EVALUATION OF THE EFFECT OF *ALLIUM SATIVUM, LINN* (GARLIC) IN THE PREVENTION OF PASTEURELLOSIS IN JAPANESE QUAILS

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BY

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DEPARTMENT OF VETERINARY MEDICINE
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MARCH, 2017

DECLARATION

I hereby declare that the work in this Dissertation entitled **"Evaluation of the Effect of *Attium Sativum, Linn* (Garlic) in the Prevention of Pasteurellosis in Japanese Quails"** has been carried out by me in the Department of Veterinary Medicine under the supervision of Professor P.A. Abdu, Professor C. A. Kudi and Dr. M. Bello. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any other Institution.

Thomas AMAL1	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This Dissertation entitled ***"Evaluation of the Effect of Allium Sativum, Linn (Garlic) in the Prevention of Pasteurellosis in Japanese Quail"*** by Thomas AMALI meets the regulations governing the award of the Degree of Master of Science of Ahmadu Beilo University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Dissertation is dedicated to God Almighty and my family to come. You all will enjoy the dividend of this work to the fullest.

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ABSTRACT

Indiscriminate use of antibiotic in animals and human to control bacterial disease create resistant bacteria. It is therefore imperative to seek alternative treatment for the control of Pasteurellosis caused by *Pasteurella multocida mitltocida* A:4 in quails. Quail meat and eggs are renowned for their high quality protein and low caloric values. Garlic is bactericidal, cheap and available without toxicological effects. One hundred, four (4) week old Japanese male and female quails *(Coturnix coturnix japonicd)* were randomly divided into ten groups (A, B, C, D, E, F, G, H, I and J) of ten birds each. Group A was not inoculated with *Pasteurella multocida multocida* serotype A:4 at 10^4 colony forming unit (cfu/ml), thus serving as negative control. Group B was inoculated with *Pasteurella multocida multocida* serotype A:4 as positive control for comparism without treatment, Group C was inoculated and treated with ciprofloxacin, group D, E and F were inoculated and treated with garlic in feed at 3%, 5% and 10% respectively, groups G to J were inoculated and treated with garlic in water at 1%, 1.67%, 3.3% and 3.3% respectively. Ciprofloxacin in water and garlic infeed or water were administered three (3) days before inoculation. All the groups B to I were inoculated with *Pasteurella multocida mulocida* serotype A:4 at 10^4, (cfu)/bird except group J that was inoculated with 10^6cfu/bird. Half of the infective dose was administered intranasally while the second half was given intramuscularly (pectoral muscle). Clinical signs, morbidity and mortality rates were monitored for 13 more days. The values of packed cell volume (PCV), total plasma protein (TP) body weight gain (BWG) were determined. Gross and microscopic lesions in sections of heart, liver and spleen were evaluated on days two (2) and 13 post inoculation in all the groups for comparism. Daily cloacal temperature were also taken. The effect of ciprofloxacin, garlic concentrations in-feed, and water were determined based on mortality at the end of the experiment. The results showed that there was loss of appetite, somnolence and hurdling together were initially noted a day post

inoculation in groups B, F, G and I. The value of PVC were significantly reduced ($p<0.05$) in all the inoculated groups. However, there was significant ($p<0.05$) increment in PCV after 4-5 days post inoculation. There was significant increase ($P<0.05$) in temperature in all the groups that were inoculated as compared to the negative control (group A). However, these temperature dropped to normal values including the negative control (group A) except in the positive control (Group B). Generally, there was increase in body weight gain in all groups. On day three (3) post inoculation, microscopic sections of the spleen of quails inoculated with *Pasteurella multocida muhocida* A:4 showed depletion of lymphocytes at the germinal centres. In the liver, there were fatty degeneration, congested sinusoids and distorted hepatic architecture while the heart had mononuclear cellular infiltration. However, at 13 days post inoculation, the spleen, liver and heart showed normal photomicrograph. Mortality started a day post inoculation and ended five (5) days post inoculation. The highest reduction of mortality was observed in ciprofloxacin treated water at 10% concentration (mortality rate 20%) followed by garlic treated water at 3.3%. Concentration (mortality rate 30%). Garlic at 3.3% concentration in water reduced the effect of pasteurellosis.

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LIST OF ABBREVIATIONS AND SYMBOLS

AD	Anno Domini
BC	Before Christ
Bwg	Body weight
Cfu	Colony forming units
Cipro	Cipro floxacin
cm	Centimetre
CTCR	Centre Technique de Coopertion Agricole et Rurale
DNA	Deoxyribonucleic acid
e.g	For example
EDTA	Etheylene diether tetra acetic acid
etc	et cetera
FAO	Food and Agriculture Organization
FDA.	Food and Drug Administration
g	Gram
G	Guage
g/dL	Gram per decilitere
g/1	Gram per litres
g/mol	Gram per mole
h	Hour
H and E	Haematoxylin and Eosin
Histo	Histopathology
LD	Lethal Dose
M	Metres
Ma	Mortality
MDR	Multidrug resistance
ME	Metabolisable energy
mg/g	Milligram per gram -
MJ	Mega Joules
Ml	Mililitres
mm	Millimetres
Mo	Morbidity

°C	Degree Celsius
PCV	packed cell volume
PM	Postmortem
RNA	Ribonucleic acid
S.E.M	Standard error of the mean
TP	Total protein
USD A	United State Department of Agriculture
W/V	Weight per volume
WHO	World Health Organisation
%	Percentage
<	Less than
>	Greater than
ul	Microlitres
fig	Microgram

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The use of plants in the treatment of livestock diseases problems dates back to several centuries as ancient traditions of raising livestock employed the use of ethnoveterinary medicine (Wanzala *et al.,* 2005). Historical records of the use of ethnoveterinary practices are over 14,000 years old. For example, the use of Ayurvedic medicine in Sri Lanka in the treatment of horses and elephants in 3,000 BC (Githiori *et al.,* 2005; Wanzala *et al.,* 2005). Many of the active ingredients in chemically manufactured drugs were originally derived from plants. For instance, morphine was isolated from the opium poppy (*Papaver somniferum*) in 1806 by Frederick Surturner (Schiff, 2002) and salicylic acid in 1839 (Schiff, 2002; Wanzala *et al.,* 2005). Thus, plants used in traditional medicine constitute an important source of new biologically active compounds (Masoko *et al.,* 2007).

Recently, interest in ethnoveterinary medicine, especially with regards to medicinal plants is on the increase. This is due to the rapid emergence of new diseases and organisms which are resistant to available drugs (Wanzala *et al.,* 2005; Masoko *et al.,* 2007). Gammaniel (2000), reported that people who employ the use of medicinal plants in the treatment of disease believe that medicinal plants are in their natural state and less toxic thus, making them safer than manufactured drugs, in addition to being relatively cheap when compared to manufactured drugs. In developing countries, medicinal plants are still used on a regular basis (Wanyama, 2000). This is because they are more accessible than manufactured drugs especially where modern techniques are non-existent and or even when available are too expensive (Wanyama, 2000; Alawa *et al.,* 2002; Wanzala *et al.,* 2005).

