SEROPREVALENCE AND PATHOLOGY OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* IN LOCAL CHICKENS IN THREE LOCAL GOVERNMENT AREAS IN NIGER STATE, NIGERIA

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AUGUST, 2012

DECLARATION

I hereby declare that this work entitled, "Seroprevalence and pathology of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in local chickens in three local government areas in Niger State,Nigeria", was performed by me in the Department of Veterinary Pathology and Microbiology under the supervision of Profs. S.B. Oladele, L.Sa'idu and M.Y.Fatihu.

The works of other investigators referred towere duly acknowledged.No part of this thesis has been submitted to any other university for a degree or diploma.

James Sa'iduAHMED, ---------- ----------
Signature Date

CERTIFICATION

This thesis entitled 'SEROPREVALENCE AND PATHOLOGY OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* IN LOCAL CHICKENS IN THREE LOCAL GOVERNMENT AREAS IN NIGER STATE, NIGERIA', by James Sa'iduAHMED, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is passionately dedicated to the Almighty God,and to my late parents Mr Joseph Lawal Ahmed and Mrs Grace Teni Ahmed. With thanks to my wife Victoria and our four children Tony, Josephine, Joseph and Jonathan. Also, to my only brother(Israel Sadauki Ahmed) and sisters (Christiana Adama Ahmed, Comfort LadiKolo, Patience Larai Ahmed, Elizabeth RakiyaGana(late) and Eunice AsabeOtegbola).

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ABSTRACT

Mycoplasmosis is one of the important diseases of exotic and local poultrybreeds in Nigeria. The disease causes decreased feed efficiency, poor carcass quality and sub-optimal egg production in layers. Seroprevalence of *Mycoplasma gallisepticum*(MG) and *M. synoviae*(MS) infection in local chickens in three Local Government areas (L.G.A.) of Niger State was determined between August and December, 2010. A total offive hundred and fifty two blood samples were collected. Serum plate agglutination (SPA) test and a combined MG/MSEnzyme-linked immunosorbent assay(ELISA)techniques were used to determine the presence of antibodies against *M. gallisepticum* and *M. synoviae*. The overall seroprevalence of MG was 85.86%, while the prevalence for MS was 79.71% with SPA test. The ELISA gave a seroprevalence of 91.84% for both MG and MS. There was highly significant difference ($P<0.001$) between the mean red blood cell (RBC), haemoglobin (Hb) and packed cell volume (PCV)($3.28 \pm 2.23\times10^6$/ml, 14.45 ± 7.85g/dl, 44.85 ± 30.15%,respectively) of the chickens affected by MG and MS in Kontagoraand the corresponding mean RBC, Hb and PCV ($2.36 \pm 1.55\times10^6$/ml, 12.6 ± 7.65g/dl, 32.05 ± 23.85%, respectively)in Minna. The results of the serum biochemistry obtained during this study were lower than the reports of previous works by Durotoye*et al.* (2000) but similar to the report of Ladokun*et al.*(2008) who also reported serum total protein range of 4.63 - 4.81 mg/dl, cholesterol 31.3 – 32.45mg/dl and albumin 3.28 - 3.48 g/dl in Nigerian chickens. Thehistopathological changes of some lung tissues collected from apparently healthy chickens revealed varying degrees of cellular infiltrations in the lungs. It was concluded that*M. gallisepticum* and *M. synoviae*are in circulation among local chickens in three Local Government Areas surveyed and they could serve as major reservoirs of these organisms in Niger State and in Nigeria as a whole.

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LIST OF ABBREVATIONS

ANOVA	Analysis of variance
DNA	Deoxyribonucleic acid
DNA G+C	Deoxyribonucleic acid Guanine and cytocine
EDTA	Ethylene diamine tetra acetic acid
et al.	and others
FAO	food and agricultural organization
Fl	fento litre
g/dl	gram per decilitre
H &E	Haematoxilne and eosine
Hb	Haemoglobin concentration
I.F	Immnuoflourescent technique
Ig A	Immunoglobulin A
Ig G	Immunoglobulin G
Ig M	Immunoglobulin M
Kb	Kilobase
kD	Kilo Dalton
LGA	Local government area
MCHC	Mean corpuscular hemoglobin concentration
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MG	*Mycoplasma gallisepticum*
mg/dl	milligram per decilitre
MG/MS ELISA	*Mycoplasma gallisepticum* and *Mycoplasma synoviae* Elisa kit

MI	*Mycoplasma iowe*
MM	*Mycoplasmameleagridis*
MS	*Mycoplasma synoviae*
NAD	Nicotinamide adenine dinucleotide
ND	Newcastle Disease
NK	Natural killer
NPIP	National poultry improvement plan
O.I.E	Office international des epizootics
PCR	Polymerase chain reaction
PCV	Packed cell volume
Pg	Picogram
pH	Hydrogen ion concentration
PPLO	Pleuro pneumonia like organism
RBC	Red blood cells
RFLP	Restriction fragment length polymorphism
RIM	Resource inventory and management
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SPA	Serum plate agglutination
SPA.MG	Serum plate agglutination for *Mycoplasma gallisepticum*
SPA.MS	Serum plate agglutination for *Mycoplasma.synoviae*
TNF	Tumour necrotizing factor
USA	United states of America.
WBC	White blood cells

CHAPTER 1

1.0 INTRODUCTION

Avian mycoplasmosis occurs in a variety of bird species (Nascimento *etal*, 2005). The most important mycoplasmas for chickens and turkeys are *M. gallisepticum*, *M. synoviae*, and *M.meleagridis*(Nascimento *et al*, 2005; Barua *et al.*, 2006), *Mycoplasma iowe* is an emerging pathogen in turkeys, but of little concern for chickens (Barua *et al.*, 2006). Mycoplasmas are bacteria that lack cell wall and belong to the class Mollicutes (Bradbury, 2001; Barua *et al.*, 2006).

Avian mycoplasma can potentially be present in backyard poultry flocks (McBride *et al*., 1991). As in other poultry producing countries, mycoplasmosis is one of the important disease problems for poultry in Nigeria, forboth commercial exotic and local breeds (Abdu *et al.,* 1983).Avian mycoplasma infection causes decreased egg production in chickens, turkeys and other avian species (Ley and Yoder, 1997). In addition to the overt disease, the infection causes decreased feed efficiency, poor carcass quality and sub-optimal egg production in layers (Ley and Yoder, 1997; Hossain*et al.,* 2007). The infection appears to be world-wide in distribution (Ley and Yoder, 1997). Avian mycoplasma infections are transmitted both horizontally and vertically and it can remain in the flock constantly as a subclinical form (Bencina *et al*., 1988 a, b).

Avian mycoplasmosis may be diagnosed by different methods, such as morphology of causal agents, cultural characteristics; physical, biochemical and serological properties (Ley and Yoder, 1997). Serology is the only reliable tool for detecting subclinical infection in a flock and among serological tests, the serum plate agglutination (SPA) test could be used as a tool for quick detection of avian mycoplasma infection(Pradhan, 2002).

Poultry population in Nigeria from Resource Inventory and Management (RIM) report (1993) consists of 150 million poultry, out of which 102.8 million are indigenous. Distribution pattern of both local and exotic breeds of chickens inNigeria revealed that the majority of local chickens are found in the northern parts ofthe country. On the other hand, the majority of the exotic chickens (83%) are found in the southern parts of Nigeria (Majiyagbe and Lamorde, 1997).

1.1 STATEMENT OF RESEARCH PROBLEM

The indigenous chickens, which constitute the majority of the local chicken population of northern Nigeria is mainly kept under extensive management system, where they roam freely and scavenge for food (Olabode *et al.,* 1992). Their movement is uncontrolled and they hardly receive any prophylactic treatment or vaccination (Adu *et al.,* 1986; Olabode *et al.,* 1992). It is believed that these free roaming chickens act as reservoirs and carriers of infections to themselves and susceptible exotic breeds in commercial enterprises (Adegboye, 1983).

Until recently less research work has been published on rural poultry health, despite the fact that up to 80% of the poultry population in Africa and Asia is kept by the households as free range chickens (Minga *et al.,* 1989; Aini, 1990).In addition, detailed epidemiology of avian mycoplasmosis in the village situation is largely unknown (Yongolo, 1997). However, some studies have shown that other diseases are present in scavenging poultry communities (Bell *et al.,* 1990; Cumming, 1991; Bell, 1992; Sa'idu *et al*., 1994; Chrysostome *et al.,* 1995; Permin *et al.,* 1999; Sa'idu *et al*., 2005).

1.2 JUSTIFICATION OF THE RESEARCH

In view of the increasing importance of poultry meat in Nigerianpopulace,it was estimated that the country is losing millions of Naira yearly on medication costs, poor feed conversion ratio, etc., among mycoplasma infected flocks, and it was proposed that a nationally- coordinated control program be instituted (Adegboye,1983). Losses attributed to mycoplasmosis, mainly *M. gallisepticum* and *M. synoviae* infection, are due to decrease in egg production and egg quality, poor hatchability (high rate of embryonic mortality and culling of day-old birds), poor feed efficiency, increase in mortality and carcass condemnations, besides medication costs (Mohammed *et al*., 1986; Yoder, 1991; Ley and Yoder, 1997).

Abdu *et al*.(1983) in their work reported a seroprevalence of 47.5% for *M. gallisepticum* and 49.4% for *M. synoviae* in Zaria.In a cultural study of chronic respiratory disease of chicken in Zaria, *M. gallisepticum* was isolated by Etukudo and Adegboye(1983) and Molokwu(1985).

To the best of our knowledge, there has not been any report on seroprevalence of mycoplasmosis in chickens in Niger State before this time.Therefore, it is pertinent to investigate the seroprevalence of *M. gallisepticum* and *M. synoviae* in local chickens in Niger State.

1.3 AIM OF STUDY

The aim of the study was to determine the seroprevalence of avian mycoplasmosis among local chickens in Niger State.

1.3.1 Objectives of the study

The objectives of the study were to:

1. Determine the prevalence of *M. gallisepticum* and *M. synoviae* among local chickens in3 Local Government Areas of Niger State using ELISA and Serum plate agglutination tests.
2. Determine the haematological and serum biochemical changes of seropositive chickens samples.
3. Isolate mycoplasmafrom tracheal swabs from the local chicken that were sampled for serology.
4. Study the gross and histopathologic changes in lung tissues of affected chickens.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 AVIAN MYCOPLASMA

Mycoplasmas are the smallest known living organisms (Baseman and Tully, 1997; Vogl *et al*., 2008). They belong to the family *Mollicutes* (*molis*, soft; *cutis*, skin, in Latin) that includes all cell wall-less prokaryotes (Baseman and Tully, 1997). The cytoplasm of mollicutes are encased solely

by a plasma membrane composed of approximately 60% membrane proteins and 40% membrane lipids, 4 to 20% of which are steroids (Razin *etal*.,1998). Mycoplasmas also have an unusually high percentage of lipoproteins when compared to other eubacteria (Razin *et al*., 1998).

The minute size and lack of a cell wall of mycoplasma baffled scientists about their identification for decades. Nocard and Roux (1898), first cultivated mycoplasmasuccessfully *in vitro*. The condition designated as "chronic respiratory disease" was described by Delaplane and Stuart (1943). In the early 1950's, Markham and Wong (1952) and Van Roekel and Olesiuk (1953) reported the successful cultivation of the organisms from chickens and turkeys and noted their similarity, and suggested they were the members of the pleurpopneumonia group. Edward and Freundt (1956) proposed a system for classification and nomenclature of organisms of the pleuropneumonia group, according to which all species were arranged into the genus *Mycoplasma*, Family *Mycoplasmataceae*, and order *Mycoplasmatales*. Edward and Freundt (1957) also proposed *Mollecutes* as a name of the class for the order*mycoplasmatales*. Different strains of pleuropneumonia like organisms of avian origin have been described, which are now designated as different species of *Mycoplasma* (Adler *et al*., 1957).

