, placed with plastic material between them into test tubes which were sealed and kept frozen at -25° C.

Sections of each tissue sample were cut 6 to 8 microns thick in a cryostat microtome and mounted on coverslips, two sections per coverslip. All of the section were dried for 30 minutes at 37° C, placed in acetone for 10 minutes at room temperature, air dried, and stored frozen in coverslip boxes at -25° C until stained. The coverslip preparations were stained with Mg, Tu or PMY fluorescein isothiocynate conjugated antibody. The technique of fluorescent antibody staining and examination of the sections was described by Corstvet and Sadler (11).

Test Design - Chickens 24-days-old were used. Duplicate groups of 50 chickens each were inoculated intratracheally with 3.2×10^3 Mg organisms, with 4.4×10^3 Tu organisms and with a combination of 1.6×10^3 Mg plus 2.2×10^3 Tu organisms. Two weeks later PMY, EID_{50} of $2 \times 10^{5.6}$ viral units, was inoculated intratracheally into one of each of the above groups and into one group

which had not been previously inoculated. Sixteen days after mycoplasma inoculation, the chickens were showing respiratory signs of disease and one-half of the chickens in each group received 12.5 mg tylosin subcutaneously in the back of the neck the next day.

Four control chickens were killed at the time of viral inoculation. Two chickens from each group and two control chickens were killed daily for three days after viral inoculation. Then four chickens (two of which had received tylosin) from each group and two controls were killed daily for six days, then every other day, excluding Sunday, for two weeks.

CHAPTER IV

RESULTS

The fluorescent antibody technique (FAT) was compared with the culture method to detect Mycoplasma gallisepticum (Mg) and Mycoplasma species (strain Tu) in chicken tissues. The two methods were comparable for the detection of Mg in the trachea of chickens, while the culture method was more efficient for the detection of Mg in the air sacs (Table I). These results differ slightly from those of Corstvet and Sadler (13,14) who found the FAT superior to the culture method for the detection of Mg in the trachea. Tu was detected in the trachea of 3 chickens by the culture method and in the tracheal mucus of 3 other chickens by the FAT. In addition the tracheal exudate was found infected in 5 other chickens by the FAT. The results of the culture method and the FAT for the detection of Mg are tabulated in Table I.

Mg was found in 6 of 29 of the uninoculated control chickens by the culture method but not by the FAT. As a result, imprints of suspect Mg colonies from the sinus and trachea of groups not inoculated with Mg were checked for the presence of Mg. No Mg was detected in these groups.

TABLE

RESULTS OF THE CULTURE METHOD (CM) AND THE FLUORESCENT ANTIBODY TECHNIQUE

Test Group	Tissue	Days Post Mg 13 CM	13 FAT	14 CM	14 FAT	15 CM	15 FAT	16 CM	16 FAT	17 CM	17 FAT	18 CM	18 FAT	19 CM	19 FAT
Mg	Sinus*			-		-"		-		a		-		-	
	Trachea			a'	a,b	-	b	a	a,b	a,b	a	a	a	a	a
	LAT**			-	-	-	-	-	-	-	-	-	-	-	-
	RAT**			-	-	-	-	a	-	b	-	b	-	a	a
	Abd.**			-	-	-	a	-	-	b	-	-	-	-	-
Mg-Tu+	Sinus			b		a		-		a		-		-	
	Trachea			b	b	a,b	a,b	b	a,b	-	-	a,b"	-	-	-
	LAT			-	-	a"	-	b	-	-	-	-	-	-	-
	RAT			-	-	a	-	b	-	-	-	b	-	c	-
	Abd.			-	-	a	-	-	-	-	-	-	-	c	-
Mg-PMY+	Sinus			-		-		-		-		-		-	
	Trachea			b	a,b	a	a	-"	a,b	a,b	a,b	a,b	a,b	a,b,c	a,c
	LAT			-	b	-	b	-	-	d	a	c	-	-"	-
	RAT			-	b	-	-	-	-	c,d	-	c	-	d	-
	Abd.			-	b	-	-	-	-	a	-	-	-	c	-
Mg-Tu-PMY	Sinus			-		-		-		a,b		a		a	
	Trachea			-	a,b	-	b	a,b	a,b	a,b	a,b	b	a,b	a,b	a,b
	LAT			-	-	-	-	-	-	a,b	-	-	-	d	-
	RAT			-	-	a	-	b	-	a,b	-	-	-	-	b
	Abd.			-	-	-	-	-	-	-	-	b	-	-	b
Control	Sinus	-		-		-		-		-		-		-	
	Trachea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	LAT	-	-	-	-	-	-	-	-	-	-	-	-	a	-
	RAT	-	-	-	-	-	-	-	-	-	-	-	-	b	-
	Abd.	-	-	-	-	a	-	-	-	-	-	-	-	-	-

* No sinus smears were examined by the FAT

** Left anterior thoracic, right anterior thoracic, and abdominal air sacs

' Bird no. as shown in the appropriate table of Tables II through VIII

" Not determined for one chicken in the group

\+ Mycoplasma species (strain Tu), paramyxovirus strain Yucaipa

I

(FAT) FOR THE DETECTION OF MYCOPLASMA GALLISEPTICUM (Mg) IN CHICKEN TISSUES

Inoculation 20		21		22		25		27		29		32		34	
CM	FAT	CM	FAT	CM	FAT	CM	FAT	CM	FAT	CM	FAT	CM	FAT	CM	FAT
a		-		b,c,d		a		a		-		-		-"	
a,b	a,d	b	b	b	b	a,b	a,b,c	b,d	a,b	a	a	b	b	a,b	a
a	-	-	-	b,c	b	a	b	-	-	b	-	-	-	a	-
-	-	-	-	-	b	a	c	a	-	-	-	-	-	-	-
-	-	-	-	c	b	-	-	-	-	-	-	-	-	-	-
a		-		-		d		-		-		-		-	
b	-	a,b,c	a,b,d	b	b	b,d	a,b	a	a	-	-	b	-	a"	-
-	-	a	-	b	-	c	-	-	-	-	-	-	-	-	-
-	-	a,b	a,d	-	-	-	-	-	-	-	-	-	-	-	-
-	-	a	b	-	-	-	-	-	-	-	-	-	-	-	-
a		-		a		b		-		-		-		-	
a,b	a,b	a	a,b,d	a,b	b	a,b,c	a,b,c	a,b,d	-	a	-	a	a	a,b	a
-	-	-	-	a,d	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	b	a	-	-	-	-	-	-	-	-
-	-	-	-	-	-	b	a,b	-	-	-	-	-	-	-	-
-		-		-		d		a		-		-		-	
a,b	a	a,b	a,b	a,b	a,b,c,d	b,c	b	a,b	a	a,b	b	a	-	a	-
-	a	-	-	-	b	b	-	-	-	-	-	-	-	-	-
-	a	-	-	-	-	c	-	-	-	-	-	-	-	-	-
-	-	-	-	-	b	-	-	-"	-	-	-	-	-	-"	-
-		-		-		-		-		-		-		-	
-	-	-	-	b	-	-	-	-	-	-	-	-	-	-	-
-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	a,b	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Examples of positive and negative colony imprints used in the culture method are found in Figures 1 and 2.

Mycoplasma gallisepticum

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma gallisepticum (Mg) were correlated (Table II). Slight to moderate rales were heard in the flock beginning 16 days postinoculation and lasted for 5 days. Tracheal exudate was found in varying degrees throughout the experiment. The gross pathologic changes seen in the anterior and posterior thoracic air sacs were thickening and/or the presence of exudate.