The World Health Organization has estimated that perhaps 80% of the inhabitants of the world rely chiefly on traditional medicines (WHO, 1993). It therefore approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of these products (Wolfgang, 2005; Saxena, 2010).

Quail farming was introduced into Nigeria in 1992 to boost sources of animal protein for the populace (Haruna *et al.,* 1997). The short generation interval, fast growth and lower feed requirement made it an ideal bird and an additional source of revenue for low and medium income poultry farmers. Quails are considered to be more resistant than chickens and turkeys to common poultry diseases (Naveen and Arun, 1992).

Despite their relative resistance to infection there have been reports on the susceptibility of quails to salmonellosis, ulcerative enteritis, botulism, colibacillosis, fowl cholera, Marek's disease, aspergillosis and candidiasis (Sharma and Kaushik, 1986; Naveen and Arun, 1992). Of the bacterial diseases affecting quails, avian pasteurellosis (fowl cholera) caused by *Pasteurella multocida multocida* is the most economically detrimental because of its rapid onset and potential to cause high morbidity and mortality (Odugbo *et al.*, 2004).

Pasteurellosis was first reported in commercially raised bob white quails (*Colinus virginarus*) in 1982 (Panigraphy and Glass, 1982). It has also been reported in captive California valley quail (*Lophortyx caflfornicus*) in 1943 (Hinshaw and Emlen., 1983) and Japanese quails (*Coturnix japonica*) in the USA (Glisson *et al.,* 1989), India (Chandran *et al.*, 1995) and Japan (Goto *et al.*, 2001).

Fowl cholera was first reported in Nigeria in quails 1999 and since then there have been 11 reported outbreaks (Odugbo *et al.,* 2004). The disease occurred in peracute and acute forms in which large numbers of quails were usually found dead in good body condition, with no

premonitory clinical signs. In a few cases, noticeable clinical signs included marked depression, anorexia, fetid diarrhoea with apparent dehydration and lethargy, with death occurring a few hours after the onset of clinical signs (Odugbo *et al.,* 2004).

Postmortem examination showed that gross lesions were absent in most of the birds. Although few that showed lesions of vascular disturbance were associated with the acute disease including general hyperaemia, and swollen liver with multiple small areas of necrosis; other lesions include thrombi in the lungs and liver, petechial and ecchymotic haemorrhages involving the epicardium of the heart and increased pericardial and peritoneal fluids (Odugbo *et al.,* 2004).

1.2 Statement of the Research Problem

One of the important challenges of quail (*Coturnix coturnix japonica)* producers is preventing disease outbreaks. Quails are susceptible to a variety of non-infectious, infectious and parasitic diseases. Because they are related to chickens and turkeys, many of the diseases in quails are similar to those in poultry (Myint and Carter, 1988). The three most common diseases that have been reported to occur in quails include bronchitis, ulcerative enteritis and quail pox (Dozie and Bramwell, 2002). Other conditions reported in quails include salmonellosis, botulism, colibacillosis, fowl cholera, Marek's disease, aspergillosis and candidiasis (Khare *et al.,* 1975; Sharma and Kaushik, 1986; Naveen and Arun, 1992), Newscatle disease (Higgins and Wong, 1968; Lima *et al.,* 2004; Oladele *et al.,* 2008) and *Pseudomonas aeruginosa* infection (Badr *et al.,* 2006). In a survey between 1999 and 2002, eleven outbreaks of pasteurellosis occurred in Nigeria involving flocks of about 7,000 – 10,000 Japanese quails (Odugbo *et al.,* 2004). The farm suffered high mortalities involving 250 – 500 deaths of 4 week old quails in each outbreak. The isolates from the outbreaks were identified as *Pasteurella multocida multocida* serotype A4. In Nigeria, fowl cholera is a

major constraint to poultry production and outbreak could cause high mortalityrate of up to 80% (Abdu, 1990; Ambali *et al.,* 2003; Odugbo *et al.,* 2004; Akpavi *et al.,* 2011).

Indiscriminate use of antibiotics in animals and humans create resistant bacteria (Javandel, *et al.,* 2008). There is increasing pressure to reduce the use of antibiotics in poultry feed due to negative human health issue arising from antibiotic resistance (Javendel, *et al.,* 2008).

It is imperative to seek for alternative treatment of fowl cholera in quails, because the birds are consumed by human beings. The mechanism of transfer of drug from animals to human via the food chain is still incomplete (Janvan, 1998). Human physicians however believed that the use of antibiotic in animal health contribute substantially in this resistance (Janvan, 1998). Researching on garlic as alternative to antibiotics for the treatment of fowl cholera in quails prompted this research.

There is no research work or report in the literature on the use of garlic for the treatment of fowl cholera in chicken in Nigeria. This research therefore is designed to provide information on the efficacy of garlic for the treatment of fowl cholera in quails.

1.3 Justification of the Study

Poor animal health is an important factor limiting animal productivity in most developing countries. In many of these countries there has been a decline in funding for veterinary services and animal health care in general (CTCAR, 1987; Alawa *et al*., 2002). Traditional remedies on the other hand, are more readily available and cheaper than orthodox veterinary treatment. Ethnoveterinary medical health care is the only alternative to “western” veterinary therapy (Alawa *et al.,* 2002). Traditional medicine as defined by WHO (2000) is the sum total of the knowledge and practices, whether replicable or not, used in diagnosis, prevention and elimination of physical and mental or social imbalance and relying exclusively on practical

experience. There is also an increasing awareness of the use of medicinal plants in treating many livestock disease (Abu *et al.,* 2009).

Ethnoveterinary medicine or traditional animal health care comprises all practices, methods and indigenous knowledge which are applied to alleviate livestock diseases. Modern veterinary services and allopathic drugs are expensive and unavailable in some communities. Hence resource limited rural and peri-urban livestock farmers resort to the use of herbal remedies (Abu *et al.,* 2009).

Garlic is widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases ranging from infectious to heart disease. Raw garlic (*Allium Sativum*) used in Nigeria as a vermifuge was shown to have some activity against *Ascaridia galli* in chickens and to inhibit the embryonation of the eggs of *Necator americanus* and *Ancylostoma caninum* (Dalziel, 1937; Bastidas, 1970; Das and Thakuria, 1977). Kolawole *et al*. (2007), reported that crushed garlic mixed with feed for local chicken suffering of diarrhoea were cured in about seven days.

Japanese quail farming was introduced more than a decade ago in Nigeria (Haruna *et al.*, 2008). They are reared by many farmers in different parts of the country and accepted by the populace especially because of their prolific nature, lean meat and low level of cholesterol in both meat and egg (Haruna *et al.,* 2008). With a population of over 140 million people, Nigeria has over the years not been able to meet the Food and Agricultural Organization recommended minimum protein intake requirement of 65 g per person/day. (Haruna *et al.,* 2008). This is partly because the expansion of cattle population cannot progress at a satisfactory rate to cope with the increasing demands for meat. This has given rise to the increase in commercial poultry production. However, since 1986, poultry production has been on the decline mainly due to high cost of feed, inadequate supply of day old chicks,

diseases, high cost of drugs and biological. This led to the search for alternative cheaper sources of poultry production and subsequent introduction of quails in Nigeria (Haruna *et al.*,2008).