2.2 CLASSIFICATION OF MYCOPLASMA

The mycoplasmas belong to a class of microorganisms known as mollicutes (Table 2.1) (Jordan, 1972). They have the following distinctive features (a) The absence of cell wall; (b) presence of trilaminar membrane; (c) small size (as the smallest free -living microbes just within the limits of resolution of the microscope); (d) possession of a few cellular organelles, particularly those for metabolism and reproduction; and (e) a small genome of about 5 x 10^8 daltons (Freundt and Edward, 1979; Razin, 1981).The classification includes eight genera, Acholeplasma, Anaeroplasma, Mesoplasma, Entomoplasma, Spiroplasma, Mycoplasma, Asteroplasma and

Ureaplasma (Dybvig and Voelker, 1996) (Table 2.1). Classification of any isolate within the family depends on biochemical and serological tests (Tully *et al*., 1993). The characteristics of mycoplasma species isolated from avian sources are presented in Table 2.2.

2.3 AETIOLOGY OF AVIAN MYCOPLASMOSIS

Avian mycoplasmosis is caused *by* several pathogenic mycoplasmas of which*M. gallisepticum*and *M. synoviae*are the most important; they are the only ones listed by the Office International Des Epizootics (OIE, 2008). *Mycoplasmagallisepticum*and *M. synoviae*represent a pathogenic species within the genus Mycoplasma and stain well with Giemsa, but is weakly Gram negative. It is generally coccoid and approximately 0.25-0.5 μm (Razin and Freundt, 1984). The organism shows a filamentous or flask-shaped polarity of the cell body, which is due to the presence of terminal organelles or bleb (Maniloff and Quinlan, 1973). It has now been established that these terminal organelles govern motility, attachment to the host cell and pathogenicity (Levisohn and Dykstra, 1987).

Table 2.1: Classification and major features of members of the class of *Mollicutes*

Order	Family	Genus	Genome size (kbp)	%GC	Cholesterol requirement	$O2^1$	Habitat
Mycoplasmatales	*Mycoplasmataceae*	*Mycoplasma*	580-1350	23-40	Yes	FA	animals and humans
		Ureaplasma	760-1170	27-30	Yes	FA	animals and humans
Entomoplasmatales	*Entomoplasmataceae*	*Entomoplasma*	790-1140	27-29	Yes	FA	plants and insects

		Mesoplasma	870-1100	27-30	No	FA	plants and insects
	Spiroplasmataceae	*Spiroplasma*[2]	940-2220	25-31	Yes	FA	plants and insects
Acholeplasmatales	*Acholeplasmataceae*	*Acholeplasma*	1500-1650	25-36	No	FA	animals, plants and insects
		Phytoplasma[3]	640-1185	23-29	ND	ND	plants and insects
Anaeroplasmatales	*Anaeroplasmataceae*	*Anaeroplasma*	1500-1600	29-34	Yes	OAN	bovine and ovine rumen
		Asteroleplasma	1500	40	No	OAN	bovine and ovine rumen

source: Stakenborg Tim (2005).

Table 2.2: Characteristics of avian mycoplasmas

Species	Usual host	Glucose Fermentation	Arginine hydrolysis	Phosphate activity
A. laidlawii[a]	various	+	-	+ or –
M. anatis	Duck	+	-	+
M. anseris	Hawk	-	+	-
M. buteonis	Goose	+	-	-
M. cloacale	Buteo hawk	-	+	-
M. Columbinasale	Turkey	-	+	-
M. columbinum	Pigeon	+	-	-
M. columborale	Pigeon	+	-	-
M. corogypsi	Black vulture	+	-	-
M. falconis	Saker falcon	-	+	-
M. gallinaceum	Chicken	+	-	-
M. gallinarum	Chicken	-	+	-
M. gallisepticum	Chicken, Turkey	+	-	-
M. gallopanovia	Turkey	+	-	-

M. glycophilum	chicken	+	-	+or -
M. Gypis	Griffon vulture	-	+	+
M. imitans	duck, goose Partridge	+	-	-
M. iners	Chicken	-	+	-
M.iowae	Turkey	+	+	-
M. lipofaciens	chicken	+	+	-
M. meleagridis	Turkey	-	+	+
M. Pullorum	Chicken	+	-	-
M. synoviae	Chicken, Turkey	+	-	-
U. gallorale[b]	Chicken	-	-	-

Source: (www.ncbi.nlm.nih.gov/htbin-post/Taxonomy/wgetorg) (2007)
Key:
[a]Acholeplasma species do not require sterols for growth.
[b]Ureaplasma species are characterized by splitting of urea.

Mycoplasmas require a protein-rich medium for their growth, containing 10-15% added animal serum. A medium described by Frey *et al.* (1968) or a medium described by Bradbury (1978) is commonly used for the cultivation of avian mycoplasmas. Growth generally is optimal in medium at approximately pH 7.8 incubated at 37- 38^{o}C. They produce tiny, smooth, circular translucent colonies on agar plate with a dense, raised central area (Frey *et al*., 1968; Kleven, 1994; Ley and Yoder, 1997).

2.4 TRANSMISSION OF AVIAN MYCOPLASMOSIS

Mycoplasmas may be transmitted horizontally through infectious aerosols coughed and sneezed by infected birds, and through contaminated feed, water, contact,personnel and communicant animals, mainly birds(Jordan, 1985). The most frequent source of mycoplasma infections of

poultry appears to be ovarian (vertical) transmission (MacOwan*et al*., 1984;Bencina *et al*., 1988a, b). Infection of the ovary or oviduct with *M. gallisepticum*and *M. synoviae*may be by haematogenous spread or by direct spread from infected air sacs (Charlton *et al*., 1996). Shedding in the egg can vary; egg transmission is more frequent in birds infected during laying than in birds infected before they mature(Charlton *et al*., 1996). Infected birds carry *M. gallisepticum* for life, and can remain asymptomatic carriers until they are stressed (OIE, 2008).

Horizontal transmission can occur from bird to bird by aerosol, droplets in respiratory infections with *M .gallisepticum*and *M. synoviae*from birds in the incubation period of disease, clinically affected ones, or carriers (Jordan, 1985; Nascimento *et al*., 2005). Olson *et al.* (1964) and Olson and Kerr (1967) have demonstrated *M. synoviae* in the respiratory tract of contact birds about 1 – 4 weeks after infection of inoculated birds. Lateral spread has also been demonstrated by Hemsley (1965), and Kerr (1965) between groups of chicken kept up to 45 feet apart. Wyeth (1975) reported lateral spread to in-contact birds from birds infected by the intravenous and respiratory routes.

Airborne and contact transmission had been described previously (Fahey and Crawley 1954; Jordan, 1985; Bradbury, 2001). Both eggs and airborne transmission may be influenced by concurrent infections and probably by other non-infectious factors which stimulate multiplication of the mycoplasma (Jordan, 1985). Arthropod transmission has been reported by Turner*et al.* (1963)who demonstrated mycoplasma in the blood of mosquitoes and mites. Rodents frequently visit chicken farms in search of food which may also be responsible for the mechanical transmission between flocks and farms (Johnson *et al*., 1981; Soliman *et al*., 1988), particularly in intensive or semi intensive systems.

In ducks and guinea fowls, high SPA antibodies titre had been demonstrated (Onunkwo and Onoviran, 1978; Adesiyun and Abdu, 1985). These birds, particularly those kept in various households could mix freely and readily with other free-range domestic poultry and establish or maintain a definite cycle of infection (Adesiyun and Abdu, 1985). They therefore, constitute yet another major reservoir for these organisms.

It has also been noted that infection with *M. gallisepticum* by transovarian or via the respiratory tract rarely results in tenosynovitis and arthritis which may be produced by foot pad inoculation (Kerr and Olson, 1967a,b). Grimes and Rosenfield (1972) infected fowls via the infra-orbital sinus; lesions were produced in the turbinate mucosa, sinus and air sacs but not in the trachea. Wyeth (1975) found localized lesions and minimal spread of *M. synoviae* to susceptible birds after intravenous inoculation. There was increased spread of the infection after intrasinus or aerosol exposure accompanied with no lesions. Olson*et al*. (1964) showed that *M. synoviae* may persist in the absence of lesions for seven weeks in the turbinates of chickens infected via the sinuses whereas Springer (1971) found that lesions resulting from airsac inoculation remained localized. Pruthi *et al*. (1985) and Kempf *et al.* (1989) noted that the yoke sac route was more effective than the tracheal route of *M.gallisepticum*infection in establishing in day-old chicks. However, natural infection of ducks and geese with *M.gallisepticum*and *M. synoviae*had been reported (Bencina *et al*., 1988a;b).

2.5 INCUBATION PERIOD OF AVIAN MYCOPLASMOSIS

Experimentally, infected poultry develop symptoms such as conjunctivitis, coughing and sneezing after 6 to 21 days(Bozman *et al*., 1984; Luttrell *et al.*, 1996). In natural infections, the

incubation period is variable; infected birds may be asymptomatic for days or months until stressed (Bozman *et al*., 1984; Jordan 1985). In finches, incubation periods of 4 to 14 days have been reported (Luttrell *et al*., 1996).

2.6 PATHOGENESIS OF AVIAN MYCOPLASMOSIS

It is presumed that avian mycoplasma enters the respiratory tract by inhalation of aerosols or via the conjunctiva, but it is not clear how it surmount bird's natural defence mechanism (Bradbury, 2001). *Mycoplasma gallisepticum* attaches to mucosal cells by its well-organized terminal organelles or blebs, which govern motility or host-pathogen interactions and ultimately pathogenicity (Levisohn and Dykstra, 1987). Cell division of *M. gallisepticum*occurs through binary fusion, which is synchronouswith DNA replication (Quinlan and Maniloff, 1973). *Mycoplasma gallisepticum* antigens, especially those with adhesin or haemagglutinin properties, may play key roles in the pathogenesis of, and immune response to, infection (Markham *et al*., 1992; Avakian and Ley, 1993; Kheyar *et al*., 1995). Mycoplasmahas sophisticated ways of varying its surface antigens and that these changes may permit subpopulations to avoid the immune response and persist in the host for long periods (Bradbury, 2001). Markham *et al*. (1992) described a haemagglutinin of 67 kD from the S6 strain of *M. gallisepticum*, which was found to be encoded by a family of genes, whosehypothetical function is immune evasion.

Although it is not confirmed how the lesions and clinical signs of *M. gallisepticum* infection originated, many of the lesions seem to result from the host immune and inflammatory response rather than the direct effects of the organism (Razin *et al*., 1998). Sometimes, chickens may not show the signs of the disease, but they remain as carriers for many months after the acute phase of the infection (Bencina and Dorrer, 1984).

*Mycoplasma gallisepticum*and *M. synoviae*have been considered extracellular organisms, but Vogl *et al*. in 2008 proved that mycoplasmas invade cells.

In order to survive within the hostto induce disease and evade the host immune system, mycoplasmas use some pathogenicity mechanisms.The mechanisms include adherence to host target cells, mediation of apoptosis, innocent by-stander damage to host cell due to intimate membrane contact, molecular mimicry that may lead to tolerance, and mitotic effect for B and/or T lymphocytes (Vogl *et al*., 2008). The mitotic effect for B and/or T cells could lead to suppression of function and/or production of cytotoxic T cell.Mycoplasma also produces by products, such as hydrogen peroxide and superoxide radicals (Vogl *et al*., 2008). Moreover, mycoplasma has the ability to stimulate macrophages, monocytes, T-helper cells and natural killer (NK) cells, resulting in the production of substances, such as tumour necrotizing factor (TNF-a), interleukin (IL-1, 2, 6) and interferon (a, b, g)(Razin and Tully, 1995; Razin *et al*., 1998). These mechanisms may explain the transient suppression of humoral and cellular immune responses during mycoplasma infection in birds, the immune tolerance and auto immune diseases, as well as the massive lymphoid cell infiltration in the respiratory tract and joint tissues of infected fowls (Razin and Tully, 1995; Razin *et al*., 1998).

Besides these mechanisms that may be used by *M. gallisepticum*, *M. synoviae* and *M. meleagridis*, latency is common to avian mycoplasmas (Razin *et al.,* 1998). Thus, these pathogens induce disease after the host is affected by other disease-causing organisms, such as bacteria and viruses and/or after an episode of host weakness (Yoder, 1991; Whitford *et al*., 1994). The latent status, i.e., when the mycoplasma is not recognized by the host immune system, may be explained

by its intracellular location due to environmental pressure, as can be exemplified by the presence of antimicrobials in host tissues for the treatment of *M. gallisepticum*, *M. synoviae* or *M. meleagridis* infection of birds (Razin *et al.,* 1998). Silva*et al.*(2003) and Nascimento *et al.*(2005) in their works provided experimental evidences that *M. synoviae* causes immune depression by affecting the chicken cellular and humoral immune system.