Results of the survey of the trachea for Mg by the fluorescent antibody technique (FAT) showed that the organism was not present in all areas of the trachea and that the extent of the circumference involved varied greatly during the 34 day period. Mg was seen to multiply almost entirely extracellularly in the layer of mucus nearest the epithelial cells. Where the multiplication was intracellular, it was in the epithelial cells lining the lumen of the trachea. The Mg multiplication began as small microcolonies and continued until the microcolonies became confluent (Figures 3, 4, 5). No correlation was found in the amount of tracheal circumference involved, the area of trachea infected and the time after inoculation of Mg. Mg was usually detected in the trachea of chickens

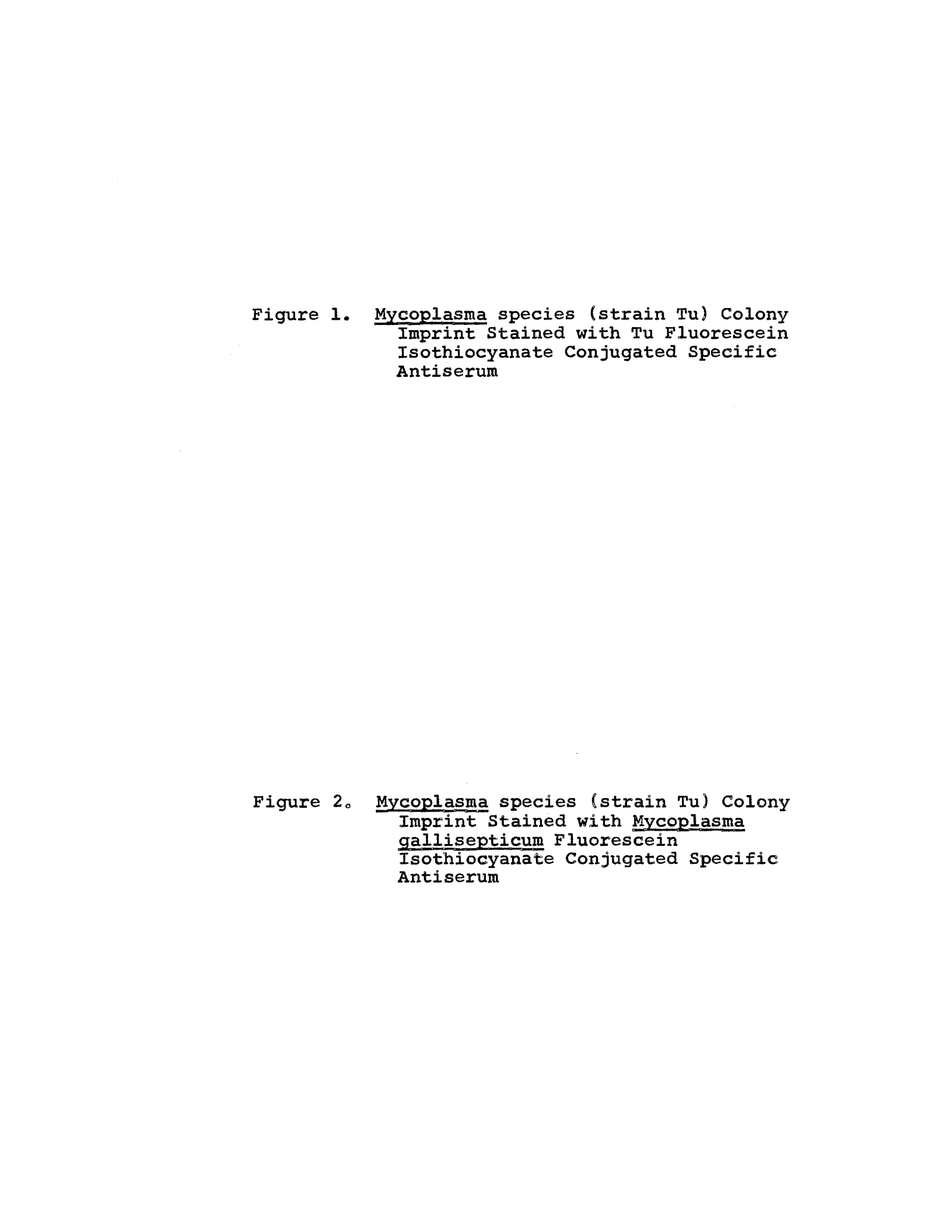
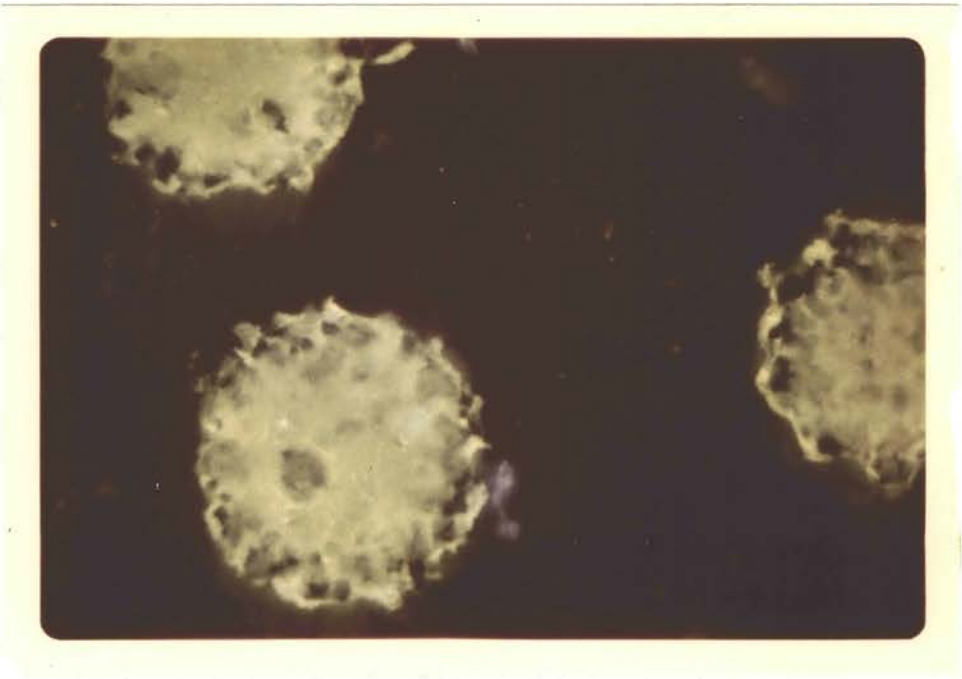
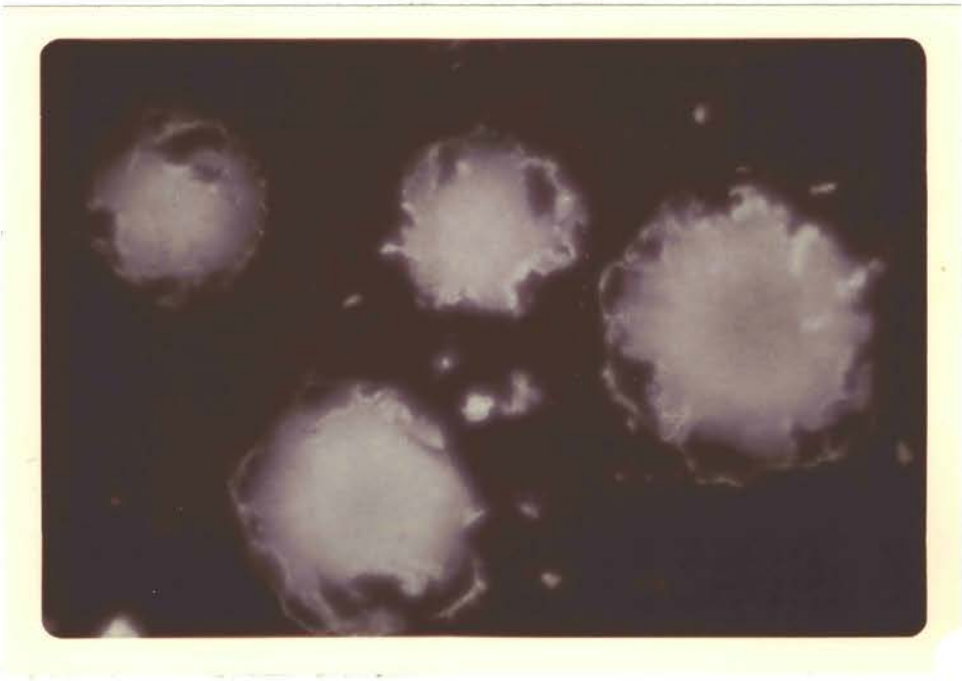


Figure 1. Mycoplasma species (strain Tu) Colony Imprint Stained with Tu Fluorescein Isothiocyanate Conjugated Specific Antiserum

Figure 2. Mycoplasma species (strain Tu) Colony Imprint Stained with Mycoplasma gallisepticum Fluorescein Isothiocyanate Conjugated Specific Antiserum