Quail meat and eggs are renowned for their high quality protein. The biological value and low caloric values makes it a choice of meat in hypertensive-prone individuals (Haruna *et al.*, 1997). Garlic has medicinal value, it is cheap and available. Researching into its curative and prophylactic properties is beneficial not only in quail production but in poultry. Knowledge of the possible effects of the use of garlic on haematological parameters and pathological change will add information to the toxicological information of the herb which is widely consumed by human beings.

1.4 Aim of the Study

The aim of the study was to evaluate the efficacy of garlic in the prevention of pasteurellosis in Japanese quails

1.5 Objectives of the Study

The objectives of the research were to;

1. Evaluate the efficacy of garlic in quails inoculated with *Pasteurella multocida multocida* serotype A:4,
2. Determine the effects of garlic on some haematological values in quails inoculated with *Pasteurella multocida multocida* serotype A:4,
3. Evaluate the pathological lesions in quails inoculated with *Pasteurella multocida multocida* serotype A:4 under treatment with garlic garlic.

1.6 Research Questions

1. Is garlic effective in preventing disease in quails inoculated with *Pasteurella multocida multocida* serotype A:4
2. What are the effects of garlic on PCV, TP, body weight cloacal temperatures
3. Is there any pathological lesion in tissue of quails inoculated with *Pasteurella multocida multocida* A:4

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical of Medical Plant

2.1.1 Plants and herbal medicine

Plants that have healing properties are termed as medicinal plants or herbs. Medicinal plants can be simply classified as trees, shrubs, woody perennials, annuals and biennials and climbers (Everaldo, 2005). Medical herbalism is the practice of healing with medicinal plants. Modern western treatment is different from medical herbalism, but at some point these two merge. For example, the use of friar's balsam or benzoin tincture for treating colds, the use of *Aloe vera* gel for treating sun burn and bruises and the use of cascara or senna to relieve constipation. The tendency in modern medicine is to use synthetic drugs that eventually were modeled on compounds obtained mainly from plants. Therefore, whether the plants are used as a whole or extracts or their synthetics, their discovery originated from the long term practice of medical herbalism (Everaldo, 2005).

2.1.2 Liliaceae family

The lily family is composed of large number of plants with medicinal virtue. Most of these are herbs and rarely shrubs. Examples from this plant family include asphodel (*Asphodelus aestivus*), wild asparagus (*Asparagus aphyllus*), seaside squill (*Drimia maritima*) mediterranean smilax (*Smilax apera*), greater butcher's broom (*Ruscus hypophyllum*), butcher's boom (*Ruscus awleatus*), tassel hyacinth (*Muscari comosum*). Madonna lily (*Lilium candidum*), bluebell (*Hyacinthus orientalis) (Aloe vera),* garlic (*Allium sativum*), garden onion (*Alium cepa*), meditterranean meadow saffron (*Colahium cupani*), meadow saffron (*Colchium cupani*), meadow saffron (*Colctium autunnale*) (Evaraldo, 2005).

Garlic (*Allium sativum*) is a rather cultivated plant that originated from central Asia. It flowers between May and June. The bulb of the plant is used medicinally, in the fresh, dried or processed state. It contains an essential oil and allin that is broken down into allicin as the tissue is disrupted on cutting or pressing. These constituents make garlic strongly antiseptic, hypertensive and expectorant. Externally it can be applied to boils, insect bites and broken chilblains (Everaldo, 2005)

2.2 Ethnoveterinary Medicine

2.2.1 Ethnoveterinary medical practices and knowledge

Ethnoveterinary medicine or traditional animal health care comprises all practices, methods, and indigenous knowledge which are applied to alleviate livestock diseases (Abu *et al.*, 2009). The era of treating ethnoveterinary medicine and any other ethno-knowledge system with suspicion and labeling it as myth, superstition and witchcraft, is long gone (Wanzala *et al*., 2005). The role of ethnoveterinary medicine in livestock development is beyond dispute (Martin *et al*., 2001; Wanzala *et al*., 2005). As wide spread as it is, the practice of ethnoveterinary medicine has lagged behind that of its counterpart (modern veterinary medicine) many times partly because the practice was secretly done and its information hidden (Mathias, 2004; Wanzala *et al.*,2005). In many native and local stock raising communities if not all, a considerable proportion of useful ethnoknowledge and some of the traditional animal health care practices remain unknown to date (Wanzala *et al.*, 2005).

2.2.2 Brief historical perspective of traditional veterinary medicine

Wynn (2001) describes the today's traditional medicine as undoubtedly the oldest form of medicine and probably evolved simultaneously with the evolution of human beings. People's association and care for animals health is a very ancient phenomenon and perhaps, stems from the Biblical times of Adam in the Garden of Eden. This becomes self-evident in the

Bible when Adam was shown herbal remedies and made the master of all animals (Genesis 1:26 – 29). Adam of course was not a professional veterinary doctor who could treat animals when they become sick. However, this mythical presentation *per se*, sends some signals of herbal application in Adam's life time in one way (Wanzala *et al.*, 2005).

Historical records reveal people's association with and treatment of animals to be over 14,000 years ago, first with a dog, followed by sheep and goats by 9,000BC in the fertile Nile valley, and then with cattle in Egypt from 4,000BC and in Anatolia around 6,000BC (Melleart, 1967). This evolution was followed by pig and horse cultures in view of their importance (Murray, 1968). For instance, at around 3,000BC horses, elephants, and other animals were highly regarded and were in good association with man in what is present day, Sri Lanka and could be treated with Ayurvedic medicine (Anjaria, 1987). These associations were based on economic, cultural, social and religious belief attached to each type of animal and it was during this time that veterinary medicine evolved specifically to take care of the health of animals, which were being domesticated (Thrusfied, 1986). For instance, veterinary therapeutic techniques of Egyptian healer (Priest-healers) are recorded in the veterinary papyrus of Kahun (1,900 BC). They combined both religious and medical roles. Literary records of similar age, describing veterinary medical activities, are exact from other parts of the world such as Indian Sanskrit texts from the Yedic period (1,800 – 1,200BC) (Thrusfield, 1986).

2.2.3 Ethnoveterinary practice in Nigeria

In recent times there has been an increased awareness of the importance of traditional medicine in the health care of human populations in developing countries (Ibrahim and Abdul 1980). It is a test that before the advent of modern medicine Nigerians were containing animal diseases through traditional medical treatment (Nwude, 1997). Naturally, some branches of traditional medicine such as psychotherapy and therapeutic occultism did not

play much role in the treatment of animal diseases but herbal medicine, surgery and preventive medicine did play a significant role (Nwude, 1997).