Moreover, mycoplasmas are more susceptible to mutations than other bacteria (Woese *et al.*, 1985), and this can be explained by their defective DNA repair system, as demonstrated in the case of *M. gallisepticum* (Ghosh *et al.*, 1977). The frequent changes on surface antigens (antigenic variations) allow mycoplasma to evade the host immune system, and facilitate their survival when adhered to the host respiratory tract, as noticed for *M. gallisepticum* (Markhan *et al.*, 1994).

2.7 AGE OF THEBIRDS

Chicks and turkey poults are generally more susceptible than older birds and resistance is said to increase with age (Jordan, 1979). However, Kerr and Olson (1964) found no marked increase in age susceptibility to *M. gallisepticum*infection up to 20 weeks as assessed by morbidity and mortality patterns.

2.8 CONCURRENT INFECTIONS OF AVIAN MYCOPLASMA WITH OTHER DISEASE AGENTS

Pathogens that have been encountered concurrently with *M. gallisepticum* are infectious bronchitis virus (MacOwan*et al.*, 1983; Soeripto *et al.*, 1989a). Newcastle disease virus (Sato, 1970), influenza A virus (Ranock*et al.*, 1970), reovirus (Subgamanya and Pomeroy, 1960), *Hemophilus gallinarum* (Kato, 1965), and *E. coli* (Batra and Singh, 1980). Bradbury (1975) noted

that a previous infection of chicken with *M. synoviae* reduces the severity of disease and the antibody titre produced by later infection with *M. gallisepticum*.

For *M. synoviae*, organisms that have been found in concurrent infection include Newcastle disease virus (Villegas *et al*., 1976), infectious bronchitis and *E. coli* (Springer*et al*., 1974), influenza A virus (Bahl*et al.*, 1974), *M. meleagridis* (Rhoades, 1977), reovirus (Al-Afaleg *et al.*, 1989), *Staphylococcus* spp (Moorhead *et al.*, 1967) and *Pseudomonas* spp (Peterson, 1975). Giambrone *et al.* (1977) also showed that early exposure of chicks to infectious bursal disease virus led to an increase in susceptibility to air sac inflammation.

2.9 THE ROLE OF STRESS FACTORS ON MYCOPLASMA INFECTION

Debilitating factors, such as scarification of the trachea (Corsvet and Saddler, 1964), excess ammonia (Sato *et al*., 1973) and social stress (Gross and Colmano, 1971) play active role in the exacerbation of poultry mycoplasma infections. Jordan (1972) reported that changes in intensive poultry husbandry practices, multiple breeding and increased population density are factors that enhance respiratory disease due to *M. gallisepticum* infection. It is not known whether debilitating factors are of significance in precipitating disease of synovial tissues of *M. synoviae* Yoder *et al*. (1977) showed that low environmental temperatures increased the incidence and severity of air sacculitis caused by *M. synoviae* in 3 -4 week old infected chickens.

2.10 DISTRIBUTION OF AVIAN MYCOPLASMOSIS

Mohammed *et al*. (1986) reported prevalence of 73% and 3% for *M. gallisepticum* in southern and central California, respectively. Godoy *et al*. (2001) in his study reported prevalence of 59%, 22% and 66% for *M. gallisepticum* in three municipalities in Aragua State, Venezuela by

haemagglutination inhibition test. Zhang *et al*. (2001) in his work on prevalence and pathogenicity of *M. gallisepticum* on broiler chickens in Mongolia reported that 53% of the birds were positive to *M. gallisepticum* using SPAtest. Zelenika *et al*. (1999) working in Croatia reported prevalence rates of 10% in 1997 and 13% in 1998 of *M. gallisepticum* in heavy hen hybrids. In Zimbabwe, Kelly *et al*. (1994) using ELISA reported a prevalence rate of 33% for *M. gallisepticum*. In Malaysia, Shah-Majid (1996) in his work on a seroprevalence study on village chickens using ELISA, found 26% samples positive to *M. gallisepticum*.

Observations of *M. gallisepticum* in village chickens from other countries include Bangladesh (Barua*et al.*, 2006), Benin (Chrysostome *et al.*, 1995), Botswana (Mushi *et al.*, 1999), India (Chakraborty *et al.*, 2001), Malaysia (Shah-Majid, 1996), Senegal (Arbelot *et al.*, 1997) and Zambia (Pandey and Hasegawa, 1998). The overall prevalence in these investigations ranged from 26% (Malaysia) to 73% (Benin).

2.11 DIAGNOSIS OF AVIAN MYCOPLASMOSIS

Diagnosis of avian mycoplasmosis is based on clinical signs, gross and microscopic lesions and serological tests (Jordan, 1985).

2.11.1 Clinical signs associated with *mycoplasma gallisepticum and M. synoviae*

Infection with *M. gallisepticum* can cause a wide variety of clinical manifestations from sub-clinical infection to chronic respiratory disease in chickens, characterized by nasal exudates, coughing, sneezing and dyspnoea. Infectious sinusitis in turkeys is characterized by sinusitis with profuse nasal exudates, coughing, sneezing and mild conjunctivitis (Stripkovits and Kempf, 1996; Ley and Yoder, 1997).

Coryza, characterized by swelling of one or both intra-orbital sinuses, or conjunctivitis, is generally more severe in turkeys (Jessup, 1984). Facial oedema and nasal discharge were reported in broilers and day old chicks (Uchida *et al.,* 1990). Other signs include ataxia in turkeys associated with brain lesions and lameness with lesions of the hock in chickens. Natural uncomplicated infections may result either in disease with clinical signs or be inapparent or very mild and can be exacerbated when complicated by intercurrent infections or non-infectious debilitating factors (Jordan, 1985). Under experimental condition, clinical manifestations may depend on factors like route of infection, dose and nature of infecting organisms (Yoder *et al.*, 1977). None of the signs is said to be pathognomonic in the chicken or turkey (Jordan,1979).

The clinical signs seen in chickens infected by *M. synoviae* include paleness of the face and comb, lameness and retarded growth (Kemps and Gibson, 1981). There are swellings around joints, especially in the areas of the hock and footpad, breast blisters are also common (Shi, 1988). Birds become listless, dehydrated and emaciated (Jordan, 1975). Lameness is usually associated with hot, painful, swollen hocks or foot joints, and in very severe cases all the body joints may be affected (Morrow *et al.,* 1990). In the respiratory form, chickens may show slight rales in 4-6 weeks or may be asymptomatic (Olson, 1972). Mortality is low, varying from 2-6% but reports of morbidity vary greatly between 2-75% with an average in natural infections of 20% (Olson, 1972). In turkey, there is sinusitis (Jordan, 1975).

2.11.2 Gross lesions associated with *mycoplasma gallisepticum*and *M. Synoviae* infectionin chickens

Lesions of the respiratory tract may be very mild, or consist only of excess mucous or catarrhal exudates in the nares, sinusitis, trachea and lungswith oedema of the airsac walls (Yagihashi*et al.*, 1988). Caseous exudates may appear later particularly in the airsacs. Fibrinous or fibrinopurulent pericarditis and perihepatitis are usually present when infections are complicated with pathogenic strains of *E. coli* (Gross, 1961; Fabricant and Levine, 1963). Salpingitis has also been reported (Domermuth and Gross, 1962). Arthritis and synovitis, particularly of the hocks have been seen in chickens (Uchida, 1984), and in turkeys (Fabricant, 1969). Lesions in the brain which appear as pale areas of 1-2mm in the cerebrum have been reported (Varley and Jordan, 1978a; b). Acute swelling of the capillary endothelium has been described (Thomas *et al.*, 1966).

The association of *M.synoviae* with arthritis in chicken has been reported (Sentíes-Cué *et al.*, 2005). In the early stages of the infection, vicious creamy exudate appears in the synovial space of the keel (Morrow *et al.*, 1990). As infection progresses, the exudates becomes caseous (Sells, 1971). In chronic cases, inhibition of the sesamoid bone development in the tendons and degenerative osteoarthritis may also occur (Olson and Kerr, 1970). Kerr and Olson (1970) described lesions encountered in 2- week old chicks inoculated via foot pad and found that 80% of the chickens inoculated had gross lesions in the pelvic limbs, while only 27% of contact-infected birds developed lameness and tendo-vaginitis.

Occasionally, considerable mucus is found in the trachea following intranasal inoculation of *M. synoviae*(Olson, 1972). It has been reported that *M. synoviae* on its own is capable of causing airsacculitis (Rhodes, 1978). It could cause the condition when concurrently infected with Newcastle disease and /or infectious bronchitis virus (Kleven *et al.*, 1975). Ghazikhanian *et al.*(1973) observed thickened air sacs containing non adherent yellow exudates in turkeys seven days after inoculation via the air sacs. Bradbury and Howell (1975) reported air sac inflammation

in 44% of birds infected *in vivo* with *M. synoviae*. Endocarditis and valvular lesions had been reported in chickens affected between 2 and 20 weeks of age (Kerr and Olson, 1967 b) and pericarditis, epicarditis and myocarditis in chick embryo (Kerr and Bridges, 1970). Cardiac lesions have also been observed in chickens following footpad inoculation (Kerr, 1971). Kerr and Olson (1970) found that cardiac lesions in contact – infected chickens paralleled those in inoculated chickens. Splenomegaly followed in some infected chickens by hepatomegaly and nephromegaly (Olson, 1972). Kawakubo *et al*. (1990) recorded atrophy of the bursa of Fabricius and thymus in experimentally infected birds. Jordan (1979) noted that none of the clinical pathological manifestations was pathognomonic of mycoplasmosis in the turkeys.

Sentíes-Cué*et al*. (2005) in their necropsy work on broilers reported that the livers were mildly to severely enlarged with a prominent reticular pattern, and occasionally had a greenish tinge, the spleens were moderately to severely enlarged with white mottling. Sentíes-Cué *et al.* (2005) also observed that there were mildly to moderately enlarged hocks with an accumulation of fibrinous and/or caseous exudate in five birds. Other lesions included a mild to severe accumulation of fibrinous to caseous exudate in the air sacs, pericardium and capsule of the liver; congestion of the lungs; moderate to severe atrophy of the bursa of Fabricius and thymus; moderately enlarged and pale kidneys; moderately thickened proventricular wall and a moderate increase in mucus in the trachea.

2.11.3 Histopathology associated with *mycoplasma gallisepticum a*nd *m. Synoviae*infection in chickens

The respiratory tract lesions seen in dead chicks as a result of infection with *M. gallisepticum* consist of hypertrophy and hyperplasia of the epithelial tissue with deciliation and some oedema and infiltration of sub-epithelial tissue with mononuclear cells (Dykstra *et al.*, 1985; Salami,

1986). Monocytes aggregated into lympho-follicular foci with germinal centres occurring throughout the respiratory system (Kerr and Olson, 1967a). Hyperplasia of the mucous glands is seen, and in the trachea, they become long and narrow, extending the whole width of the thickened mucosa (Garside, 1962). In the lungs, there is massive infiltration of monocytes and heterophils (Kerr and Olson, 1967a). Heterophils may be found in large numbers in joint fluids and bursa exudates. Polyarthritis of the local arteries and arterioles has been described (Lamas Da Silva and Adler, 1969). Glavits *et al.* (1986) reported lympho-histiocytic rhinitis, multiple focal pleuritis with air sacculitis accompanied by lymphocytic and histiocytic infiltration.

Oedema of soft tissue with heterophil infiltration of tendon sheaths and synovial membranes followed by marked thickening caused by infiltration of mononuclear cells and plasma cells had been reported (Sentíes-Cué *et al.*, 2005). Considerable hyperplasia has been observed in the heart, liver, spleen and to a lesser degree in the kidneys, joints, lungs, gastrointestinal tract and brain (Sentíes-Cué *et al*., 2005). Poveda *et al*. (1986), and Sentíes-Cué *et al.*(2005) reported intense inflammatory reactions together with epithelial hyperplasia and lymphocyte infiltration in the respiratory tract.

2.12 THE AVIAN IMMUNE RESPONSE TO MYCOPLASMA INFECTION

The response to microbial infection involves interaction of both innate and acquired immunity. The development of the immunity involves both T and B cells in eliminating infection. However, this varies with disease (Nighot *et al.*, 2002).