TABLE

MYCOPLASMA GALLISEPTICUM (Mg) [NOCULATION OF 24-DAY-
OF CLINICAL SIGNS, GRO.S PATHOLOGIC CHANGES,

Days post-inoculation of Mg	Bird No.*	Clinical Signs	Trachea		Right thoracic	
			Pathologic lesions: exudate	% of Circumference infected (X/Y/Z)**	Pathologic lesions	
					Exudate	Thickened
14	a	-	Mod.	30/50/20	-	-
	b	-	Mod.	70/60/30	+	+
15	a	-	-	-/-/-	-	-
	b	-	Sl.	15/5/1	-	-
16	a	-	Sl.	50/60/10	-	-
	b	-	-	5/1/-	-	-
17	a	Rales	Sl.	1/5/-	-	-
	b	-	-	-/-/-	+	-
	c	-	-	-/-/-	+	-
	d	-	Mod.	-/-/-	-	-
18	a	-	Mod.	5/1/-	-	-
	b	-	Mod.	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
19	a	-	Mod.	15/70/70	+	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	+	-
	d	-	-	-/-/-	-	-
20	a	-	-	-/1/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	1/-/-	-	-
21	a	-	-	-/-/-	-	-
	b	-	-	5/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
22	a	-	-	-/-/-	-	-
	b	-	Mod.	80/70/15	+	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
25	a	-	Mod.	40/30/20	-	-
	b	-	Sl.	30/-/-	-	-
	c	-	-	1/1/1	+	+
	d	-	-	-/-/-	-	-
27	a	-	Sl.	10/-/-	-	-
	b	-	-	10/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
29	a	-	-	5/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
32	a	-	-	-/-/-	-	-
	b	Congested	Mod.	-/70/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
34	a	-	-	15/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	Sl.	-/-/-	-	-

* Birds c and d received tylosin subcutaneously

** This refers to the respiratory epithelium, in histologic section, proximity to the epithelial surface. X, Y, and Z are portions of

Sl.=slight; Mod.=moderate; Sev.=severe; 0=sample not taken

II

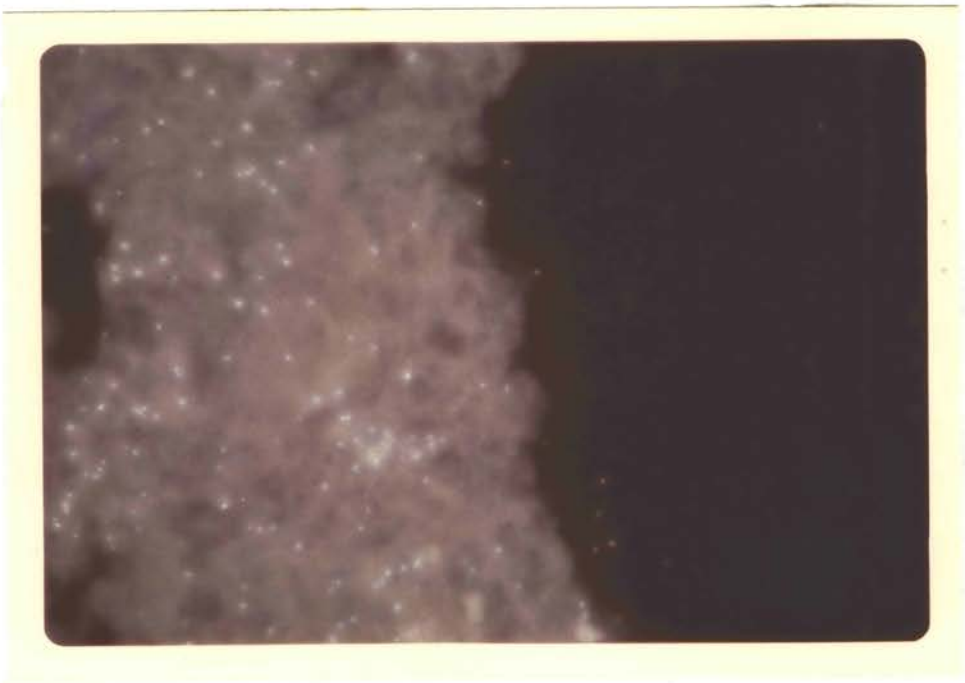
OLD CHICKENS WITH MYCOPLASMA GALLISEPTICUM]: CORRELATION SEROLOGIC RESPONSE, AND DISPERION OF THE PATHOGEN

anterior air sac	Left anterior thoracic air sac			Abdominal air sac			
	Pathologic lesions			Pathologic lesions			
% of Circumference infected	Exudate	Thickened	% of Circumference infected	Exudate	Thickened	% of Circumference infected	Serologic response: aggn. titer
-	+	+	-	-	-	-	20
-	-	-	-	-	-	-	10
-	+	-	-	-	-	-	10
-	-	-	-	-	-	1	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	20
-	+	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	+	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	10
1	-	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	40
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	40
-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	20
-	+	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	20
0	-	-	-	-	-	-	20
1	-	-	5	-	-	1	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	+	-	-	-	-	-	40
-	-	-	1	-	-	-	20
1	-	-	-	-	-	-	10
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	40
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	+	-	-	-	-	-	20
-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	+	-	-	20
-	-	-	-	-	-	-	20
-	-	-	-	-	-	-	10
-	-	-	-	-	-	-	10

in which the pathogen was intracellular or extracellular, or both, in close the trachea, upper, middle, and lower, respecitvely.

Figure 3. Uninfected Area of Tracheal Mucosa Stained with Mycoplasma gallisepticum Fluorescein Isothiocyanate Conjugated Specific Antiserum

Figure 4. Early Mycoplasma gallisepticum Infection in the Trachea



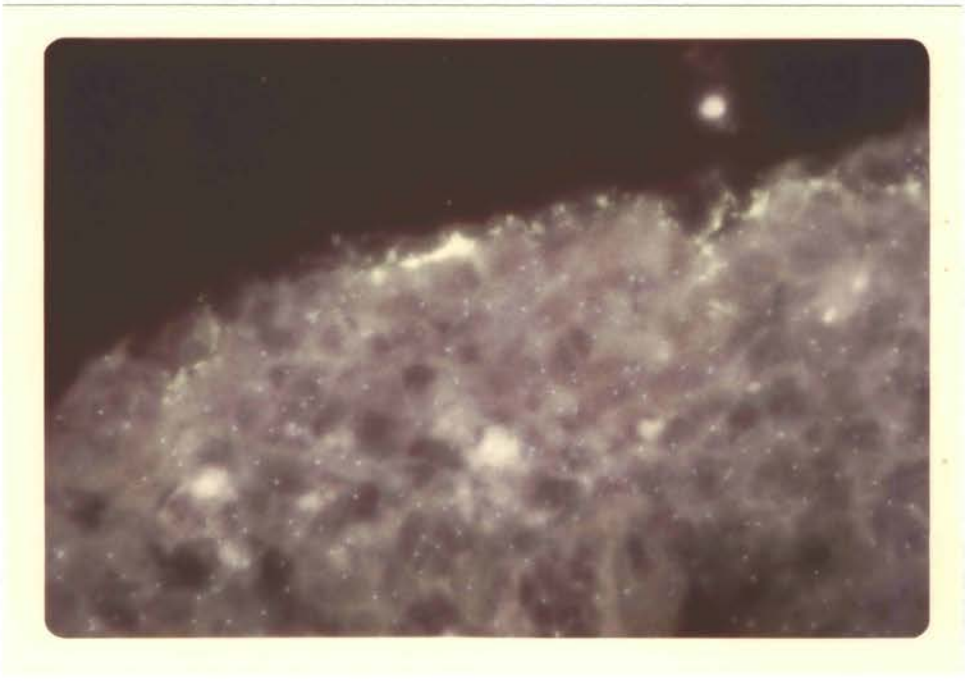
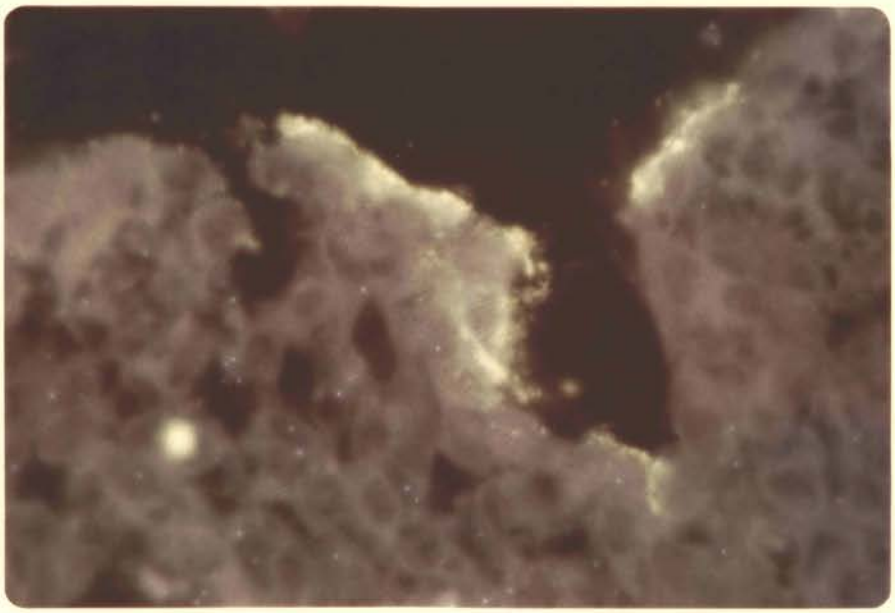


Figure 5. Later *Mycoplasma gallisepticum* Infection in the Trachea



(Mg inoculated groups) which had tracheal exudate at the time of necropsy. Mg antigen was detected by the FAT in the air sacs of 5 chickens as compared to 11 chickens with air sac infection by the culture method (Table I). The infection was seen more often in the thoracic air sacs than in the abdominal air sacs.