Although, in Nigeria, there were no professional traditional veterinary medical practitioners, as such, herds men, village elders and others who kept animals were experienced in diagnosing and treating animal diseases. Sometimes the same herbs used in treating human diseases were also used in treating corresponding animal diseases, but there are some herbs that are used mainly for veterinary purposes and some diseases peculiar to animals that had to be treated with herbs (Nwude, 1997). Current veterinary therapy in Nigeria is suffering from both scarcity and the high cost of drugs. The danger exists that the cost of veterinary care may grow beyond the reach of the Nigerian livestock owner. The easiest and most rational solution to the problem is to develop acceptably effective drugs from reasonably inexpensive sources for use as supplements to commercial drugs (Nwude, 1997).The local remedies and drugs for the various diseases as well as the mode of administration of these remedies and vernacular names are presentedin table 2.1 (Nwude and Ibrahim, 1980).

2.2.4 Ethnoveterinary medical practices in poultry

Smallholder poultry production systems which are common in African rural areas have been described by various workers (Sonaiya, 1990; Gueye and Bessei, 1996; Gueye, 1998). Birds kept under these conditions experience high mortality resulting from accidents, predation and disease. However, the high incidence of disease is one of the principal constraints to these production systems (Sonaiya, 1990; Gueye, 1997, 1998).

The general resource poor village poultry farmers in Africa do not have money for or acess to chemical medicine or to other cost effective control measures. They rely on ancestral indigenous knowledge to control various poultry diseases (Bizimana, 1994; Gueye, 1997). In ethnoveterinary medicine the treatment and/or in some cases the prevention of disease of

poultry through ethnoveterinary medicine has been recorded. In the Gambia, one preventive measure (vaccination) traditionally used by farmers against Newcastle disease consist of blending the excreta from any wild bird with goat milk and giving the resulting mixture to village fowl to drink (Bonfoh, 1997). Chavunduka (1976) reported that *Annona senegalensis* roots are soaked in water and the fluid is sprinkled in the pen to repel snakes. In Nigeria, poultry owners grow certain plants (e.g *Euphorbia* spp and lemon grass) or place sliced garlic (*Allium sativum*) around pen houses to repel snakes (Ibrahim, 1996).

Abu *et al.* (2009) reported that the stem bark and fruit of *Adansonia digitata* in the family *Bombacacae*, is used to treat coccidiosis and fowl cholera in chickens. Also, in ruminants it is used to treat trypanosomosis, diarrhoea, and poor milk flow. Ibrahim and Abdu (1996) reported that to protect chicks and keet against hawk attacks the spiny fruit of *Cucumis pustulatus* are placed in birds drinking water. In village duck farms in Northern Nigeria several old farmers have reported lameness, respiratory disorders and cholera as the principal disease problem. To protect ducks against various diseases a preparation from the fresh leaves of *Lannea acida*, *Momordica charantia*, the fruit of sweet pepper and *Olerefera subdarfa* grounded together and dissolved in water is given to ducklings up to seven days of age (Hassan and Aliyu, 1996).

2.2.5 Garlic as an ethnomedicinal plant

Raw garlic (*Allium sativum*) used in Nigeria as a vermifuge was shown to have some activity against *Ascaridia gali* in chickens and to inhibit the embryonation of the eggs of *Necator americanus* and *Ancylostoma caninum* (Dalziel, 1937; Bastidas, 1970; Das and Thakuria, 1997). Kolawole *et al.* (2007) reported that crushed garlic mixed with feed for local chicken suffering diarrhoea were cured within 7 days. Chopped bulb of *Allium sativum* and *Capsium annuum* (red pepper) were used to control fever in local chicken given orally (Nwude and Ibrahim, 1980). Sliced*Allium sativum* bulb was used as a repellant against snake (Ibrahim,

1996).Garlic inclusion rates of 2% to 4% in feeds had a protective effect on chickens subjected to experimental candidiasis and feed containing 5% of the garlic eliminated Candidiasis (Palmer and Mckean, 1997). The aqueouct of onion and garlic at concentration of 1 and 5% respectively have measureable bacteriacidal activity with maximum bateriacidal effect at 5 and 10% (Michael and Reese 1969).

2.3 Garlic

2.3.1 Uses and composition fo garlic

Many of the spices and herbs used today were known to the people of the ancient cultures throughout the world and they were valued for their preservative and medicinal powers as well as their flavour and odour qualities (Zaika, 1988). Garlic is one of the edible plants, which has generated a lot of interest throughout human history as a medicinal panacea. Garlic (*Allium sativum* Linn.) a member of the *Liliaceae* family is used as public spice extensively in many parts of the world (Baghalian *et al.,* 2005). Many species of genus *Allium* have been used for thousands of years as vegetables, spices and as medicinal plant for the treatment of various diseases (Baruchin *et al.,* 2001; Haciseferogullari *et al.,* 2005; Sharma and Prasad, 2001). Avecina, the famous ancient Persian physician, had suggested the usage of garlic extract in the curing of numerous infectious diseases (Hosseini, 1988).

The various powerful flavours of these *Allium* species plants and their possible medical applications have attracted the attention of chemists and plant physiologists (Akgul, 1993). Most of garlic therapeutic effects are attributed to specific oil and water-soluble organosulfur compounds which are responsible for the typical odour and flavour of garlic (Sivam, 2001).

Garlic has an unusually high concentration of sulfur-containing compounds (l-3%) and its therapeutic properties are largely due to one particular class of sulfur-containing compounds, the thiosulfinates (Koch and Lawson, 1996; Lawson, 1996).The thiosulfinate structure

appears to be essential for the bactericidal, antifungal and antiprotozoal properties of garlic, likely reacting with SH-containing enzymes of these pathogens (Reuter *et al.,* 1996).

One of the important memberof these thiosulfinates is allicin, which name was taken from the Latin name of the garlic plant, *Allium sativum* (Ankri and Mirelman, 1999). Other studies were also carried out on the antimicrobial and antioxidant properties of garlic and its derivatives such as essential oil and oleoresin (Zaika, 1988; Akgul, 1993). These bioactive components have been isolated from aqueous, ethanolic and fermented extracts of crushed garlic and have the potential to interact with a number of cellular targets, particularly those exhibiting reactive sulfhydryl moieties, whose functions range from control of cell cycle to expression of crucial antioxidant and detoxification enzymes. Interactions with these processes may underlie garlic's putative therapeutic potential (Cooper and Pinto, 2005).

2.3.2 Origin and distribution of garlic

Allium sativum grows in the areas where it has become naturalized; it probably descended from the species *Allium longicuspis,* which grows wild in south-western Asia (Salunkhe and Kadam, 1998). The "wild garlic", 'crow garlic" and field garlic" of Britain are the species *Allium ursinum, Allium vineale* and *Allium oleraceum*, respectively (Salunkhe and Kadam, 1998). In North America, *Allium vineale,* known as "wild garlic" or "crow garlic" and *Allium canadense,* known as "meadow garlic" or "wild garlic" and "wild onion", are common weeds in fields (McGee, 2004). One of the best known "garlic", the so-called elephant garlic, is actually a wild leek (*Allium ampeloprasum*). In northern Nigeria, the Hausa called garlic "*tafarnuwa"* and in southern Nigeria, the Yoruba called it "*Ayo"* and while in eastern Nigeria the Igbo called it *Ayu* (Dalziel, 1937).