It has been reported that the first antibody population to appear after *M. gallisepticum* infection in chickens is IgM (Roberts, 1967). In mycoplasma infected chickens, Kuniyasu *et al*. (1967) reported that antimycoplasma IgM persist for 77 days. These antibodies are followed by IgG antibodies (Kuniyasu, 1969; Roberts, 1969; Leslie, 1975).Antibodies against *M. gallisepticum* in chicks which react with plate agglutination test are associated with both IgM and IgG (Rhoades, 1978).

2.13 LABORATORY DIAGNOSIS OF AVIAN MYCOPLASMOSIS

Several serological tests have been utilized for the detection of antibodies against poultry mycoplasmas. The most commonly used procedures are the serum plate agglutination test (SPA), tube agglutination (TA), haemagglutination-inhibition test (HI) and enzyme-linked immunosorbent assay (ELISA) (OIE, 2008).

2.14 SEROLOGICAL IDENTIFICATION OF AVIAN MYCOPLASMA

Serological tests provide an essential basis for identification and classification of avian species of mycoplasma isolated from different field cases by comparing them with standard species.Reliable serological tests are prerequisites for any control measures, for determining the magnitude of the problem, monitoring the effectiveness of eradication techniques and for preventing the re-establishment of infection (Snell and Cullen, 1978).

Various procedures developed over the years are rapid serum plate and tube agglutination, haemagglutination-inhibition, agar gel precipitation and direct identification by specific fluorescent antibodies. All these serological methods are for the identification of serotypes (Whitford *et al*., 1994). Three serotypes of major concern have already been given separate species designation: *M.gallisepticum, M.synoviae* and *M. meleagridis*(Bradbury, 1982).

Serological tests for identification of antibodies are used routinely in the control programmes for chicken and turkeys in the United States of America and as a part of the National Poultry Improvement Plan (NPIP) (Yoder, 1975).

Mycoplasma infections of poultry may be readily confused with other respiratory diseases and in fact are frequently observed only in conjunction with Newcastle disease or infectious bronchitis virus infection from field exposure or vaccination (Springer *et al.*, 1974). Secondary bacterial invasion associated with airsacculitis is often significant and *E. coli* is the species isolated most frequently (Springer *et al.*, 1974). This makes it important to employ appropriate cultural and serological procedures for the diagnosis of mycoplasma infections.Serological tests form an essential part of identification and classification of a new mycoplasma isolate (Rozina, 2000).

2.14.1 Serum agglutination test.

Serum agglutination test was described by Adler (1954), and subsequently, used by other investigators for the detection of antibodies (Engureanu and Jonita-Jonescu 1986; Salem *et al.*, 1986; Salami, 1986; Thayer and Beard, 1998). The basic principle of the test is to detect the presence of antibodies in the sera of birds. It is simple, sensitive and fast, however it has the disadvantage of non-specific reactions (Wise and Fuller, 1975). The test has been described to work best with fresh clear serum and easier to read if conducted soon after serum collection (Rocke*et al.*, 1985). Several factors associated with the serum and/or the antigen can influence the specificity and sensitivity of the test (Woods and Wood, 1984; Rhoades, 1984) and the test is considered to be of greatest value for indicating flock rather than of individual bird infection (Jordan, 1979).

Serum plate agglutination test has also been used for characterizing the serotypes of different mycoplasmas.The use of saline diluted yolk from fresh eggs as a source of antibodies employed with rapid plate test antigen is described by Devos *et al.*(1968).

2.14.2 Tube agglutination test

Test tube agglutination test was found more reliable than the plate test by Jungher *et al*. (1955). The antigen prepared for the plate test can also be used in the tube agglutination test with some modifications (Rhoades, 1978; Salami, 1986).

2.14.3 Haemagglutination inhibition (HI)

Haemagglutination inhibition test has been used by several workers for detecting antibodies in the sera of birds (Newnham and Audrey, 1964; Yoder and Hofstad, 1964; Dierks *et al.,* 1967). It can also be used for differentiating between two species (Vardaman and Yoder, 1970). It is laborious and time consuming test and mainly performed as a confirmatory test on sera reacting positively to the serum agglutination test. Chabra and Goel (1980) studied the immune response of chicken against *M.gallisepticum* and the antibody response was determined by the HI test.

2.14.4 Agar gel precipitation test (AGP)

Species of mycoplasma are readily typed by the agar gel precipitin test (Nonomura and Yoder, 1977). The precipitin reaction is highly specific. Aycardi *et al*. (1971) devised a relatively simple agar gel precipitin test (AGP) and classified various serotypes of avian mycoplasma. The AGP test could be used for the identification of mycoplasma isolates from field flocks (Nonomura and Yoder, 1977).

2.14.5Enzyme-linked immunosorbent assay technique

Enzyme-linked immunosorbent assay has been developed for the diagnosis of avian mycoplasma infections (Talkington *et al.,* 1985), but the first commercial kits did not show the specificity and sensitivity desired for routine serological diagnosis of *M. gallisepticum* infection (Avakian *et al.,* 1988). Yagihashi and Tajima (1986) used enzyme- linked immunosorbent assay to measure antibodies in sera and respiratory secretions from chickens infected with *M. gallisepticum*. Enzyme-linked immunosorbent assay kits vary between manufacturers, therefore non-specific reactions may occur (Avakian and Kleven, 1990).

2.14.6 Immnuoflourescent technique

Immunoflourescent technique (I.F.)has proved useful in the identification of mycoplasma and detection of antibodies against them (Bencina and Dorrer, 1983). Many investigators have used immunoflourescent technique as a serological method for examining mycoplasma. Immunoflourescent technique is an excellent procedure because it allows rapid identification and simultaneous detection of mixed cultures (Bencina and Dorrer, 1983). It should be realized that serological techniques are not equally valuable unless carried out with pure cultures, which are not easily possible to obtain without adequate biological characterization and determinations of culture purity. However, this is not the case with the immunoflourescent technique as it can be used in mixed cultures (Al-Aubaidi and Fabricant, 1971).

Immunoflourescent technique has been used rather extensively in the identification of mycoplasmas by many researchers (Ertel *et al.*, 1970). Both direct and indirect methods of immunoflourescent technique have been used. The indirect method requires preparation of only one antispecies conjugate, however, Corstvet and Sadler (1964) reported that indirect immunoflourescent technique was not as useful for identifying avian mycoplasmas. Baas and

Jasper (1972) noted that direct immunoflourescent technique was more suitable for the agar block technique. Other researchers have tested mammalian mycoplasmas by direct and indirect immunoflourescent technique (Rosendal and Black, 1972) but the avian strains have not been examined as thoroughly as the mammalian species. Talkington and Kleven (1983) also reported the successful use of the direct immunoflourescent test for identifying *M. gallisepticum* and *M. meleagridis* isolates. Buys (1976) described the use of the direct test with conjugates for 10 avian *Mycoplasma species*for identifying such isolates in situ on agar plates. There were reports of the use of indirect immunofluorescence to examine serological relationship between the avian serotype, but there were no reports on the application of this technique for identifying field isolates (Bradbury, 1982).

Direct immunoflourescent staining of mycoplasma colonies grown on agar medium has also been used (Stewart, 1967; Al-Aubaidi and Fabricant, 1971). In another technique, mycoplasma colonies were transferred from agar blocks onto glass slides by submerging the agar blocks and slides in boiling water and the colonies were heat fixed to the slides while, the agar melted (Lemeka and Leach, 1968; Karbe and Helmboldt, 1968). Other researchers grewcolonies on coverslips to eliminate the necessity of transferring agar grown organisms, centrifugation preparation and use of chick embryo (Ertel *et al.*,1970).

Clark *et al*. (1963) studied the direct fluorescent antibody technique for the identification of mycoplasma in broth cultures and in tissue culture fluid and they concluded that this method has the distinct advantage of rapid identification and determination of mycoplasma growth. Perreau *et al*. (1969) also used this technique to classify 13 murine mycoplasma strains and concluded that it was clearly the most useful and distinct criterion for classifying the murine serotypes.

M.bovimastidis, a bovine type, was successfully identified by the Immunoflourescent technique.Studies on the identification of *M.mycoides var. mycoides, M.agalatae, M. capri,* and other strains by the immunoflourescent method have reported to be satisfactory (Kleven *et al.*, 1972).

2.14.7Nucleic acid recombinant technique

A recent approach to diagnosis of mycoplasma infections involve the use of nucleic acid probes (Razin, 1985). Specific recombinant DNA probes have been developed for the rapid detection of *M.gallisepticum* (Hyman *et al*., 1989; Levisohn *et al*., 1989).

2.14.8 Polymerase chain reaction

The use of PCR is very beneficial in the rapid diagnosis of clinical disease(Nascimento *et al*., 1993). The PCR seems to be not only specific but a sensitive method for amplifying low amounts of nucleic acid (Nascimento *et al*., 1993). In early infections or during carrier stage when conventional serological and cultural procedures may fail, this sensitive method for detecting the organism would facilitate diagnosis of the infection (Nascimento *et al.*, 1993). The ability of the PCR to detect extremely low levels of DNA however requires some caution on the use of this method for diagnosis of *M. gallisepticum*. *Mycoplasma gallisepticum*-PCR is 1000-10,000 times more sensitive than *M. gallisepticum*DNA probe technology (Nascimento *et al*., 1993). It is a simple and rapid procedure that does not require sophisticated equipment. *Mycoplasma gallisepticum*PCR product can be visualized by agarose gel electrophoresis within 7 hours. The routine method of *M. gallisepticum* isolation and identification takes about 10 days or more (Yoder, 1991; Nascimento *et al*., 1993).

2.15 PREVALENCE AND ISOLATION OF AVIAN MYCOPLASMA IN NIGERIA

Addo (1976) screened 1,690 sera from the native fowls of Northern Nigeria using the SPA test and obtained a seropositive rate of 32.4% for *M. gallisepticum*. Onunkwo andOnoviran (1978) found a seropositivity rate of 69% for *M. gallisepticum* and 22% for *M. synoviae* among 1,604 chickens killed in Jos market, Plateau State. They further recorded a seropositive rate of 29% for *M. gallisepticum* and 15% for *M. synoviae* among 234 guinea fowls and also reported that 5 out of 14 ducks were positive for *M. gallisepticum*. Nawathe *et al*. (1982) screened 175, 117, 165, 61, 61, 30 and 92 chicken sera from Lagos, Anambra, Imo, Benue, Kaduna, Plateau and Kano States of Nigeria, respectively. For *M. gallisepticum*and the seropositive rates they obtained were of 45.5%, 39%, 48%, 19.7%, 34.6%, 70% and 40% respectively and for *M. synoviae* they recorded the following corresponding prevalenceof 66.1%, 35.3%, 54%, 45%, 40%, 40% and 58.5%. At a market place in Zaria, Kaduna State, Kujul (1983) reported prevalence of 61.5% for *M. gallisepticum*, and 80% for *M. synoviae* among 200 local birds. Etukudo and Adegboye (1983) purchased 230 chickens from 14 clinically affected flocks in Zaria and obtained a prevalence of 71.3% for *M. gallisepticum*. Also, Abdu *et al.* (1983) working in the same environment, screened 32 apparently healthy flocks for *M. gallisepticum* and *M. synoviae* and found that chickens kept under either the free-range or intensive management system had antibodies to mycoplasma. He reported seroprevalence rates of 66.3%and 81.9% for *M. gallisepticum*and *M. synoviae* respectively for the extensive system of management. While the rates for the semi-intensive systems were 38.1% and 24.4% for *M. gallisepticum* and *M. synoviae,*respectively. The rates for intensive system were 38.1% and 41.9% for *M. gallisepticum*and *M. synoviae*respectively. The overall seroprevalence in the 32 flocks were 47.5% for *M. gallisepticum* and 49.4% for *M. synoviae*. Abdu *et al.* (1984) in Zaria found that 99% of the 93 chickens sampled from 37 flocks were seropositive for both *M. gallisepticum* and *M.synoviae*. In the same area, Adesiyun and

Abdu (1985) working on four domestic avian species, recorded for *M. gallisepticum*, a seropositivity rate of 7.8% among 115 pigeons, 16.3% among 60 ducks, 47.6% among 126 guinea fowls. Molokwu (1985), also in Zaria reported seroprevalence of 50% and 5.6% from chickens surveyed for *M. gallisepticum* and *M. synoviae*infections, respectively. In an outbreak of infectious sinusitis of turkeys in Ibadan, Oyo State, Fatumbi (1983) recorded 100% seroprevalence for *M. gallisepticum* from 30 turkeys with severe infections.