Antibodies to Mg were first detected at 14 days post inoculation, the first time the chickens were tested. The titers varied throughout the test with no consistant increase or decrease with time. No correlation could be seen between the titer and the degree of infection. The only uniformity of titer was seen on days 21 and 22 post inoculation (Table II).

Chickens which received tylosin subcutaneously had less tracheal exudate at the time of necropsy and little Mg infection of the trachea was found by the FAT. The tylosin had no visible affect on the production of antibodies to Mg.

Mycoplasma species (strain Tu)

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma species (strain Tu) were correlated (Table III). No signs of respiratory distress were heard in the flock throughout the 34 day experiment. No tracheal exudate was seen at necropsy. The left posterior and anterior thoracic air

TABLE

MYCOPLASMA SPECIES (STRAIN Tu) [INOCULATION OF 24-DAY-
OF CLINICAL SIGNS, GROSS PATHOLOGIC CHANGES,

Days post inoculation of Tu	Bird No.*	Clinical Signs	Trachea		Right thoracic	
			Pathologic lesions: exudate	% of Circumference infected (X/Y/Z)**	Pathologic lesions	
					Exudate	Thickened
14	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
15	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
16	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
17	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
18	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
19	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
20	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-⁜/-⁜	-	-
21	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
22	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
25	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
27	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
29	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	0/-/0	-	-
32	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
34	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously

** This refers to the respiratory epithelium, in histologic section, proximity to the epithelial surface. X, Y, and Z are portions of

Sl.=slight; Mod.=moderate; Sev.=severe; 0=sample not taken

⁜ 1+ infection in the exudate

III

OLD CHICKENS WITH MYCOPLASMA SPECIES (STRAIN Tu)]: CORRELATION
SEROLOGIC RESPONSE, AND DISPERSION OF THE ORGANISM

anterior air sac	Left anterior thoracic air sac			Abdominal air sac			Serologic response: aggn. titer
% of Circumference infected	Pathologic lesions: Exudate	Pathologic lesions: Thickened	% of Circumference infected	Pathologic lesions: Exudate	Pathologic lesions: Thickened	% of Circumference infected	
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	+	+	0	-	-	0	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-

in which the organism was intracellular or extracellular, or both, in close
the trachea, upper, middle, and lower, respectively.

sacs of one chicken were thickened and the anterior thoracic air sac contained exudate.

Results of the survey of the trachea for Tu by the FAT revealed no infection in the tracheal mucus. However, Tu was found in the tracheal exudate of one chicken. No Tu antigen was detected in the air sacs. No Tu agglutinins were detected during the test period.

Paramyxovirus strain Yucaipa

The clinical signs, gross pathologic changes, serologic response, and the presence of paramyxovirus strain Yucaipa (PMY) were correlated (Table IV). No signs of respiratory distress were heard in the flock throughout the experiment. A slight amount of tracheal exudate was found in 2 chickens. No gross pathologic changes were seen in the air sacs.

The survey of the trachea by the FAT revealed small areas of infection in 6 chickens. No infection was found after 5 days postinoculation when detectable levels of hemagglutination inhibition (HI) antibodies had been produced. By 7 days postinoculation all chickens had detectable levels of HI antibodies. The titers appeared to be peak at 320 HI units between 9 and 14 days post-inoculation then decline slightly.

TABLE

PARAMYXOVIRUS STRAIN YUCAIPA (PMY) [INOCULATION OF 37-DAY-
OF CLINICAL SIGNS, GROSS PATHOLOGIC CHANGES,

Days post-inoculation of PMY	Bird No.*	Clinical Signs	Trachea		Right thoracic	
			Pathologic lesions: exudate	% of Circumference infected (X/Y/Z)**	Pathologic lesions	
					Exudate	Thickened
1	a	-	-	5/10/1	-	-
	b	-	-	5/1/1	-	-
2	a	-	-	1/1/1	-	-
	b	-	-	25/10/5	-	-
3	a	-	-	-/-/-	-	-
	b	-	-	-/1/-	-	-
4	a	-	-	1/1/1	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
5	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
6	a	-	-	-/-/-	-	-
	b	-	Sl.	-/-/-	-	-
	c	-	Sl.	-/-/-	-	-
	d	-	-	-/-/-	-	-
7	a	-	-	0/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
8	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
9	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
12	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
14	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
16	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
19	a	-	-	-/-/-	-	-
	b	-	-	-/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-
21	a	-	-	-/-/-	-	-
	b	-	-	0/-/-	-	-
	c	-	-	-/-/-	-	-
	d	-	-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously

** This refers to the respiratory epithelium, in histologic section, proximity to the epithelial surface. X, Y, and Z are portions of

Sl.=slight; Mod.=moderate; Sev.=severe; 0=sample not taken

IV

OLD CHICKENS WITH PARAMYXOVIRUS STRAIN YUCAIPA]: CORRELATION SEROLOGIC RESPONSE, AND DISPERSION OF THE PATHOGEN

anterior air sac	Left anterior thoracic air sac			Abdominal air sac			
	Pathologic lesions			Pathologic lesions			
% of Circumference infected	Exudate	Thickened	% of Circumference infected	Exudate	Thickened	% of Circumference infected	Serologic response: B-HI titer
-	-	-	1	-	-	-	-
-	-	-	-	-	-	1	-
-	-	-	1	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-
-	-	-	-	-	-	0	80
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	80
-	-	-	0	-	-	-	320
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	320
-	-	-	0	-	-	-	160
-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	80
-	-	-	-	-	-	0	320
-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	160

in which the pathogen was intracellular or extracellular, or both, in close
the trachea, upper, middle, and lower, respectively.

Mycoplasma gallisepticum - Mycoplasma species (strain Tu)

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma gallisepticum (Mg) and Mycoplasma species (strain Tu) were correlated (Table V). Slight to moderate rales were heard in the flock beginning 16 days postinoculation and lasted for 5 days. Tracheal exudate was found at various times throughout the experiment. The gross pathologic changes seen in the anterior and posterior thoracic air sacs were thickening and/or the presence of exudate.

Results of the survey of the tracheal sections for Mg and Tu by the FAT showed that the Mg infection was not present in all areas of the trachea and the extent of the circumference involved varied greatly. Approximately one-half as many chickens had infected tracheas as in the Mg only group. The Mg multiplication was again almost entirely extracellular in the layer of mucus nearest the epithelial cells. There was no correlation in the amount of the tracheal circumference involved, the area of the trachea infected, and the time after inoculation of Mg. Mg antigen was detected in the air sacs of 2 chickens, one of which had gross pathologic changes in an air sac that was not found infected. Tu was seen to infect very small areas in the tracheal mucus of 3 chickens and the tracheal exudate of 6 chickens.

TABLE

MYCOPLASMA GALLISEPTICUM (Mg) - MYCOPLASMA SPECIES
MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SPECIES
GROSS PATHOLOGIC CHANGES, SEROLOGIC

Days post-inoculation: Mg-Tu	Bird No.*	Clinical Signs	Trachea: Pathologic lesions: exudate	Trachea: % of Circumference infected (X/Y/Z)**: Mg	Trachea: % of Circumference infected (X/Y/Z)**: Tu	Right thoracic: Pathologic lesions: Exudate	Right thoracic: Pathologic lesions: Thickened
14	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	50/-/-	-/-/-	-	-
15	a	Rales	Sl.	80/50/10	-✱/-✱/-	+	-
	b	-	Mod.	90/80/60	-/-/-	-	-
16	a	-	Mod.	10/5/10	-✱/-/-	-	-
	b	-	Mod.	5/-/-	-✱/1✱/-	+	-
17	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	Sl.	-/-/-	-/-/-	+	-
	d	-	-	-/-/-	-/-/-	+	-
18	a	-	Sl.	-/-/-	-/1✱/-	-	-
	b	-	Mod.	-/-/-	-/-/1	+	+
	c	Rales	Mod.	-/-/-	-/-/-	-	-
	d	Rales	-	-/-/-	-✱/-✱/-	-	-
19	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	Mod.	-/-/-	-/-/-	+	-
	d	-	-	-/-/-	-/-/-	-	-
20	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/-/-	-/-/-	+	-
21	a	-	Sl.	60/40/20	-/-/-	+	+
	b	-	Sl.	-/-/-	-/-/-	+	-
	c	-	-	1/-/-	-/-/-	-	-
	d	-	-	1/-/-	-/-/-	-	-
22	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	1/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/-/-	-/-/-	+	+
25	a	-	-	1/1/5	-/-/-	-	-
	b	-	Sl.	70/1/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/-/-	-/-/-	-	-
27	a	-	Sev.	-/10/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/0/-	-/0/-	-	-
29	a	-	Sl.	-/-/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	+	-
	d	-	-	-/-/-	-/-/-	-	-
32	a	-	-	-/-/-	-/-/-	-	-
	b	-	Sl.	-/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/-/-	-/-/-	-	-
34	a	-	-	-/-/-	-/-/-	-	-
	b	-	-	-/-/-	-/-/-	-	-
	c	-	-	-/-/-	-/-/-	-	-
	d	-	-	-/-/-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously

** This refers to the respiratory epithelium, in histologic section, in proximity to the epithelial surface. X, Y, and Z are portions of the

Sl.=slight; Mod.=moderate; Sev.=severe; 0=sample not taken

✱ 1+ infection in the exudate

V

(STRAIN Tu) [INOCULATION OF 24-DAY-OLD CHICKENS WITH (STRAIN Tu)]: CORRELATION OF CLINICAL SIGNS, RESPONSE AND DISPERSION OF THE PATHOGEN

anterior air sac		Left anterior thoracic air sac				Abdominal air sac				Sero-logic res-ponse: aggn. titer	
% of Cir-cumfer-ence in-fected:		Pathologic lesions		% of Cir-cumfer-ence in-fected:		Pathologic lesions		% of Cir-cumfer-ence in-fected			
Mg	Tu	Exu-date	Thick-ened	Mg	Tu	Exu-date	Thick-ened	Mg	Tu	Mg	Tu
-	-	-	-	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	40	-
-	-	-	-	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	80	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	+	+	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	+	-	-	-	-	-	-	-	40	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	1	-	-	-	-	-	40	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	1	-	20	-
-	-	-	-	1	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	+	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	0	0	+	-	-	-	10	-
-	-	+	+	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	+	+	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	+	-	-	-	-	-	-	-	20	-
0	0	-	-	0	0	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	+	-	-	-	-	-	-	-	20	-
-	-	-	+	-	-	-	-	-	-	10	-
-	-	+	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	40	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	+	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	5	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	10	-

which the pathogen was intracellular or extracellular, or both, in close trachea, upper, middle, and lower, respectively.

No agglutinins to Tu were detected. The Mg agglutinin level varied throughout the test period. Again no correlation between the amount of Mg infection and the Mg antibody titer was seen.

Chickens which received tylosin had little or no tracheal exudate at necropsy and little Mg infection of the trachea was found by the FAT. One of the chickens with Tu infected tracheal exudate had received tylosin. Tylosin had no visible affect on the production of Mg agglutinins.

Mycoplasma gallisepticum - Paramyxovirus strain Yucaipa

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma gallisepticum (Mg) and paramyxovirus strain Yucaipa (PMY) were correlated (Table VI). No signs of respiratory distress were heard in the flock. Tracheal exudate was seen at various times during the experiment. The frequency and amount of exudate present was no more than that seen in the group inoculated with Mg alone. Gross pathologic changes in the air sacs consisted of thickening and/or the presence of exudate.

Results of the FAT survey of tracheal sections for Mg showed infection at various times throughout the experiment. Mg infection was usually found in chickens that had tracheal exudate at necropsy. Not all areas of the trachea

TABLE

MYCOPLASMA GALLISEPTICUM (Mg) - PARAMYXOVIRUS STRAIN
MYCOPLASMA GALLISEPTICUM AND THEN PARAMYXOVIRUS
GROSS PATHOLOGIC CHANGES, SEROLOGIC

Days post-ionc-ulation:		Bird	Clin-ical	Trachea			Right thoracic	
				Patho-logic	% of Cir-cumference infected (X/Y/Z)**		Pathologic lesions	
Mg	PMY	No.*	Signs	lesions: exudate	Mg	PMY	Exu-date	Thick-ened
14	1	a	-	Sl.	5/-/-	1/5/5	-	-
		b	-	Mod.	70/75/90	5/25/1	-	-
15	2	a	-	Sl.	25/-/3	-/1/1	-	-
		b	-	-	-/-/-	1/5/5	-	-
16	3	a	-	Mod.	25/10/5	50/50/20	-	-
		b	-	-	80/80/25	-/-/-	-	-
17	4	a	-	Sl.	30/20/40	-/-/-	+	-
		b	-	Sl.	1/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
18	5	a	-	Mod.	80/40/10	1/-/-	-	-
		b	-	-	60/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/1	-	-
		d	-	-	-/-/-	-/-/-	-	-
19	6	a	-	Mod.	1/20/5	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	Mod.	30/-/-	-/-/-	-	-
		d	-	Sl.	-/-/-	-/-/-	-	-
20	7	a	-	-	50/-/-	-/-/-	-	-
		b	-	-	25/5/1	1/-/1	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
21	8	a	-	Sl.	85/95/95	-/-/-	-	-
		b	-	-	1/-/-	-/-/-	-	-
		c	-	Sl.	-/-/-	-/-/-	-	-
		d	-	-	5/1/-	-/-/-	-	-
22	9	a	-	Sev.	-/-/-	-/-/-	-	-
		b	-	Sl.	-/5/1	0/-/-	-	-
		c	-	Sl.	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
25	12	a	-	Sl.	10/-/-	-/-/-	-	-
		b	-	Sl.	5/15/5	-/-/-	-	-
		c	-	-	20/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
27	14	a	-	Mod.	-/-/-	-/-/-	-	-
		b	-	Sl.	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
29	16	a	-	Sl.	-/-/-	-/-/-	-	-
		b	-	Sl.	-/-/-	-/-/-	-	+
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
32	19	a	-	-	5/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	+	-
		d	-	-	-/-/-	-/-/-	+	-
34	21	a	-	Sl.	-/5/5	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously

** This refers to the respiratory epithelium, in histologic section, in proximity to the epithelial surface. X, Y, and Z are portions of the

Sl.=slight; Mod.=moderate; Sev.=severe; 0=no sample taken

VI

YUCAIPA (PMY) [INOCULATION OF 24-DAY-OLD CHICKENS WITH STRAIN YUCAIPA]: CORRELATION OF CLINICAL SIGNS, RESPONSE, AND DISPERSION OF THE PATHOGENS.

anterior air sac		Left anterior thoracic air sac				Abdominal air sac					
% of Circumference infected:		Pathologic lesions		% of Circumference infected:		Pathologic lesions		% of Circumference infected:		Serologic response: aggn. B-titer	HI
Mg	PMY	Exudate	Thickened	Mg	PMY	Exudate	Thickened	Mg	PMY	Mg	PMY
-	3	+	-	-	25	-	-	-	1	10	-
1	-	-	-	1	1	-	-	1	-	40	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	1	-	-	-	-	5	-
-	-	-	+	-	1	+	-	-	10	40	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	+	-	1	1	-	-	-	1	320	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	-	-	-	-	-	10	-
-	-	-	-	-	1	-	-	-	10	10	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	80
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	+	-	-	10	20	160
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	160
-	-	-	-	-	-	-	-	-	1	20	-
-	-	-	-	-	-	-	-	-	-	20	-
-	-	-	-	-	-	-	-	-	-	20	80
0	0	-	-	-	-	-	-	-	-	20	160
-	-	-	-	-	-	-	-	-	-	20	160
-	-	-	-	-	-	-	-	-	-	20	160
-	-	-	-	-	-	-	-	-	-	20	320
-	-	-	-	-	-	-	-	-	-	10	80
-	-	-	-	-	-	-	-	-	-	20	640
-	-	-	-	-	-	-	-	-	-	10	-
-	-	+	-	-	-	-	-	-	-	20	640
-	-	-	-	-	-	-	-	-	-	20	320
-	-	+	-	-	-	-	-	-	-	10	320
-	-	-	-	-	-	-	-	-	-	5	160
1	-	+	+	-	-	-	-	1	-	40	160
-	-	-	-	-	-	+	-	1	-	20	640
-	-	-	-	-	-	-	-	-	-	5	320
-	-	-	-	-	-	-	-	-	-	5	160
-	-	-	-	-	-	-	-	-	-	20	640
-	-	-	-	-	-	-	-	-	-	10	80
-	-	-	-	-	-	-	-	-	-	5	160
-	-	+	-	-	-	-	-	-	-	10	640
-	-	-	-	-	-	-	-	-	-	10	160
-	-	+	-	-	-	-	-	-	-	40	160
-	-	-	-	-	-	-	-	-	-	10	320
-	-	-	-	-	-	-	-	-	-	5	320
-	-	-	-	-	-	-	-	-	-	20	160
-	-	+	-	-	-	-	-	-	-	80	160
-	-	-	-	-	-	-	-	-	-	20	320
-	-	-	-	-	-	-	-	-	-	10	160
-	-	-	-	-	-	-	-	-	-	40	80
-	-	+	+	-	-	-	-	-	-	10	160
-	-	-	-	-	-	+	-	-	-	40	160
-	-	-	-	-	-	-	-	-	-	10	160

which the pathogen was intracellular or extracellular, or both, in close trachea, upper, middle, and lower, respectively.

were infected and the extent of the tracheal circumference involved varied greatly. No correlation was found between the area and extent of the infection and the time after inoculation. Mg antigen was detected in the air sacs of 5 chickens by the FAT while 8 chickens had air sac infection by the culture method (Table I).