2.3.3 Cultivation and collection of garlic

Garlic is cultivated in well-drained moderately clay and loamy soil, it needs cool moist climatic conditions during the growth and dry period during maturity. Garlic is a hardy perennial with narrow flat leaves and bears white small flowers and bulbils. The cultivation of bulb is done by planting bulbs generally in the month of September to late October in northern Indian. It takes about four months for harvesting. It is also taken as an alternate crop with many other vegetables. For cultivation, about 300kg of bulbs per hectare are required, and yield per hectare is about 8,000 kg (Kokate *et al.,* 2007).

2.3.4 Chemical properties of garlic

Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of allyl sulfides. However, it was not until 1944 that Cavallito and Bailey (1944) isolated and identified the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur molecule, which they termed allicin, from the Latin name of the garlic plant, *Allium sativum*.

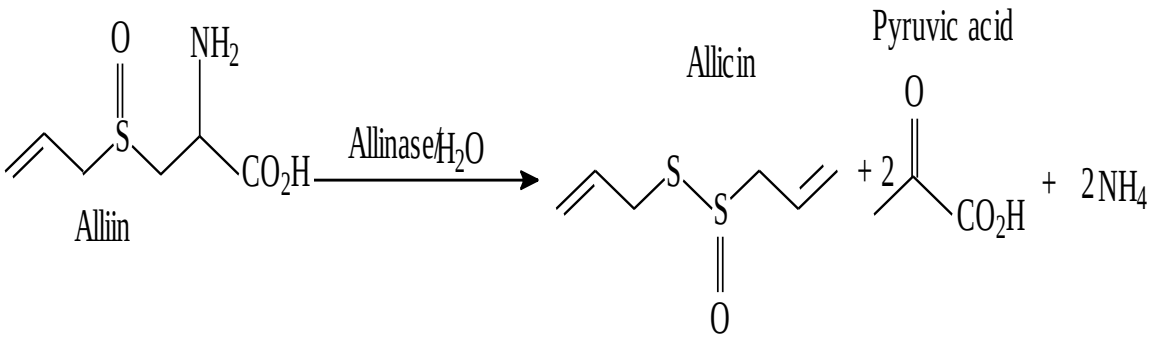
The debate on the presence of allicin in crushed garlic cloves versus its absence in odourless intact cloves was resolved after Stoll and Seebeck isolated, identified, and synthesized oxygenated sulfur amino acid that is present in large quantities in garlic cloves and which they named allin (Figure 2.1) (Stall and Seebeck, 1951). Allin was found to be the stable precursor that is converted to allicin by the action of an enzyme termed allinase, which is also present in the cloves (Figure 2.1) (Ellmore amd Feldberg, 1994). The transformation of allin into the biologically active allicin molecule upon crushing of a garlic clove is extremely rapid, being complete in seconds. The enzyme responsible for this conversion is allinase, which is present in unusually large amounts in garlic cloves: at least 10% of the total protein content (10 mg/g fresh weight) (Ellmore and Feldberg, 1994).

Garlic cloves are odour-free until crushed or processed when garlic supplements are manufactured and cross-section studies have indicated that the substrate alum and the enzyme allinase are located in different compartments (Ellmore and Feldberg 1994; Koch and Lawson, 1996). This unique organization suggests that it is designed as a potential defense mechanism against microbial pathogens in the soil. Invasion of the cloves by fungi and other soil pathogens causes the interaction between alum and allinase that rapidly produces allicin and which in turn inactivates the invader. The reactive allicin molecules produced have a very short half-life, as they react with many of the surrounding proteins, including the allinase enzyme, making it into a quasi-suicidal enzyme (Koch and Lawson, 1996).This very efficient organization ensures that the clove defense mechanism is only activated in a very small location and for a short period of time, whereas the rest of the alum and allinase remain preserved in their respective compartments and are available for interaction in case of subsequent microbial attacks.

2.3.5 Medicinal properties of garlic

2.3.5.1 *Cardiovascular Properties of garlic*

Successful clinical use of garlic for treating elevated blood pressure and arteriosclerosis has been known since the early part of this century. It has been reported that regular garlic intake causes both a prolonged lowering of hypertension and an improved sense of well-being in patients. As early as 1928, definite blood pressure decreases were achieved as well as increases in productive heart power with garlic therapy, not only in older patients, but also in younger hypertonic patients (Cellini *et al.*, 1996).



Allin + Allinase enzyme and Water = Allicin + Pyruvate

Figure 2.1: Generation of allicin in a garlic clove. Source: Koch and Lawson (1996)

2.3.6 Antibacterial properties of garlic

The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus* and *Clostridium.* Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic (Uchida *et al.,* 1975). Garlic extracts are also effective against *Helicobacter pylori,* the cause of gastric ulcers, and garlic extracts can also prevent the formation of *Staphylococcus* enterotoxins A, B and Cl as well as thermonuclease (Gonzalez-Fandos *et al.,* 1994; Cellini *et al.,* 1996). Cavalito and Bailey (1994) were the first to demonstrate that the antibacterial action of garlic is mainly due to allicin (Block, 1985). The sensitivity of various bacterial and clinical isolates to pure preparations of allicin is very significant (Holzgartner *et al.,* 1992). As shown in (Table 2.1)**,** garlic has a wide broadspectrum antibacterial effect, the antibacterial effect of allicin is broad spectrum. In most cases the 50% lethal dose concentrations were somewhat higher than those required for some of the newer antibiotics. Interestingly, various bacterial strains resistant to antibiotics such as methicillin resistant *Staphylococcus aureus* as well as other multidrug-resistant enterotoxicogenic strains of *Escherichia coli, Enterococcus, Shigelia dysenteriae, S. fiexnenl* and *S. sonnel* cells were all found to be sensitive to allicin (Holzgartner *et al*., 1992).

2.4 Toxicity of Garlic

There is little literature about the toxicity of the widely used garlic (Mikail, 2010). Acute toxicity studies were conducted following subcutaneous administration of graded doses of garlic extract in experimental rabbits. LDs_0 of 3,034 mg/Kg of garlic was found, and maximum tolerated dose was 2,200 mg/Kg (Mikail, 2010). In acute toxicity tests carried out in wistar rats and ddY mice, the LDjo value of garlic extracts were estimated over 30 ml/Kg in both animals. No specific signs due to garlic extracts were observed (Nakagawa *et al.,*

1984). The influence of garlic extract in chronic toxicity tests were examined orally in wistar rats for 6 months. There were no toxic symptoms due to garlic extracts even at the dose level of 2,000 mg/Kg for five (5) times a week during six (6) months. High dose of garlic extracts did not inhibit body weight gain (Nakagawa *et al.*, 1984).

2.5 Ciprofloxacin

2.5.1 Use of ciprofloxacin as an antibiotic

Ciprofloxacin (INN) is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class (Kawahara S., 1998; Nelson *et al.*, 2007). It is a second-generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops synthesis of DNA and of protein (Kawahara, S., 1998). Ciprofloxacin is marketed worldwide with over three hundred different brand names. In the United States, Canada, and the UK, it is marketed as*Baycip, Ciloxan, Ciflox, Cipro, Cipro XR, Cipro XL, Ciproxin, Prociflor***,** and most recently, *Proquin.* It is also marketed as *Ciprex* in India and Russia, and "*Cetraxal*" in Spain. In addition, ciprofloxacin is available as a generic drug under a variety of different brand names and is also available for limited use in veterinary medicine (Kawahara S., 1998).