Mohan *et al.* (1979) isolated *M. meleagridis* from an outbreak of airsacculitis and bad leg syndrome in turkeys on the University Farm at Nsukka, Anambra State. Etukudo (1982) isolated a total of 59 mycoplasma isolates from 580 chickens consisting of 230 clinically ill ones and 350 received from postmortem examination. Two (3.4%) were identified as *M. gallisepticum*, 28(47.5%) as *M. gallinarum* and the rest were either lost during storage or remained untypable with available antisera. Fatumbi (1983) isolated *M. gallisepticum* from an outbreak of infectious sinusitis in turkeys at Ibadan, Oyo State. He further reported that thirty of the seventy imported broad breasted white turkeys with severe sinusitis were culturally positive for *M. gallisepticum*. Molokwu (1985) provided additional report of isolation of *M. gallisepticum* and also documented the isolation of *M. gallisepticum*, *M. synoviae*, *M. columbianum*, *M. gallinarum*, *M. columborale* and *Acholeplasmalaidlawii* from chickens in Zaria. Etukudo (1982) and Molokwu (1985) noted that mycoplasma could be isolated from several seropositive birds. Mycoplasma can be detected in tissue fragments of affected organs like trachea, air sacs and lungs. Besides synovial, ocular and infraorbital sinus exudates, good sources are swabs from trachea and air sacs, and pipped embryos (Sentíes-Cué *et al.*, 2005; Vogl *et al.*, 2008).

Swabs from trachea and choanal cleft constitute excellent specimens, mainly for isolation or PCR, which are used as confirmatory tools for monitoring *M. gallisepticum* and *M. synoviae* infections in live birds (Salami, 1986).

2.16 ISOLATION AND IDENTIFICATION OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE*

Isolation,propagation, description, and characterization in avian mycoplasmology have received a noticeable advancement. Any of the cultural procedures, namely artificial media, embryonated eggs and tissue culture or a combination of these may be employed for the isolation and propagation of mycoplasma from suspected tissues, eggs and exudates (Bencina *et al.*, 1988 a, b). Extraneous bacterial contamination is usually controlled by the inclusion of thallium acetate and penicillin in the original inoculums of medium used (Adegboye, 1977).

Frey's medium (Frey *et al.*, 1968) or a modification of Frey's medium (Kleven, 1994) is commonly used for isolation of *M. gallisepticum*. Direct plating of exudates or tissue swabs onto agar medium (Kleven, 1994) may give rise to colonies after 4-5 days of incubation, but initial enrichment in broth is generally a more sensitive method. Cultures are incubated at 37^{o} C for 5-7 days. Growth may not be evident, but 2 or 3 serial passages at 3- to 7-day intervals may increase the number of isolations (Ley and Yoder, 1997).

Confirmation of mycoplasma isolates as *M. gallisepticum* is commonly done by antibody-based procedures. Direct immunofluorescence employing colonies on the surface of agar plates, or

colony imprints, has been very effective for culture and identification (Talkington and Kleven, 1983). These techniques and their modifications are particularly useful for identification of *M. gallisepticum* in cultures containing other mycoplasma species (Bencina and Bradbury, 1992). The agar gel precipitin test is also used to identify cultures (Nonomura and Yoder, 1977). *Mycoplasma gallisepticum* strains can be differentiated from one another by direct comparison of protein banding patterns resulting from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Khan *et al*., 1987), or restriction fragment length polymorphism (RFLP) (Kleven *et al*., 1988). These methods are especially useful for identification of vaccine strains of *M. gallisepticum* (Thomas *et al*., 1991) and for epidemiologic investigations of *M. gallisepticum* outbreaks (Ley and Yoder, 1997). Deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid (rRNA) gene probes have also been used to speciate *M. gallisepticum* and identify vaccine strains (Nascimento *et al*., 1991;Dohms *et al*., 1993; Farnandez *et al*., 1993).

Polymerase chain reaction (PCR) technique has increased the sensitivity of organism detection based on specific sequences of nucleotides (Sentíes-Cué *et al*.,2005).Vogl *et al*.(2008) have developed and used nested PCR to detect avian mycoplasma in chicken blood.

2.17 TREATMENT AND CONTROL OF AVIAN MYCOPLASMOSIS

Mycoplasmosis can be successfully treated using a range of antibiotics, although resistance to some antibiotics has been recorded(Nascimento *et al*., 1999). Mycoplasmas are resistant to antibiotics that act on cell wall, such as penicillin, but are sensitive to tetracyclines (oxytetracycline, chlortetracycline and doxycycline), macrolides (erythromycin, tylosin, spiramycin, lincomycin, and kitasamycin), quinolones (imequil, norfloxacin, enrofloxacin and

danofloxacin) or tiamulin(Nascimento *et al*., 1999). Drugs that accumulate in high concentrations in the mucosal membranes of the respiratory and genitourinary tracts, such as tiamulin and enrofloxacin (Nascimento *et al*., 1999), are often preferred (Stipkovits and Kempf, 1996).

Areas of control of avian Mycoplasmosis should include careful choice of source of chickens, good management and hygiene practices, periodic serological testing of chickens and maintaining of flocks free of the organisms. Flock testing and eradication programmes have been applied and found to be valuable as a control measure in some countries thathave been ableto pursue the measure diligently (Yamamoto *et al*., 1983).

Vaccination is another possible means of controlling*M. gallisepticum* and *M. synoviae*. There have been some vaccination attempts with both live and inactivated vaccines against avian mycoplasma infections (Hayatu *et al*., 1975; Lam *et al*., 1983; Lam and Lin, 1984; Yoder *et al.*, 1984; Kleven *et al*., 1984 and Soeripto *et al*., 1989b). None of these vaccines afforded complete protection but some were effective in suppressing the multiplication of the organisms resulting in less tissue damage followed by faster recovery (Hildebrand *et al*., 1983; Soeripto *et al*., 1989b; Salami, 1994).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1STUDY AREA

Niger State is situated in the North Central geopolitical zone of Nigeria, and lies between longitude 8'00 and 13'30 N and latitude 4'20 and 8'40 E.Livestock and animal husbandry constitute a major economic activity of the people. Niger State shares boundaries with Republic of Benin (West), Zamfara State (North), Kebbi State (North-West), Kaduna (North-East), Kogi (South), the Federal Capital Territory, Abuja (South-East) and Kwara State (South-West).

There are three senatorial zones in Niger State namelyZone A (eight Local Government Areas), Zone B (nine L.G.As) and Zone C (eight L.G.As) (Ngex^{TM}, 2009).

3.2 SAMPLE SIZE

Sample size was determined using the standard formula adopted from Araoye(2004):

$N=z^2pq/d^2$

Where

N= sample size

Z=1.96confidence interval

P=prevalence(66.3% for MG Abdu *et al.*, 1983)

D=5% allowable error

Q=1-p

This gives the minimum sample size of 334 for the local chickens for the three senatorial zones in Niger State.

3.3 BLOOD SAMPLING

A total of 552 blood samples were randomly collected from local chickens at the live bird market (LBM) by exsanguination. One hundred and eighty four (184) bloodsamples were collected per zone. Six millilitres of blood were collected aseptically from each exsanguinated local chicken in to a vacutainer tube from each bird and then shared into two containers; one with EDTA for haematological analysis using a BC-3000 plus auto haematology analyser (Shenzhen Mindray Biomedical company limited) and the other container was slanted and allowed to clot for serum to form. The sera were decanted into labelled ependoff tubes after the blood has clotted and kept on ice packs, and thereafter, analysed in the Mycoplasma Research Laboratory N.V.R.I., Vom.

3.4 SERUM PLATE AGGLUTINATION TEST

The serum plate agglutinationtest was performedat room temperature by mixing equal amounts (20 µl each) of crystal violet stained *M. gallisepticum and M. synoviae* commercial antigens (obtained from Soleil Sarl^{R}, France), and the serumon a thoroughly cleaned white tile.A stirring rod was used to mix the antigen and serum and the mixture spread over a circular area of approximately 1.5 cm diameter, then the tile was rocked for 2 minutes and then observed for any agglutination (Jordan, 1979).

3.5 ENZYME-LINKED IMMUNOSORBENT ASSAY

A commercial *M. gallisepticum and M. synoviae* antibody ELISA kits were purchased from Biochek^{R} veterinary diagnostics (2010) Holland and used according to the manufacturer's recommendations. The micro titre plates have been pre-coated with inactivated MG/MS antigens.

The chicken serum samples were diluted and added to the micro titre wells where any anti-MG/MS antibodies ifpresent will bind and form an antigen-antibody complex. Nonspecific antibodies and other serum proteinswere then washed away using the wash buffer provided. Anti-chicken IgG labelled with the enzyme alkaline phosphatase was then added to the wells tobind to any chicken MG/MS antibodies bound to the antigen. After another wash to remove unreacted conjugate, substrate was added in the form of pNPP chromogen.

Test procedures:

1. Preparation of the substrate reagent: two tablets of substrate was dissolved into 12mls of substrate diluent (all provided in the kit).
2. Preparation of wash buffer: one sachet of the wash buffer powder (provided) was dissolved into one litre of deionised water.
3. One millilitre of the diluent (provided) was dispensed into 92 empty well labelled vials and then 2µl of the test sera was added to make the required 1:500 dilution.
4. The MG/MS coated plate was removed from a sealed bag and the location of samples on the template were recorded. Wells A1 and B1 were marked for negative controls, while wells C1 and D1 were marked for positive controls.
5. One hundredmicrolitres (100µl) negative controls weredispensed into wells A1 and B1 while 100µl of positive control was dispensed into wells C1 and D1.
6. One hundred microlitres (100µl) of diluted test sera was dispensed in other wells and then covered with a lid and incubated for 30 minutes at room temperature. The plates were emptied and washed 4 times with wash buffer and emptied.
7. One hundred microlitres (100µl) of conjugate (provided) were added to all the wells, then covered with a lid and incubated at room temperature for 30 minutes. The plate was emptied and washed 4 times with wash buffer and emptied.

8. One hundred microlitres (100µl) of substrate reagent was then dispensed into all wells and covered with a lid and then incubated at room temperature for 15 minutes.
9. The stop solution (100µl) was then added to each well.
10. The plate was then placed on the ELISA reader and read at 405nm.

A yellow colour developed due to presence of Mg/Ms antibody and the intensity is directly related to the amount of Mg/Ms antibodies present in the sample.

3.6 SERUM BIOCHEMISTRY

Two millilitres of serum from each chicken sampled was used to measure the serum triglyceride, albumin, cholesterol and total protein levels in the clinical chemistry laboratory in N.V.R.I., Vom.

3.6.1 Assay for serum albumin.

Pipette into three small labelled tubes'A(reagent)' 0.01ml of distilled water and 3.0 ml of BCG reagent were mixed thoroughly. In the second tube labelled 'A(standard)', 0.01ml of standard and 3.0ml of BCG reagentwere mixed thoroughly. In the third tube'A(serum)', 0.01ml of local chicken serum and 3.0 ml of BCG reagent. The samples were mixedthoroughly and incubated at 20 – 25^{o}C for 5 minutes. The absorbance of each tube was measured using a spectrophotometer(model 6400/6405, Jenway)at 600 – 650 nm against the reagent blank.

$$\text{Albumin concentration (g/dl)} = \frac{\text{A(serum)} \times \text{concentration of standard}}{\text{A(standard)}}$$

3.6.2Assay for serum cholesterol

Ten microlitres (10µl) of distilled water and 1000 µl of reagent in a cuvette labelled 'C(reagent)' and mixed thoroughly. A cuvette labelled 'C(serum)' containing 10 µl of serum and 1.0 ml of reagentwere mixedthoroughly. Thereafter, 10 µl of standard was pipetted into a cuvette and labelled 'C(standard)' whichwas also thoroughly mixed and incubated at 37^{o}C for 5 minutes. The absorbance of each cuvette against 'C(reagent blank)' within 60 minutes was measuredusing a spectrophotometer (model 6400/6405, Jenway) at 500nm wave length.

$$\text{Cholesterol concentration (mg/dl)} = \frac{\Delta C(\text{serum}) \times \text{concentration of standard}}{\Delta C(\text{standard})}$$

3.6.3 Assay for serum triglyceride

Ten microlitres (10µl) of serum sample and 1.0 ml of reagent were measured into a cuvette and labelled 'T(serum)' and were mixed thoroughly. A cuvette labelled 'T(standard)' containing 10 µl of standard and 1.0 ml of reagent were mixed thoroughly.The 10 µl of reagent pipettedinto a cuvette was labelled 'T(reagent)'. The contents were mixed thoroughly and incubated at 20-25^{o}C for 10 minutes. The absorbance of 'T(serum)' and'T(standard)' was measuredagainst reagent blank within 60 minutes in a spectrophotometer (model 6400/6405, Jenway) at 500nm wave length.

$$\text{Triglyceride concentration (mg/dl)} = \frac{T(\text{serum}) \times \text{concentration of standard}}{T(\text{standard})}$$

3.6.4 Assay for serum total protein

About 0.02ml of distilled water and 1.0 ml of reagent were pipetted into three small tubes and mixed thoroughly. In the second tube labelled 'P(standard)', 0.02ml of standard and 1.0ml of reagent were mixed thoroughly. In the third tube 'P(serum)', 0.02ml of local chicken serum and 1.0 ml of reagent were thoroughly mixed and incubated at 20 – 25^{o}C for 30 minutes. The

absorbance of P(serum) and P(standard) were measuredusing a spectrophotometer (model 6400/6405, Jenway) at 600 – 650 nm against the reagent blank.

$$\text{Total protein concentration (g/dl)} = \frac{\text{P(serum)} \times \text{concentration of standard}}{\text{P(standard)}}$$

3.7 ISOLATION OF *MYCOPLASMA GALLISEPTICUM* AND*MYCOPLASMA SYNOVIAE*

3.7.1 Specimens.

A total of 138 tracheal swabs were randomly taken from local chickens at the live bird market for culture and isolation as follows:

From Kontagora and Minna, 50 tracheal swabs each were taken, while only 38 swabs were taken from Bida before slaughter.