As revealed by the FAT survey, PMY infected small areas of the tracheal mucosa of 8 chickens. One of these chickens was found infected 2 days after HI antibodies to PMY were detected. PMY multiplied in the epithelial cells at various depths in the tracheal mucosa, most often near the outer or lumenal edge of the mucosa. The foci of infection became larger as the virus multiplied (Figures 6, 7, 8). PMY was found in the air sacs of 8 chickens, 4 of which had infected tracheas. The air sac infection was found in 6 of the chickens before antibodies to PMY were detected.

Agglutinins to Mg were detected throughout the experiment. The titers were in the same range as those in the single Mg infection, but in the Mg-PMY group the titers were more consistant on a given day than those in the Mg group. HI antibodies to PMY were detected at 5 days postinoculation. There was more variation in the titers on a given day than in the PMY group but as in the single infection the titers began to decline slightly at 16 days postinoculation after several chickens had attained antibody levels of 640 HI units.

Figure 6. Uninfected Area of Tracheal Mucosa Stained with Paramyxovirus strain Yucaipa Fluorescein Isothiocyanate Conjugated Specific Antiserum

Figure 7. Early Paramyxovirus strain Yucaipa Infection in the Tracheal Mucosa

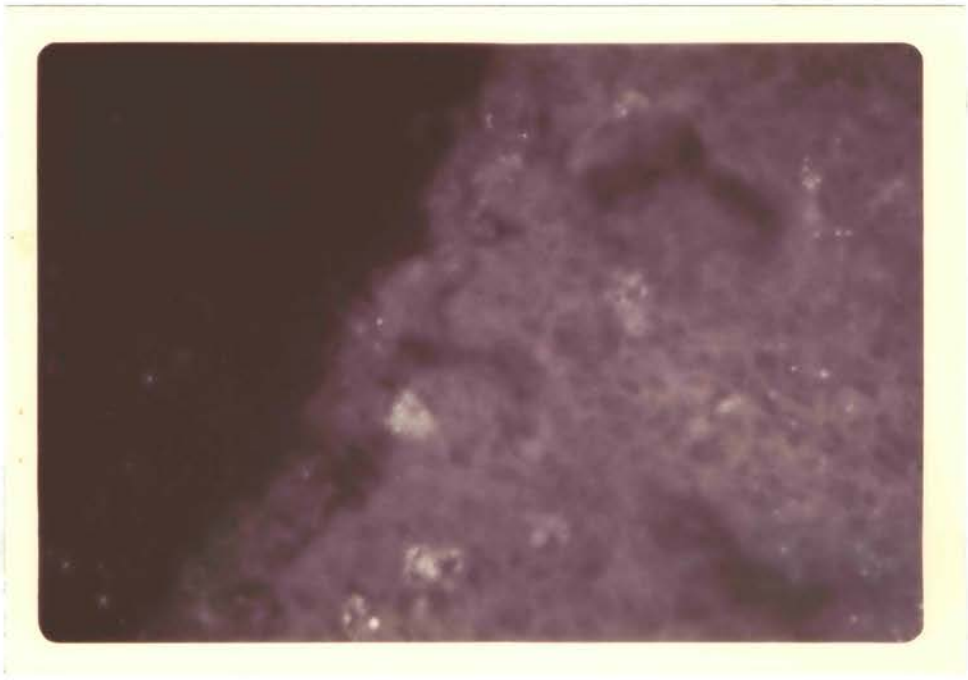
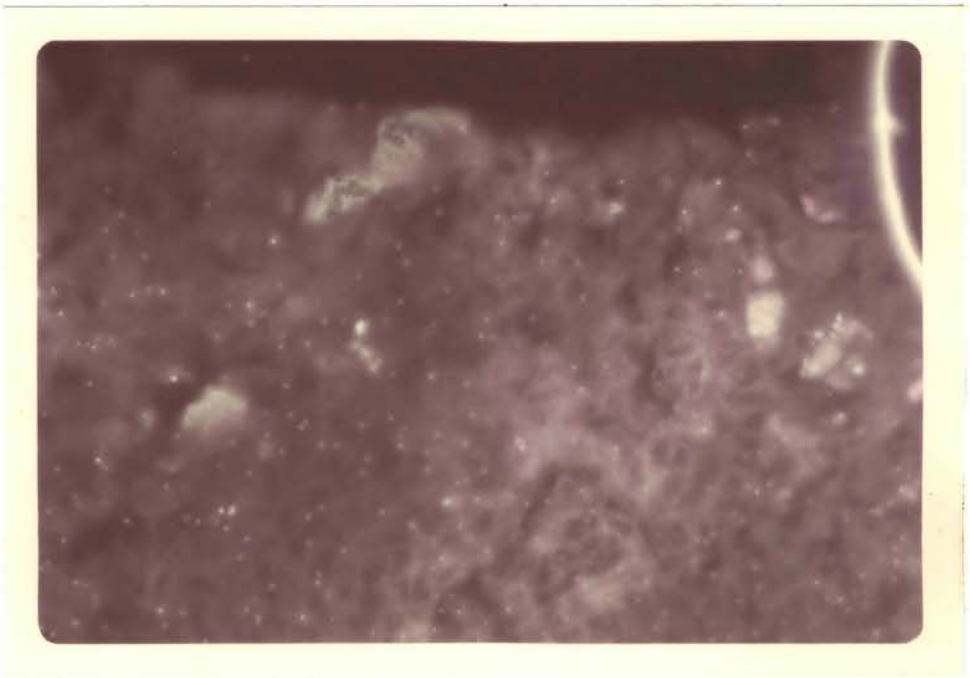


Figure 8. Later Paramyxovirus strain Yucaipa Infection in the Tracheal Mucosa



Tracheal exudate was seen in a few chickens which received tylosin while many chickens which did not receive the antibiotic had tracheal exudate. Most of the chickens with Mg infected tracheas had not received tylosin and they were more severely infected than those chickens which had received it. Tylosin had no visible affect on the production of Mg agglutinins.

Mycoplasma species (strain Tu) - Paramyxovirus strain Yucaipa

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma species (strain Tu) and paramyxovirus strain Yucaipa (PMY) were correlated (Table VII). No signs of respiratory distress were heard in the flock during the experiment. Tracheal exudate was not seen in any of the chickens at necropsy. The right anterior thoracic air sac of one chicken was thickened.

The FAT survey of tracheal sections revealed a small foci of Tu infection in the layer of mucus nearest the epithelial cells of one chicken. Tu antigen was not detected in the air sacs of any chicken. Small foci of PMY infection were found in 5 chickens, 3 of which also had infected air sacs. Two other chickens with no detectable PMY tracheal infection had infected air sacs.

No agglutinins to Tu were detected. HI antibodies to PMY were first detected 5 days postinoculation with nearly

TABLE

MYCOPLASMA SPECIES (STRAIN Tu) - PARAMYXOVIRUS STRAIN
MYCOPLASMA SPECIES (STRAIN Tu) AND THEN PARAMYXO-
SIGNS, GROSS PATHOLOGIC CHANGES, SEROLOGIC

Days post-inoculation:		Bird	Clinical	Trachea			Right thoracic	
				Pathologic lesions:	% of Circumference infected (X/Y/Z)**		Pathologic lesions	
Tu	PMY	No.*	Signs	exudate	Tu	PMY	Exudate	Thickened
14	1	a	-	-	-/-/-	1/3/1	-	-
		b	-	-	-/-/-	1/1/-	-	-
15	2	a	-	-	-/-/-	15/7/5	-	-
		b	-	-	1/-/-	-/-/-	-	-
16	3	a	-	-	-/-/-	1/1/1	-	-
		b	-	-	-/-/-	-/-/-	-	-
17	4	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	1/1/1	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
18	5	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
19	6	a	-	-	-/-/-	-/-/-	-	+
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
20	7	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
21	8	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
22	9	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
25	12	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
27	14	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
29	16	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
32	19	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-
34	21	a	-	-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously.