2.5.2 History of ciprofloxacin

Patent history for ciprofloxacin makes reference to a 1982 European Patent (patent number 0049355), as well as German patent dated 21 January 1986. Bayer A.G. introduced ciprofloxacin in 1987. It was later approved by the U.S. FDA on 22 October 1987 for use in the United States to treat specific bacterial infections (Cooper *et al*., 2005). It has 12 FDA-approved human uses and other veterinary uses.In 1991, the intravenous formulation was introduced. Ciprofloxacin interacts with other drugs, herbal and natural supplements, and thyroid medications (Cooper *et al,* 2005).

Table 2.1: Sensitivity of some bacteria to garlic

Bacterial Strain	Allicin concentration (LD_{50} mg/ml)	Comments
Escherichia coli	15	Sensitive to antibiotics
Escherichia coli	15	Multidrug resistant (MDR)
Staphylococcus aureus	12	Sensitive
Staphylococcus aureus	12	Methicillin resistant
Streptococcus pyogenes	3	Sensitive
Streptococcus β-hemolyticus	>100	Clinical MDR strain
Proteus mirabilis	15	Sensitive
Proteus mirabilis	>30	Clinical MDR strain
Pseudomonas aeruginosa	15	Sensitive to cefprozil
Pseudomonas aeruginosa	>100	MDR mucoid strain
Acinetobacterbaumanli	15	Clinical isolate
Klebsiella pneumoniae	8	Clinical isolate

Source: Holzgarther (1992).

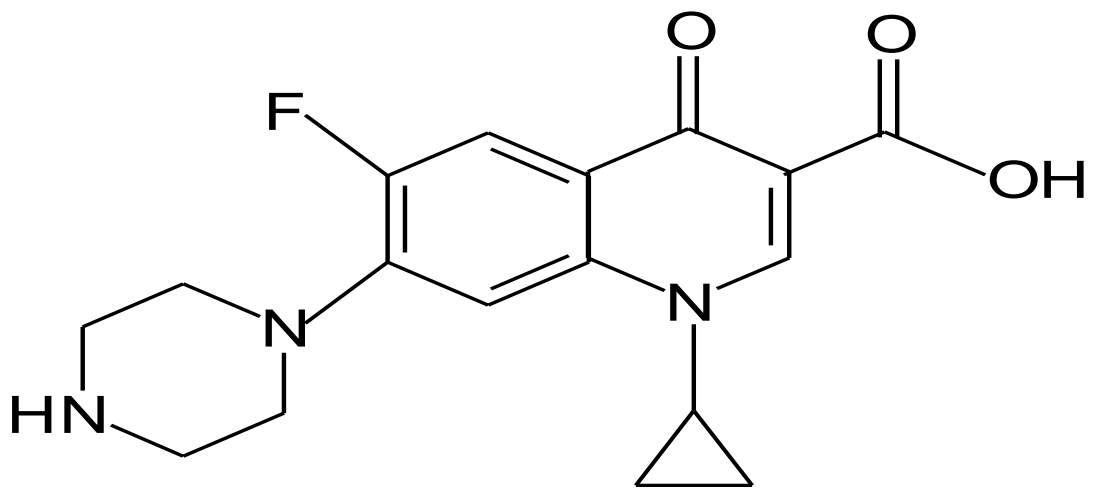
2.5.3 Mechanism of action of ciprofloxacin

Ciprofloxacin is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division (Drlica and Zhao, 1997).This mechanism can also affect mammalian cell replication. In particular, some congeners of this drug family (for example those that contain the C-8 fluorine) robins display high activity not only against bacterial topoisomerases but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and *invivo* tumour models (Sissi and Palumbo, 2003). Although quinolones are highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone-induced DNA damage was first reported in 1986 (Hussy *et al.,* 1986).

Studies have demonstrated a correlation between mammalian cell cytotoxicity of the quinolones and the induction of micronuclei (Forsgren *et al.,* 1987; Gootz *et al.,* 1990, Lawrence, *et al.,* 1993). As such, some fluoroquinolones may cause injury to the chromosome of eukaryotic cells (Elsea *et al.,* 1992; Suto *et al.,* 1992; Enzmann*et al.,* 1999; Kashida *et al.,* 2002).There continues to be debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe adverse reactions experienced by some patients following fluoroquinolone therapy (Al-Soud and Al-Masoudi, 2003; Sissi and Palumbo, 2003; Yaseen *et al.,* 2003).

2.5.4 Chemistry of ciprofloxacin

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7- (1 -piperazinyl)-3-quinolinecarboxylic acid (Kawahara S., 1998). Its empirical formula is $C_{17}H_{18}FN_3O_3$ (figure 2.2). Its molecular weight is 331.4 g/mol (Kawahara, S., 1998). It is a faintly yellowish to light yellow crystalline substance.



Ciprofloxacin

Figure 2.2: Structure of Ciprofloxacin Source: Kawahara S. (1998)

2.6 Antibiotic Resistance

The emergence of antimicrobial resistance has its roots in the use of antimicrobials in animals and the subsequent transfer of resistance genes and bacteria among animals, animal products and the environment (McEwen and Fedorka-Cray, 2002). Extra-chromosomal genes were found responsible for these antimicrobial resistant phenotypes that may impact resistance to an entire antimicrobial class. These resistance genes have been associated with plasmids which are larger, transfereable, extra-chromosomal DNA elements. Other DNA mobile elements such as transposons and integrons are present on plasmids.

These DNA mobile elements transmit genetic determinants for antimicrobial resistance mechanisms and may cause rapid dissemination of resistance genes among different bacteria (McDermott *et al.*, 2002). The emergence of multi-resistant bacteria to antimicrobial drugs has increased the need for new antibiotics or modifications of older antibiotics (Tollefson and Miller, 2000). However, herbs and their essential oil have been known for their varying degrees of antimicrobial activity (Shelef, 1983; Zaika, 1988; Beuchat and Golden, 1989; Juven *et al.*, 1994; Chang, 1995).

2.7 Fowl Cholera

2.7.1 Epidemiology, aetiology and diagnosis of fowl cholera

Fowl cholera is a contagious bacterial disease of domesticated and wild avian species caused by Pasteurella multocida multocida. It typically occurs as a fulminating disease with massive bacteraemia and high morbidity and mortality. Chronic infections also occur with clinical signs and lesions related to localized infections. The pulmonary system and tissues associated with the musculoskeletal system are often the seats of chronic infection. Fowl cholera is also called avian cholera, avian pasteurellosis, and avian haemorrhagic septicemia. It is one of the most common pasteurellosis of poultry (Derieux, 1978).