3.7.2 Culture media

Both mycoplasma transport medium (liquid) and mycoplasma agar (solid)were used. The composition, method of preparation and culturing were carried out according to procedures of Adegboye (1977; 1981).

About 10.5g of PPLO broth was weighed and poured into a conical flask, containing deionised water (appendix 1). The mixture was mixed and following complete dissolution of the mixture, 1.0ml phenol red was added and the pH was adjusted to 7.8. The solution was auto-claved at 121^{o}C and 151Ib pressure for 15 minutes. The flask was transferred to a water bath containing yeast extract, Nicotinamide adenine dinucleotide (NAD), glucose, cysteine hydrochloride,

thallium acetate, penicillin and swine serum were added aseptically in the measured amounts as above after the medium has cooled to about 45^{o}C. The medium was tested for sterility by incubating for 24 hours and checked for cloudiness. The prepared medium was kept at 4^{o}C and used within four weeks.

The 5.25g of PPLO broth and 2.5g agar Noble were weighed and dissolved in a conical flask containing 175ml deionsed water (appendix 2). The pH of the mediumwas adjustment, autoclaved and maintained at 45 – 50^{0}C in water bath while the other ingredients were added in sterile form. The molten agar was then dispensed into sterile 90mm petri-dishes and allowed to solidify. Sterility check was performed by incubating the plates in the incubatorfor 24 hours at 37^{0}C before plating. The agar plates were kept at 4^{0}c and used within four weeks.

Thallium acetate and penicillin were added to inhibit growth of bacteria, while nicotinamide adenine dinucleotide (NAD) and cysteine hydrochloride were growth promoters. Nicotinamide adenine dinucleotide is always added to batches of broth and agar to ensure that *M. synoviae* if present in the specimen would be able to grow (Salami, 1986).

3.7.3Culture procedure

Sterile cotton buds were used to swab the trachea of live birds and then cut and put in a screw capped bijou bottle containing 2ml of mycoplasma broth medium and packed in a flask and taken to the Mycoplasma Research Laboratory, N.V.R.I. and incubated at 37^{0}C for 5 days. The bottles were examined every 24 hours for change in colour of the media from alkaline (pinkish) to slightly acidic (orange-yellow). To check for colonies, 20 microliters of each liquid culture was serially diluted to 10^{-10} and the last portions were then inoculated on mycoplasma agar plates and

incubated at 37^0C in a glass anaerobic jar. The plates were examined every 24 hours for Mycoplasma like colonies using a stereoscopic microscope at a magnification of X140 to X180. Inoculated plates were incubated for about 10 days before considered negative for isolation. The Mycoplasma-like colonies were identified as organisms with opaque colonies granular with central raised area (nipple) and surrounded by a translucent periphery (Jordan, 1981). Reference Mycoplasma strains were obtained from the N.V.R.I Mycoplasma research laboratory and compared with the Mycoplasma culture to ascertain their characteristics.

3.8 POSTMORTEM EXAMINATION

Five local chickenswere purchased examined and slaughtered for post-mortem lesions.All the lungstissues were collected for histopathology.

3.9 STATISTICAL ANALYSIS.

Thehaematological and serum biochemical data obtained were expressed as mean ± SEM (standard error of the mean)and were analysed using one way analysis of variance (ANOVA) followed by tukey's positive test from graphadprism version 4.0. Chi square was also used to test for association between variables. Values of $P < 0.05$ were considered significant.

CHAPTER 4

4.0 RESULTS

4.1 RESULTS OF SEROLOGICALSTUDIES

Table 4.1 shows the seroprevalence of *M. gallisepticum* and *M. synoviae* antibodies in local chickens in Niger State. At Kontagora live bird market, 173 (94.02%) were positive for *M. gallisepticum* and *M. synoviae* antibodies using ELISA, while 158 (85.86%) and 142 (77.12%) were positive for MG and MS antibodies, respectively using SPA. Out of 184 chickens testedin Minna, 170 (92.39%), 155(84.23%) and 153(83.13%) were positive for MG/ MS antibodies using ELISA and MG and MS antibodies using SPA, respectively. At Bida, 164(89.13%) from the 184 sera samples tested were positive by ELISA, 160(86.95%) and 145(78.84%) were positive for MG and MS respectively by SPA. The overall antibody prevalence for the three live bird markets is 507(91.84%) for MG/MS antibodies using ELISA, 473(85.86%) and 440(79.71%) for MG and MS antibodies using SPA, respectively.

4.2 RESULTS OF HEMATOLOGICAL STUDIES

The mean values of RBC, WBC, Hb, MCV, PCV, MCH and MCHC were 2.605±0.054($X10^6$/µl), 4.291±0.146($X10^3$/ml), 14.45 ± 0.264(g/100ml), 144.3 ± 0.98(fl), 37.51 ± 0.75(%), 56.49 ± 0.64(pg), and 389.9 ± 3.9(g/dl), respectively from Kontagora senatorial zone. Samples from Minna had the following mean values 2.09 ± 0.094($X10^6$/ml), 4.22± 0.071($X10^3$/ml), 11.45 ± 0.5(g/100ml), 138.9 ± 0.73(fl), 29.01 ± 23.85(%), 55.30 ± 0.77(pg) and 398.4 ± 4.9(g/dl) for RBC, WBC, Hb, MCV, PVC, MCH and MCHC, respectively. Samples from Bida had mean values of 2.386 ± 0.099($X10^6$/ml), 4.102± 0.035($X10^3$/ml), 13.14 ± 0.48(g/100ml), 142.6 ± 2.91(fl), 34.84 ± 1.42%, 56.68 ± 1.16 (pg) and 386.5 ± 7.5(g/dl) for RBC, WBC, Hb, MCV, PCV, MCH and MCHC, respectively (Table 4.2).

There were highly significant differences ($p<0.05$) between the mean RBC, Hb and PCV ofKontagora and corresponding values in Minna. While the PCV between Minna and Bida was highly significant ($p<0.01$), the Hb between Minna and Bida was just significant at $p<0.05$(Table 4.2).

4.3 RESULTS OF SERUM BIOCHEMICAL ASSAYS

Table 4.3 shows results of serum total protein, cholesterol, albuminand triglyceride levels assayed. Kontagora, Minna and Bida recorded mean values of 5.020 ± 0.378(g/dl), 4.258 ± 0.223(g/dl) and 3.854 ± 0.299(g/dl) for serum total protein, respectively. The mean values for serum cholesterol level recorded were2.992 ± 0.345(mg/dl), 3.345 ± 0.149(mg/dl) and 3.499 ± 0.299 (mg/dl) for Kontagora, Minna and Bida, respectively. The mean values of albumin serum level recorded were 2.20 ± 0.132(g/dl), 6.136 ± 0.25(g/dl) and 5.078 ± 0.4(g/dl) for Kontagora, Minna and Bida, respectively. The mean values of triglyceride serum level were 5.557 ±

1.042(mg/dl), 2.701± 0.262 (mg/dl) and 3.126 ± 0.405 (mg/dl) for Kontagora, Minna and Bida, respectively.

Table 4.1: Seroprevalence of *M. gallisepticum* and *M. synoviae*in local chickens in Niger State.

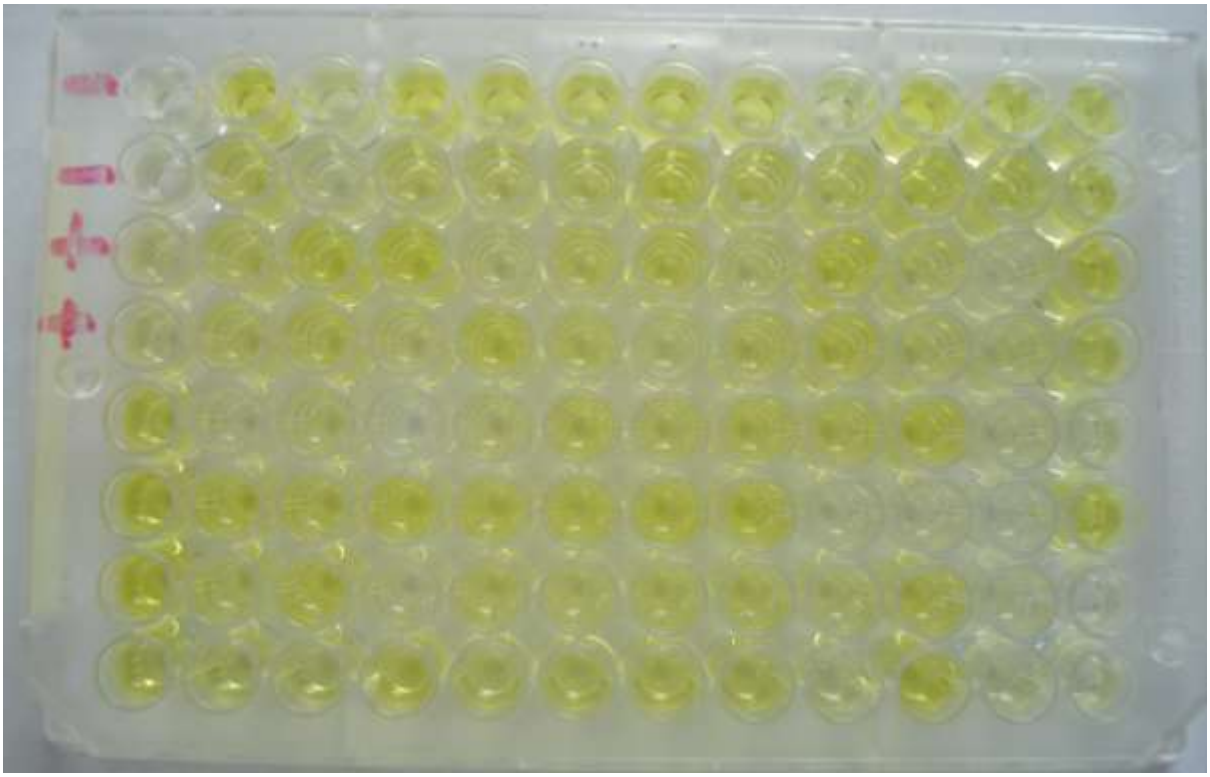
		ELISA	SPA-MG	SPA-MS
Location	n value	No.positive(prevalence %)	No. positive(prevalence %)	No. positive (prevalence %)
Kontagora	184	173 (94.02)	158 (85.86)	142 (77.12)
Minna	184	170 (92.39)	155 (84.23)	153 (83.13)
Bida	184	164 (89.13)	160 (86.95)	145 (78.84)
Total	552	507 (91.84)	473 (85.86)	440 (79.71)
		p= 0.2178;(p>0.05) X^2 = 3.049	p= 0.7553;(p>0.05) X^2 = 0.5614	p= 0.3374;(p>0.05) X^2 = 2.173

Key:

ELISA = Enzyme linked immunosorbent assay

SPA-MG = Serum plateagglutination test for *Mycoplasma gallisepticum*

SPA-MS= Serum plateagglutination test for *Mycoplasma synoviae*



1 2 3 4 5 6 7 8 9 10 11 12

A

B

C

D

E

F

G

H

PlateI: *M. gallisepticum/M. synoviae* ELISA plate.Note wells A1 and B1negative controls (marked -) and wellsC1and D1(marked +)positive controls. Yellow coloured wells indicate positive reaction of sera samplewhile the clear wells are the seronegative reaction of sera sample (from Bida).