** This refers to the respiratory epithelium, in histologic section, in proximity to the epithelial surface. X, Y, and Z are portions of the

Sl.=slight; Mod.=moderate; Sev.=severe; O=sample not taken

VII

YUCAIPA (PMY) [INOCULATION OF 24-DAY- OLD CHICKENS WITH VIRUS STRAIN YUCAIPA]: CORRELATION OF CLINICAL RESPONSE, AND DISPERSION OF THE PATHOGEN

anterior air sac		Left anterior thoracic air sac				Abdominal air sac					
% of Circumference infected:		Pathologic lesions		% of Circumference infected:		Pathologic lesions		% of Circumference infected:		Serologic response: aggn. B-titer	HI
Tu	PMY	Exudate	Thickened	Tu	PMY	Exudate	Thickened	Tu	PMY	Tu	PMY
-	-	-	-	-	1	-	-	-	-	-	-
-	1	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
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-	1	-	-	-	-	-	-	-	-	-	-
-	1	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	160
0	0	-	-	-	-	-	-	-	-	-	320
0	0	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	1280
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	1280
-	-	-	-	-	-	-	-	-	-	-	640
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	80
-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	320

which the pathogen was intracellular or extracellular, or both, in close trachea, upper, middle, and lower, respectively.

consistant titers being detected at 7 days postinoculation. The titers peaked at 1280 HI units at 14 days post-inoculation then declined slightly.

Mycoplasma gallisepticum - Mycoplasma species (strain Tu) - Paramyxovirus strain Yucaipa

The clinical signs, gross pathologic changes, serologic response, and the presence of Mycoplasma gallisepticum (Mg), Mycoplasma species (strain Tu), and paramyxovirus strain Yucaipa (PMY) were correlated (Table VIII). No signs of respiratory distress were heard in the flock during the experiment. However, at necropsy one chicken had respiratory distress. Tracheal exudate was seen at various times with about the same number of chickens having exudate as in the single Mg infection. The gross pathologic changes seen in the anterior and posterior thoracic air sacs were thickening and/or the presence of exudate.

The FAT survey of the tracheal sections for the presence of the various organisms showed findings similar to those seen in the single inoculations of each organism. Mg did not infect all areas of the trachea and the extent of the tracheal circumference involved varied greatly. Again no correlation could be made between the area and the extent of the infection and the time since inoculation. Mg antigen was detected in the air sacs of 3 chickens by the FAT and in 7 chickens by the culture method (Table I).

TABLE

MYCOPLASMA GALLISEPTICUM (Mg) - MYCOPLASMA SPECIES (STRAIN Tu) - PARA-MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SPECIES (STRAIN Tu) CLINICAL SIGNS, GROSS PATHOLOGIC CHANGES, SEROLOGIC

Days post-inoculation		Bird No.*	Clinical Signs	Trachea				Right thoracic	
				Pathologic lesions: exudate	% of Circumference infected (X/Y/Z)**			Pathologic lesions	
Mg-Tu	PMY				Mg	Tu	PMY	Exudate	Thickened
14	1	a	-	-	20/-/-	-/-/-	1/1/1	-	-
		b	-	-	25/1/15	-/-/-	1/1/-	-	-
15	2	a	-	-	-/-/-	-/-/-	1/10/5	-	-
		b	-	-	25/5/1	-/-/-	5/1/1 .	-	-
16	3	a	-	Sev.	40/70/15	-/-/-	10/15/5	-	-
		b	Rales	Mod.	40/15/-	-/-/-	-/-/-	-	-
17	4	a	-	-	60/-/-	-/-/-	2/1/-	-	-
		b	-	-	15/1/-	-/-/-	1/-/1	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	1/-/-	-	-
18	5	a	-	-	1/-/-	-/-/-	-/-/-	-	-
		b	-	Sl.	50/-/5	-/-/-	1/-/1	-	-
		c	-	-	-/-/-	-/-/-	1/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	+	-
19	6	a	-	-	5/-/-	-/-/-	-/-/-	-	-
		b	-	-	50/-/1	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
20	7	a	-	Sl.	90/80/20	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
21	8	a	-	-	30/-/-	-/-/-	-/-/-	-	-
		b	-	Sl.	10/-/-	*-/*-/*-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
22	9	a	-	Sl.	50/1/-	-/-/-	-/-/-	-	-
		b	-	Sl.	95/75/35	-/-/-	-/-/-	-	-
		c	-	-	1/0/-	-/0/-	-/0/-	-	-
		d	-	-	1/-/1	-/-/-	-/-/-	+	-
25	12	a	-	Mod.	-/-/-	-/-/-	-/-/-	-	-
		b	-	Sl.	20/-/-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	+	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
27	14	a	-	-	10/-/-	-/-/-	-/-/-	-	-
		b	-	Sl.	-/-/-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
29	16	a	-	Mod.	-/-/-	-/-/-	-/-/-	-	-
		b	-	Sl.	1/-/-	-/-/-	-/-/-	+	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
32	19	a	-	-	-/-/-	-/-/-	-/-/-	-	-
		b	-	-	-/-/-	-/-/-	-/-/-	-	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-
34	21	a	-	Sl.	-/-/-	-/-/-	-/-/-	+	-
		c	-	-	-/-/-	-/-/-	-/-/-	-	-
		d	-	-	-/-/-	-/-/-	-/-/-	-	-

* Birds c and d received tylosin subcutaneously.

** This refers to the respiratory epithelium, in histologic section, in which the epithelial surface. X, Y, and Z are portions of the trachea, upper, middle, and

Sl.=slight; Mod.=moderate; Sev.=severe; 0=sample not taken

* 1+ infection in the tracheal exudate.

VIII

MYXOVIRUS STRAIN YUCAIPA (PMY) [INOCULATION OF 24-DAY-OLD CHICKENS WITH AND THEN WITH PARAMYXOVIRUS STRAIN YUCAIPA]: CORRELATION OF RESPONSE, AND THE DISPERSION OF THE PATHOGENS

anterior air sac			Left anterior thoracic air sac					Abdominal air sac							
% of Circumference infected:			Pathologic lesions		% of Circumference infected:			Pathologic lesions		% of Circumference infected			Serologic response: aggn. B-titer		HI
Mg	Tu	PMY	Exudate	Thickened	Mg	Tu	PMY	Exudate	Thickened	Mg	Tu	PMY	Mg	Tu	PMY
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	-	-	-	-	-	-	-	-	10	-	-
-	-	1	-	-	-	-	1	-	-	-	-	1	10	-	-
-	-	-	+	-	-	-	5	-	-	-	-	-	40	-	-
-	-	-	-	-	-	-	1	-	-	-	-	1	10	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
1	-	-	-	-	-	-	-	-	-	1	-	-	5	-	80
-	-	1	-	-	-	-	-	-	-	-	-	-	10	-	640
-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	80
5	-	-	-	-	2	-	-	-	-	-	-	-	10	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	80
-	-	-	+	+	-	-	-	-	-	-	-	-	10	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-
0	0	0	-	-	-	-	-	-	-	-	-	-	20	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	160
-	-	-	-	-	10	-	-	-	-	1	-	-	10	-	640
0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	-	-	-	-	-	-	-	-	-	20	-	640
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
-	-	-	+	+	-	-	-	-	-	-	-	-	10	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	640
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	80
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	640
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	160
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	320
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	80
-	-	-	-	+	-	-	-	-	-	-	-	-	40	-	2560
-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	80

pathogen was intracellular or extracellular, or both, in close proximity to the lower, respectively.

Tu infection was seen only in the tracheal exudate of one chicken. Small foci of PMY infection were seen in the tracheal mucosa of 11 chickens, twice as many as in the single PMY inoculation. No infection was found after detectable levels of HI antibody to PMY had developed. Air sac infection was detected in 3 chickens, none of which were found to be infected with Mg.

No Tu agglutinins were detected. Mg agglutinins, as in the other Mg groups, varied throughout the experiment but tended to be present in lower amounts in this group. HI antibodies to PMY were first detected at 5 days post-inoculation but not all chickens consistantly had titers until 14 days postinoculation as compared to 7 days post-inoculation for the other PMY inoculated groups. Unlike the other PMY groups, the antibody titers never appeared to reach a peak and then decline.

Chickens which received tylosin had no tracheal exudate at the time of necropsy and very little Mg infection was found in the trachea by the FAT. Tylosin had no apparent affect on the production of antibodies to Mg.