Fowl cholera is not considered to have zoonotic potential as avian isolates are generally nonpathogenic in mammals exposed by the oral or subcutaneous routes. This disease occurs throughout the country wherever poultry is produced and has become the most hazardous infectious disease of turkeys (Derieux, 1978). The host range is extensive and includes chickens, turkeys, pheasants, pigeons, waterfowls, sparrows and other free-flying birds. Pasteurella multocida multocida can survive at least one month in droppings, three months in decaying carcasses and two to three months in the soil. Pasteurella apparently enters the tissues of the mouth and upper respiratory tract. Fowl cholera is not transmitted through the egg. Adult birds and old chickens are more susceptible to the fowl cholera. In breeder flocks, cocks are far more ‘susceptible than hens. Once the disease is introduced into a flock, it will stay until culling. Chronic carriers can always lead to re-emerging of the disease in susceptible birds (Derieux, 1978).

All avian species are susceptible to Pasteurella multocida multocida, although turkeys may be the most severely affected. Often the first sign of the disease in a flock is dead birds. Other signs include fever, anorexia, depression, mucus discharge from the mouth, diarrhoea, ruffled feathers, and drop in egg production coupled with smaller eggs, increased respiratory rate, and cyanosis of the comb and wattles at the time of death. Lesions that are often observed include congested organs with serosal haemorrhages, enlarged liver and spleen, multiple necrotic foci in the liver and/or spleen, pneumonia, mild ascites and pericardial oedema. Birds that survive the acute septicaemic stage or those infected with organisms of low virulence may develop chronic fowl cholera, characterized by localized infections. These infections often involve joints, foot pads, tendon, sheaths, sternal bursa, conjunctivae, wattles, pharynx, lungs, air sacs, middle ears, bone marrow, and meninges. Lesions resulting from these infections are usually characterized by bacterial colonisation with necrosis, fibrinopurulent exudate, and degrees of fibroplasia (Rimler and Glison, 1997).

The incubation period of fowl cholera is four to nine days. Birds are said to harbour the organism in their nasal cleft. The disease can affect all types of birds and is often fatal (Derieux, 1978; Rimler and Glison., 1997). In the peracute form, fowl cholera is one of the most virulent infectious diseases of poultry. The diagnosis depends on the identification of the causative bacterium, Pasteurella multocida multocida, following isolation from birds with signs and lesions consistent with this disease. Presumptive diagnosis may be based on the observation of typical signs and lesions and/or on the microscopic demonstration of bacteria showing bipolar staining in smears of tissues, such as blood, liver, or spleen (Rimler and Glison,1997).

2.8 Fowl Cholera in Quails

2.8.1 History of fowl cholera in quails

Fowl cholera was first reported by Charbert in 1782 and in 1836 by Mail who used the term fowl cholera. Huppe in 1886 referred to it as haemorrhagic septicemia and Lignieres in 1900 used the term avian pasteurellosis. Benjamin in 1851 gave a good description of the disease and demonstrated that it could be spread by cohabitation (Gray, 1913).Pasteur isolated the organism and grew pure cultures in chicken broth and in further studies used the organism to perform his classic experiments in attenuation of bacteria for use in producing immunity (Pasteur, 1880 and 1881).

In May 1994 the first outbreak of fowl cholera occurred in about 90 Japanese quails being bred for experimental purposes at Miyazaki University, Japan out of which 50 (56%) died of acute septicemia within a few days. At postmortem, there were no gross pathological lesions, however, severe bacteremia was observed in all cases. Bacterial examination revealed the presence of Pastenrella multocida multocida in blood and several organs in pure culture and they were of Carter’s capsular type A, Heddleston’s type 3-4 and Namioka’s type 0-8-9 (Goto et al., 2001).

2.8.2 Aetiology of fowl cholera in quails

The causative agent of fowl cholera is Pasteurella multocida multocida. In the past the bacterium was refered to asMicrococcus gallicidus 1883; Octopsis cholera gallinarum 1885; Bacterium cholera gallinarum 1886; Cocobacillus avicidus 1888; Bacterium multicidum 1899 and Pasteurlla aviseptica 1920 (Breed et al., 1957).

Pasteurella multocida multocida is composed of three subspecies that are often differentiated by fermentation of sorbitol and dulcitol. Taxonomic relationships and nomenclature for this genus have undergone considerable change throughout the years. Mutters et al. (1985), performed DNA hybridization studies that revealed three homologous groups; it was also suggested that there are different ecological niches as well as different pathogenicities for the various *Pasteurella* species (Biberstein et al., 1990).

Considerable variation has been observed among strains with respect to host predilection, pathogenicity, carbohydrate fermentation, colonial morphology and antigenic specificity.Serological differences were also observed in type specific capsular antigen of Pasteurella multocida multocida (Carter, 1955; Carter and Chenga, 1981).This resulted in the development of indirect haemagglutination test which now recognizes five distinct capsular serogroups (serogroups A, B, D, E, F) (Rhoades and Rimler, 1989).

Until recently little was known of the composition of capsular material from Pasteurella multocida multocida serogroups other than that of serogroup A which is known to be sensitive to the action of hyaluronidase (Carter, 1958). Nuclear magnetic resonance studies confirmed that the major polysaccharide component of the capsule was hyaluronic acid (Rosner et al., 1992). The capsular material of serogroups D and F has been identified primarily through the action of mucopolysaccharides (Rimler, 1994). On the basis of decapsulation, profiles of Pasteurella multocida multocida by these enzymes it was proposed that serogroups D and F possessed capsular material that contained heparin and chondroitin

sulphate respectively (Rimler, 1994). The monosaccharide analysis of serogroup B Pasteurella multocida multocida strain showed that the purified capsular polysaccharide was composed of arabinose, mannose, and galactose in the ratio of 0.5:2.0:0.8 (Rimler 1994).

2.8.3 Morphology and staining of pasteurella multocida multocida

Pasteurella multocida multocida is readily isolated, often in pure culture, from visceral organs such as lungs, liver and spleen, bone marrow, gonads or heart blood of birds that succumb to the acute bacteraemic form of the infection, or from the caseous exudate characteristic of chronic fowl cholera lesions. It is a facultative anaerobic bacterium that grows best at 37°C. Primary isolation is usually accomplished using media such as dextrose starch agar, blood agar, and trypticase-soy agar. Isolation may be improved by the addition of 5% heat-inactivated serum. Colonies range from I to 3 mm in diameter after 18-24h of incubation and are discrete, circular, convex, translucent and butyraceous. The cells are coccobacillary or short rod-shaped, 0.2-0.4 x 0.6-2.5 cm in size, stain Gram negative, and generally occur singly or in pairs. Bipolar staining is evident with Wright or Giemsa stains. Identification of Pasteurella multocida multocida is based on the results of biochemical tests, which include carbohydrate fermentation, enzyme production, and selected metabolite production (Rebers et al., 1988).

Serological characterization of Pasteurella multocida multocidastrains includes capsular serogrouping and somatic serotyping. DNA fingerprinting can differentiate among Pasteurella multocida multocida having the same capsular serogroup and somatic serotype. These characterizations require a specialised laboratory with appropriate diagnostic reagents (Rimler et al., 1997).