Table 4.2: Mean haematological values of seropositive local chickens to Mycoplasma (n= 507) inNiger State.

Parameters	Kontagora	Minna	Bida	Normal range
RBC ($\times 10^6$/ml.)	2.605± 0.054[a]	2.098 ± 0.094[a]	2.386 ± 0.099	2 – 3
Hb (g/dl)	14.45 ± 0.264[a]	11.45 ± 0.5[ab]	13.14 ± 0.48[b]	8- 13
PCV(%)	37.51 ± 0.75[a]	29.01 ± 1.29[a,b]	34.84 ± 1.42[b]	30 – 40
WBC ($\times 10^3$/ml)	4.291± 0.146	4.22± 0.071	4.102± 0.035	6-8
MCH (pg)	56.49 ± 0.64	55.3 ± 0.77	56.68± 1.16	28 – 35
MCHC (g/dl)	389.9 ± 0.64	398.4 ± 4.9	386.5 ± 7.5	27 – 34
MCV(fl)	144.3 ± 3.9	138.9 ± 0.73	142.6 ± 2.91	87 – 149

Superscripts with the same alphabet are significantly different ab $p< 0.05$

Table 4.3*:* Mean serum biochemical parametersfrom local chicken seropositive to *M. gallisepticum and M.synoviae* in Niger State.

Parameter	Kontagora	Minna	Bida	Normal range.
Total protein (g/dl)	5.020 ±0.378	4.258 ±0.223	3.854±0.299	3 -6
Cholesterol (mg/dl)	2.992±0.345	3.345±0.149	3.499±0.191	104 -123
Albumin (g/dl)	2.20 ±0.132^{ab}	6.136±0.25^{a}	5.078 ±0.40^{b}	2 – 3
Triglyceride (mg/dl)	55.57± 1.042	27.01±0.262a	31.26±0.405	68 – 85

Superscripts with the same alphabet are significantly different ab $p< 0.05$

4.4RESULTS OF MYCOPLASMAISOLATION

Out of the total 138 tracheal swabs taken,126 swabs yielded to the growth of avian mycoplasma on mycoplasma agar (Kontagora48/50, Minna46/50 and Bida32/38) (Plate II).

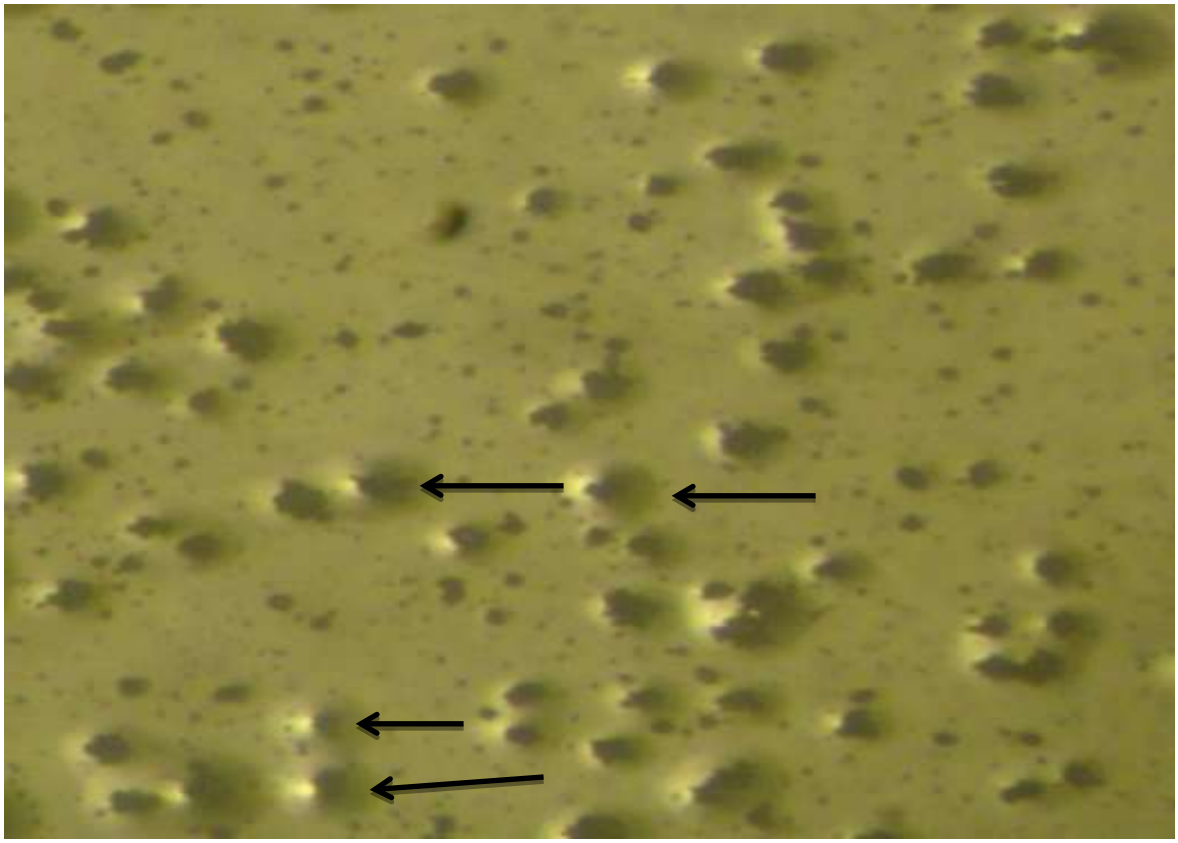


Plate II: Avian Mycoplasma colonies on PPLO agar plates. Note the round raised central dark area(nipple) and a translucent periphery, X 45.

4.5 RESULTS OF HISTOPATHOLOGICAL STUDIES.

There were varying degrees of cellular infiltrations, multifocal areas of haemorrhages within the lung parenchyma and collapse of air capillaries (from Kontagora 3/5, Minna 1/5 and Bida 0/5).

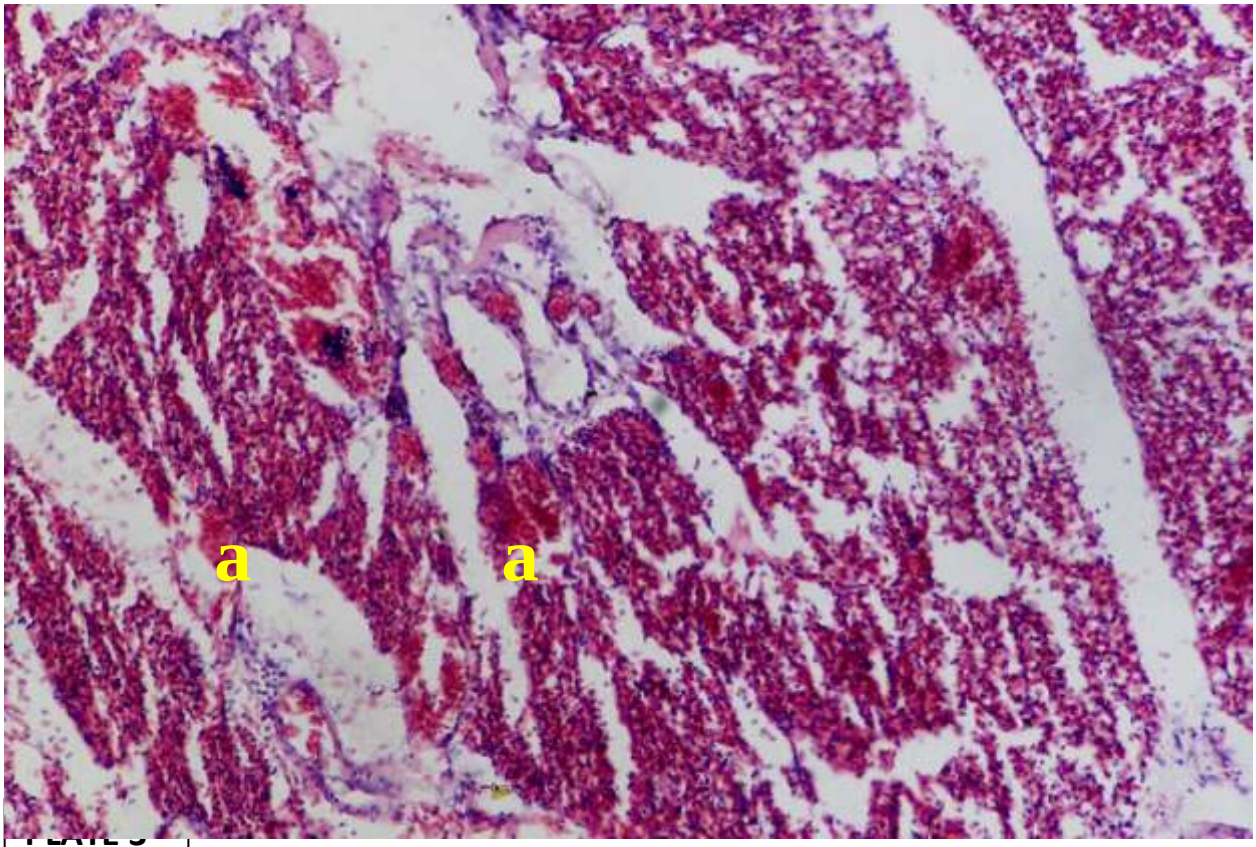


Plate III: Photograph of a microsection from lung of chicken. Note the multifocal areas of haemorrhage (**a**)withinlung parenchymaand absence of air capillaries (atelectasis). H&E. X 200.

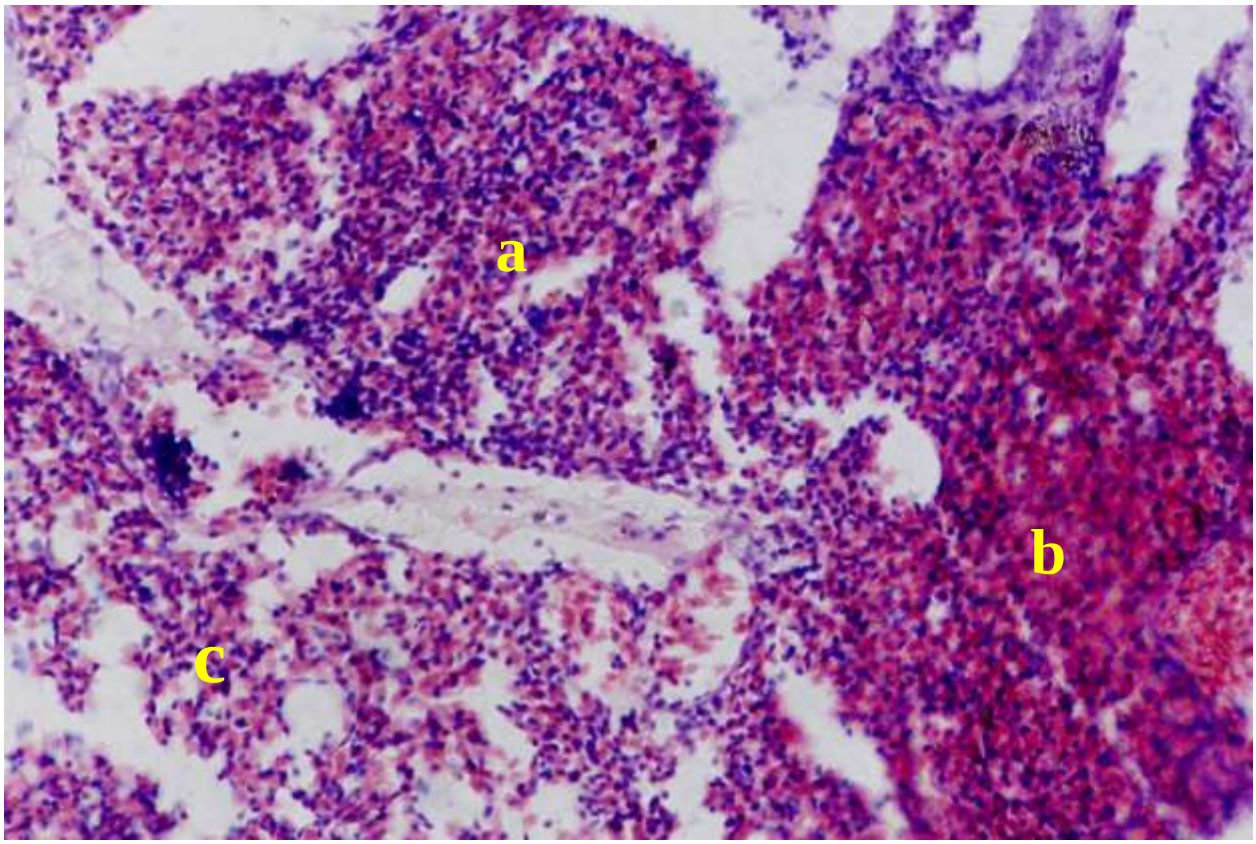


Plate IV: Photograph of amicro section of chicken lung (Kontagora). Note diffused cellular infiltrationin the lung parenchyma(a), congestion (b) and inflammation of capillary walls (c) apparentloss of the lung architecture with collapse of the air capillaries.H&E. X 200.