Controls

No signs of respiratory distress were heard throughout the experiment in the flock or in individual chickens at necropsy. There were no gross pathologic changes seen in the air sacs. In the uninoculated control chickens,

Mg was found in the air sacs of 5 chickens and in the trachea of another chicken by the culture method. No Mg, Tu, or PMY infection was found in the survey of tracheal sections by the FAT. One of the chickens with a Mg infected air sac had a Mg antibody titer of 10.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Mycoplasma gallisepticum (Mg) was recovered most often from the trachea of chickens and from the thoracic air sacs more frequently than from the abdominal air sac. The culture method was superior to the fluorescent antibody technique (FAT) for the detection of Mg in the air sacs but the two methods were comparable for detecting Mg in the trachea. The greater frequency of Mg detection in the air sacs by the culture method can be explained by the greater area surveyed in the culture method as opposed to the very small area surveyed by the FAT. Even though the FAT and culture method were comparable for the detection of Mg, the FAT was probably better since Mg does not uniformly infect the trachea. The FAT surveyed several areas of the trachea while the culture method surveyed only one area of the trachea, though admittedly a larger area. The background staining seen in the tracheal sections surveyed for Mg resulted because the conjugated antiserum was passed through coarse cellulose columns to remove the excess dye. This background staining did not interfere with the evaluation for the presence or absence of Mg infection in the tissue section.

Results of the surveys of the trachea for Mg substantiated the findings of Corstvet and Sadler (13,14). Mg multiplied in both the trachea and the air sacs. Not all areas of the trachea were infected and the extent of the tracheal circumference infected varied considerably. While infected chickens were seen at various times throughout the 34 day experiment, those necropsied at 14 to 16 days postinoculation were nearly always infected. Mg multiplied even when detectable levels of antibody were present. No correlation was seen between the time after inoculation and the antibody titer. Chickens that had tracheal exudate at the time of necropsy usually were found to have a Mg infection in the trachea by the FAT.

The gross pathologic changes in the air sacs, the culture procedure, and the FAT surveys of tracheal and air sac sections indicated that Mg migrated from the trachea to the thoracic then to the abdominal air sacs. This implies that air sac infection arises from the downward movement of the organism rather than from inhalation of the organism through the lungs directly into the abdominal air sacs.

Mycoplasma species (strain Tu) as reported by Corstvet et al. (15) did not produce clinical signs or gross pathologic lesions. The results of this experiment substantiate their findings. Tu was isolated from the trachea of 3 chickens by the culture method and was not isolated from any of the air sacs. Identical results

were found using the FAT. Tu appeared to have some affect on the establishment of a Mg infection since about twice as many chickens were infected in the Mg group as were infected in the Mg-Tu group. This may be a valid finding with the need for further investigation or it may be due to the nonuniformity of Mg infection throughout the trachea and the small sections examined by the FAT.

The results of this experiment indicate that paramyxovirus strain Yucaipa (PMY) is a virus of low virulence as reported by Bankowski and Corstvet (5). No signs of respiratory distress or gross pathologic changes were observed. Surveys of trachea for PMY by the FAT revealed a very small amount of infection. The lack of respiratory distress and a low rate of infection are characteristics of a low virulence virus. No infection was observed after detectable levels of antibody had been produced.

The low multiplication of PMY in the trachea and the low incidence of air sac infection made it difficult to comment on the migration of PMY in the respiratory tract of the chicken. However, results of the FAT surveys of tracheal and air sac sections showed that the thoracic air sacs were infected more often than the abdominal air sac, thus indicating a downward movement of the virus into the air sacs.

Respiratory distress was heard only in the groups which were inoculated with Mg and Mg-Tu and not in the

Mg-PMY or Mg-Tu-PMY inoculated groups. The only explanation seems to be that the Mg and Mg-Tu groups were kept in pens in a closed building while the Mg-PMY and Mg-Tu-PMY groups were kept in individual experimental houses which had the sides open for ventilation. Thus any respiratory distress would have been heard with ease in the closed building but have been difficult to hear in the open experimental houses.

When PMY was superimposed on Mg, no increase or decrease in the amount of Mg or PMY multiplication was observed when compared to the respective singly inoculated groups. This is the expected result since PMY damages the tracheal mucosa only to a small extent. Corstvet and Sadler (14) found that the greater the damage to the tracheal mucosa, the greater the increase in Mg multiplication. The fact that the presence of PMY did not increase the multiplication of Mg is further evidence that PMY does not cause much tracheal damage.

No change in the amount of PMY or Tu infection was seen when PMY was superimposed on Tu. This agrees with the findings of Corstvet et al. (15) that Tu did not interfere with a viral infection in chickens.

While neither Mg nor Tu had an affect on the degree of PMY infection in chickens, the combination of Mg and Tu did have an affect on the multiplication of PMY. The number of PMY infected chickens in the Mg-Tu-PMY group was twice that found in the PMY inoculated groups. It

thus appears that Mg and Tu interact in some manner to aid PMY in establishing infection. This finding seems to warrant further investigation.

The serologic response of the chickens to the various organisms was the same as those reported by other investigators (5,13,14,15). The Mg agglutinin titers varied throughout the experiment with the time after inoculation having no affect on the amount of antibody produced. Detectable amounts of antibody were present at 14 days postinoculation, the first time the titers were determined. The variations in the antibody response reflect the nonuniformity of Mg infection as found by the FAT. The nonuniformity and low levels of antibodies produced confirm the fluorescent antibody technique findings that the multiplication of Mg was not enhanced by the virus.

No Tu agglutinins were detected during the experiment, possibly indicating that the antigenic threshold had not been reached. This is supported by the fact that very little Tu infection was found by either the culture method or the FAT.

All groups which were inoculated with PMY had detectable hemagglutination inhibition (HI) antibody levels at 5 days postinoculation. Antibodies were detected in all chickens by 7 days postinoculation in the PMY, Mg-PMY and Tu-PMY groups but not until 14 days postinoculation in the Mg-Tu-PMY group. It is not known if this is a true finding or an artifact of some kind. It might indicate

the need for further study. If this is a valid finding, a possible explanation could be that initially only a few of the chickens became infected with PMY even though all were inoculated, and others became infected later after the virus had established itself and had begun to multiply in a few of the chickens.

The affect of tylosin can only be assessed against Mg since Tu did not infect the host and this antibiotic has no reported affect on viruses. Mg inoculated chickens which received tylosin had little or no tracheal exudate at necropsy and little or no Mg infection was found in the trachea and air sacs by the FAT. Chickens which were not given tylosin had varying amounts of tracheal exudate at necropsy and some had severe Mg infections in the trachea and infected air sacs when surveyed by the FAT.

The uninoculated control chickens were surveyed for Mg and Tu by the culture method and for Mg, Tu, and PMY by the FAT. Six chickens were found to be infected with Mg by the culture method. The FAT surveys of the tracheal sections revealed no infection by any of the organisms. Since other groups which had not been inoculated with Mg were checked for the presence of Mg infection by the culture method and found to be negative, it is not known why these few chickens gave positive results. If Mg spread to this uninoculated group via the air then it could have also spread to the Tu inoculated group which was kept in the same building. No Mg infection was found in the Tu

group. Under field conditions, Mg has been seen to spread to some pens and not to others located in the same area. Two other possibilities exist: (1) the Mg organism was mechanically taken into the uninoculated control group on equipment or clothing; or (2) some other mycoplasma which cross reacts with Mg was present in the chickens. Possibility (1) is very unlikely as the uninoculated control group was the first group of chickens cared for in the morning and the pen was not entered again until the next day. If (2) is the reason, there needs to be some explanation made as to why this related mycoplasma was not detected in any other group.

The conclusions drawn from this experiment are several. Mg does not uniformly infect all areas of the trachea and the extent of the tracheal circumference involved in the infection varies greatly. The multiplication of Mg was reduced one-half by the simultaneous inoculation of Tu, a nonpathogenic mycoplasma. Superimposing PMY on chickens inoculated with Mg had no affect on the multiplication of Mg.

No differences were seen between the Tu inoculated chickens and the uninoculated control chickens. Tu appeared to interfere with the multiplication of Mg when the two organisms were inoculated into chickens at the same time.

A low degree of infection was observed in chickens inoculated with PMY. Mg or Tu did not alter the

multiplication of PMY, but Mg and Tu together doubled the number of chickens found to be infected with PMY compared to the number infected when PMY was inoculated singly.

The multiplication of Mg has previously never been seen to be depressed by simultaneous inoculation of Tu, and the multiplication of a virus has not previously been seen to increase when the virus was in combination with mycoplasma. As a result, these findings indicate the possible need for further study.

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