CHAPTER 5

5.0 DISCUSSION

The results from this study revealed that the seroprevalence of local chickens from three Local Government Areas of Niger State was 91.83% by MG/MS ELISA technique. Serum plate agglutination test for *M. gallisepticum* (SPA-MG) revealed a prevalence of 85.68%, while SPA-MS for *M. synoviae* revealed a prevalence of 79.71%. These results are higher than the earlier report of 32.4% for *M. gallisepticum* by Addo (1976)who used SPA test for native fowls in northern Nigeria, and the report of Nawathe *et al.*(1982)who reported seroprevalences of 45.5%, 39%, 48%, 19.7% 34.6% 70% and 40% for*M. gallisepticum*in Lagos, Anambra, Imo, Benue, Kaduna, Plateau and Kano States, respectively, using SPA test. These workers also reported prevalences of 61.1%, 35.3%, 54%, 45%, 40%, 40% and 58.5%, respectively for *M. synoviae* in Lagos, Anambra, Imo, Benue, Kaduna, Plateau and Kano States, respectively.

In this study the prevalence recorded was higher withELISAthan SPA. This could be that the ELISA kit being a combined kit (MG/MS) will record positive for sera having either of the two antibodies present. However, the seroprevalence recorded by both test was high. This could be that there has been no control measure for avian mycoplasmosis over the years and the local chickens do not get medical attention from their owners when the chickens show any sign of sickness. The findings in this study also confirm previous reports from several countries of the world which showed the presence of infection by *M. gallisepticum*and*M. synoviae* in chickens.Mohammed *et al*. (1986) reported seroprevalence of 73% and 3% for *M. gallisepticum* in Southern and Central California, respectively, while Godoy *etal*. (2001) in their study reported prevalencesof 59%, 22% and 66% for *M. gallisepticum* in three municipalities in Aragua State, Venezuela. Zhang *etal*. (2001) reported a prevalence of 53% in broilers in Mongolia. Zelenika *etal*. (1999) working in Croatia reported prevalence of 10% in 1997 and 13% in 1998 of *M.*

gallisepticum in heavy hen hybrids. In Zimbabwe, Kelly *et al.* (1994) reported a prevalence of 33% for *M. gallisepticum*. In Malaysia, Shah-Majid (1996)in his work on seroprevalence study of *M. gallisepticum* in village chickens using ELISA, found 26% prevalence.

Among all serological tests suggested by OIE (2008)ELISA issaid to be more sensitive, highly specific and reproducible, but it is time consuming and more expensive than the SPA.

The high seroprevalence of *Mycoplasma* infection in local chickens sampled implies that there was active antibody production due to natural challenge with Mycoplasma possibly from both vertical and horizontal ways of transmission.From all the local chickens sampled in this study, none showed any obvious clinical signs associated with *Mycoplasma* infection. The absence of clinical signs and gross lesions had been reported earlier in chickens by other workers on avian Mycoplasma (Carpenter *et al.*, 1981;Salami, 1986; Lierz *et al.*, 2000; 2002;Talha, 2003; Peebles*et al*., 2004 and Lierz *et al*., 2008).

Although SPA has the disadvantage of producing false positive reactions and producing weak reactions because of the heterologous strains of Mycoplasma being used as antigens, and stronger reactions when homologous strains are used (Kleven *et al*., 2007),in this study, there were no marked variations of the prevalence for *M. gallisepticum* and *M. synoviae*on SPA, they were all high.

It is also believed that some degree of protection against diseases of the respiratory tract may be encountered in chicken and turkeys that were naturally infected (Alder, 1954). This suggests that despite the high antibody titre observed in this study, the chickens naturally had some degree of protection against Mycoplasma and this probably may suggest why there were no obvious clinical signs observed from local chickens in this study.

Furthermore, *Mycoplasma spp* have been reported to propagate intracellularly during pathogenesis (Vogl *et al.,* 2008). This intracellular persistence is believed to be the cause of chronicity of avian Mycoplasmosis. The apparent advantages of entering eukaryotic cells are protection from the host's immune system and easy access to nutrients. *Mycoplasmas* are also reported to invade chicken erythrocytes (Vogl *et al*., 2008). By so doing the *Mycoplasma* evade destruction by the immune system. A major advantage of entering erythrocytes is that it is a means to reach new sitesof infection.This systemic spread via the blood stream explains how the Mycoplasma reaches distant niches after the first colonization of the respiratory system(Vogl *et al*., 2008).

There were varying degrees of cellular infiltrations, congestion, collapse of air capillaries in the lungs (atelectasis) and inflammation of the air capillary walls which indicated that there is respiratory tract challenge. Jordan (1981) reported that following natural infection, histopathological changes occur mostly in the respiratory tracts and that the changes may include hyperplasia of respiratory and synovial epithelium; and in some cases cellular degeneration and marked monocytic infiltrations, and massive infiltration of heterophils and reticular cells as observed in this study.

The haematological data recorded for Kontagora, Minna and Bida were not higher than the normal range reported by Oyewale and Durotoye, (1988) (RBC, $2.48 \pm 0.4 \times 10^6$/ml; Hb, 10.58 ± 1.27 g/dl; PCV, $34.08 \pm 2.95\%$; MCV, 140.05 ± 19.47 fl) and Oladele (2009). There was highly significant ($p<0.05$) difference in the mean RBC, Hb and PCV values between Kontagora and Minna. Similarly,the mean PCV values between Minna and Bida were very significant ($p<0.05$).

Haematological values of avian species are also significantly influenced by poultry diseases, such as mycoplasmosis (Branton *et al.*, 1997; Burnham *et al.*, 2003; Talebi *et al.*, 2005).

In this study,the serological tests indicate that mycoplasmosis due to *M. gallisepticum* and *M. synoviae* is present in local chickens sampled in Niger State.

The results of the serum biochemistry obtained during this study were lower than the reports of previous works by Durotoye *et al.* (2000) who reported the values of 6.2 ± 0.1 mg/dl for total protein, 3.10 ± 0.60 mg/dl for albumin, 113.3± 10.1 mg/dl for cholesterol and 79.8 ± 5.9 mg/dl for triglyceride, but similar to the report of Ladokun *et al.*(2008) who also reported serum total protein range of 4.63 - 4.81 mg/dl, cholesterol 31.3 – 32.45mg/dl and albumin 3.28 - 3.48 g/dl in Nigerian chickens.

The low levels of biochemical assays recorded could result due to several factors, such as inavailability of feed, acute infections and infestations, temperature, geographical location etc. affect the physiology of birds under natural conditions (Sastry, 2004; Oladele *et al.*, 2005; Wakawa, 2007).

The low levels of serum biochemical values will affect their function in the proper maintenance of osmotic pressure between the circulating fluid and the fluid in tissue spaces and in turn hamper with the exchange of materials blood and tissue cells thus leading decreased productivity as reported earlier (Ley and Yoder, 1997; Hossain*et al.*, 2007).

It has also been established that the isolation of the organisms is the strongest evidence of aninfection (Jordan, 1981).In this study, these organisms were isolated when agar plates,

containing mycoplasma growth were observed under light microscope, colonies appeared smooth with entire margins. Colonies possessed a central dark area (nipple) and a translucent periphery identical to fried egg appearance. This implies that mycoplasmosis is present in the sampled local chickens as evidenced by the result of the two serological test in this study.

CHAPTER 6

6.0 SUMMARY

The seroprevalenceof *Mycoplasma gallisepticum* and *M. synoviae*were determined by testing sera of five hundred and fifty two local chickens using enzyme linked immunosorbent assay (ELISA) and serum plate agglutination (SPA) test.A seroprevalence of 91.84% was recorded the combined MG/MS ELISA while SPA-MG and SPA-MS recorded 85.68% and 79.71% respectively. The haematological parameters of seropositive sera were determined using a DC3000 auto haemo analyser. The range RBC, Hb, PCV and WBC obtained were 2.09 – 2.6 $x10^6$/ml, 11.45 – 14.45

g/dl, 29.01 – 37.51 % and 4.10 – 4.29 x10^3/ml respectively. The range for the serum biochemical parameters obtained for total protein, cholesterol, albumin and triglyceride were 3.8 – 5.0 g/dl, 2.9 – 3.4 mg/dl, 2.2 – 6.1 g/dl and 2.7 – 5.5 mg/dl respectively. A total of one hundred and thirty eight tracheal swabs were taken from the local chickens in the three local government areas sampled and cultured on mycoplasma agar plates. One hundred and twenty six swabs yielded mycoplasma on mycoplasma agar. The lung tissue samples collected from fifteen chickens after postmortem for histopathology revealed varying degrees of cellular infiltrations, multifocal areas of haemorrhage within the lung parenchyma and collapse of aircapillaries.

6.1 CONCLUSIONS

From this study it was concluded that:

1. *Mycoplasma gallisepticum* and *M. synoviae*infection was present among local chicken population in threeLocal Government Areas of Niger State, hence the high seroprevalence of 91.84% that was detected by MG/MS ELISA, 85.68%by SPA-MG and 79.71% by SPA-MS. This implies that there was high infection or exposure of *Mycoplasma* among local chickens in Niger State.
2. The local chickens could be a major reservoir of *M. gallisepticum* and *M. synoviae*infection in Niger State and Nigeria; because it was observed that the chickens had high seroprevalence for *M. gallisepticum* and *M. synoviae*even in the absence obvious clinical signs.

6.2 RECOMMENDATIONS

1. There is need for a national surveillance and control to combatavian mycoplasmosis in Nigeria.

2. There is need for the production of SPA antigen using local homologous mycoplasma antigen here in Nigeriain commercial quantity quick and better diagnosis of avian mycoplasmosis than using heterologous mycoplasma antigens.
3. There is need for further research on the effect of avian mycoplasmosis on the haematological and serum biochemical parameters in local chickens in Nigeria.
4. There is need to isolate and characterise various mycoplasmaspecies in local chicken and other avian species in Niger State and other States in the Country.

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APPENDIX 1

LIQUID CULTURE MEDIA (BROTH).

Pleuro-Pneumonia Like Organism (PPLO) broth	10.5 g
Yeast extracts	50.0g
Phenol red (1%)	1.0ml
Penicillin	1,000,000 iu
Thallium acetate (10%)	0.5ml
Nicotinamide adenine di nucleotide (NAD)	0.5g
Glucose	0.5ml
Cysteine hydrochloride	0.05g

Horse serum	120ml
Distilled de ionized water	1000ml.

APPENDIX 2

SOLID AGAR

Pleuro-Pneumonia Like Organism (PPLO) broth	5.25g
Agar noble	2.5g
Horse serum	30 ml
Phenol red (1%)	0.5ml
Yeast extracts	25ml
Penicillin	1,000,000iu
Glucose (10%)	2.5ml
Cysteine hydrochloride	0.025g
Nicotinamide adenine di nucleotide (NAD)	0.025g

Deionised water	175 ml.