2.8.4 Physiological properties of *Pasteurella multocida multocida*

Pasteurella multocida multocida does not produce gas but produces oxidase, catalase,peroxidaseand has a characteristic odour. It is sensitive to penicillin, easily destroyed

by disinfectants, sunlight, drying or heat. Carbohydrate fermentation reactions are essential. Those carbohydrates that are fermented include glucose, mannose, galactose, fructose, and sucrose. Those not fermented include: rhamnose, cellobiose, raffinose, inulin, erythritol, adonitol, inositol, and salicin. Mannitol is usually fermented while arabinose, maltose, lactose and dextrin are usually not fermented (Heddleston *et al.*, 1972).

Variable reactions occur with xylose, trehalose, glycerol, and sorbitol. *Pasteurellu multocida multocida* does not cause haemolysis, is not motile and only rarely grows on, MacConkey agar. It produces catalase, oxidase, and ornithinedecarboxylase, but does not produce urease, lysine decarboxylase, beta-galactosidase, or arginine dihydrolase. Phosphatase production is variable. Nitrate is reduced; indole and hydrogen suiphide are produced, and methyl red and Voges-Proskauer tests are negative (Heddleston, 1962).

2.8.5 Pathogenicity of *Pasteurella multocida multocida*

The pathogenicity of *Pasteurella multocida multocida* varies is complex and depends on the strain, host specie and variation within the strain or host and conditions of contact between the two. The ability of *Pasteureila multocida multocida* to invade and reproduce in the host is enhanced by the presence of a capsule that surrounds the organism (Manninger. 1919). Loss of ability of a virulent strain to produce the capsule results in loss of virulence (Heddleston *et al.*, 1970).

Pasteurella multocida multocida usually enters tissues of birds through mucous membranes of the pharynx or upper air passages; it may also enter through the conjunctiva or cutaneous wounds (Hughes and Pritchett, 1930). Hughes and Pritchett (1930), when testing infectivity of an isolate or susceptibility of a host said that cohabitation is the most natural method of exposure. Endotoxins are produced by all *Pasteurella multocida multocida* organisms both virulent and avirulent and may contribute to virulence; however, invasion and multiplication

of a strain are necessary for production of sufficient qualities of endotoxin *in-vivo* to contribute to pathologic processes (Priosky, 1938). An endotoxin was obtained from *Pasteurella multocida multocida* of avian origin by the trichloroacetic acid extraction procedure of biovin (Priosky, 1938).

2.8.6 Pathogenesis of *Pasteurella multocida multocida*

Pasteurella multocida multocida on being ingested enters the circulation through the mucous membrane of the pharynx and upper respiratory passages rarely through the intestinal wall. Entering into the blood the bacilli proliferate, the pathogenic activity of the organism is attributed to a toxin. The action of the toxin on the vascular endothelium is the cause of haemorrhages in the various organs and effusions into the serous cavities, and body temperature is elevated by the action of the toxin on the thermoregulatory centre (Devolt and Davis, 1932).

2.8.7 Susceptibility to *Pasteurella multocida multocida*

Most outbreaks of fowl cholera occur in chickens, turkeys, ducks or geese, however this disease also affects other type of poultry; game birds raised in captivity, companion birds, birds in zoos and wild birds. This is suggesting that all types of birds are affected with most or all of the birds dying within a few days, reduced egg production and persistent localized infection (Devolt and Davis, 1932).

2.8.8 Transmission, Carriers and Vectors of *Pasteurella multocida multocida*

Introduction of *Pasteurella multocida multocida* in a flock is often impossible to determine. Chronically infected birds are considered to be a major source of infection, the only limit to duration of chronic carrier state is the life span of the infected bird (Skidmore, 1932).

Transmission of the organism through the egg seldom occurs. Birds are said to harbour the organism in their nasal clefts, contaminated crates, feed bags or any equipment used previously for poultry may serve as a means of introducing *Pasteurella multocida multocida* into a flock. The organisms are disseminated throughout the carcasses of birds that die of acute fowl cholera (Sirnms, 1951).

The possibility that insects may serve as vectors of *Pasteurella multocida multocida* has been investigated, and the dissemination within a flock is primarily by excretion from the mouth, nose and conjunctiva of sick birds that contaminate their environment particularly feed and water (Parbs-Garnon and Soltys,1971).

2.8.9 Signs of *Pasteurella multocida multocida* infection in quails

2.8.9.1 Acute signs of fowl cholera

Clinical signs are seen only a few hours before death, and unless birds are observed the first indication of the disease is death. The clinical signs that often occur include fever, anorexia, ruffled feathers, mucus discharges from the mouth, diarrhoea and increased respiratory rate. The faeces is initially watery and whitish in colour but later become greenish and contain mucus. Birds that survive the initial acute septicemic stage may later succumb to debilitating effects of emaciation and dehydration and may become chronically infected or may recover (Rimler and Glisson, 1997).

2.8.9.2 Chronic signs of fowl cholera

Chronic fowl cholera may follow an acute stage of the disease or result from infection with organisms of low virulence. The clinical signs are generally related to localized infections; wattles, sinuses, legs or wings joint, foot pads and sternal bursae often become swollen. Exudative conjuctival and pharyngeal lesions may be observed and torticollis sometimes

occurs. Tracheal rales and dyspnoea may result from respiratory tract infection (Rimler and Glisson, 1997).

2.8.10 Gross and Microscopic Lesions due to *Pasteurella multocida multocida*

2.8.10.1 Acute lesion

Generalized hyperaemia which is most evident in veins of the abdominal viscera, petechial and ecchymotic haemorrhages is frequently found and may be widely distributed. Sub-epicardial and sub-serosal haemorrhages are common as are haemorrhages in the lungs, abdominal fat and intestinal mucosa. Increased amounts of pericardial and peritoneal fluid frequently occur. Livers of acutely affected birds may be swollen and usually contain multiple foci of coagulative necrosis and heterophilic infiltration. Heterophilic infiltration also occurs in the lungs and certain other parenchymatous organs at histopathology (Rhodes, 1964). Ovaries of laying hens are commonly affected, mature follicles often appear flaccid, yolk material from ruptured follicles may be found in the peritoneal cavity, and immature follicles and ovarian stroma are often hypaeremic (Olson, 1966).

2.8.10.2 Chronic lesions

These often occur in the respiratory tract and may involve any part including sinuses and pneumatic bones. Infections of the conjunctiva and adjacent tissues occur and facial oedema may be observed. Localized infections may also involve the hock joint, foot pads, peritoneal cavity and oviduct. Torticollis may result from localized infections of the middle ear and cranial bones with heterophilic infiltration and fibrin were consistently observed in the air spaces, middle ear and meninges while giant cells are often associated with necrotic masses of heterophils in air spaces (Olson. 1966).

2.8.11 Immunity to fowl cholera

Pasteur (1881) used an avirulent culture attenuated by prolonged growth on artificial medium and produced immunity that protected against subsequent exposure. In field use, his method did not prove practical because uniform attenuation could not be obtained and heavy losses sometimes occurred in vaccinated flocks (Bairey, 1975).

A substantial but not absolute immunity can be induced in fowl using killed *Pasteurella multocida* vaccines under controlled conditions (Bairey, 1975). The killed, *Pasteurella multocida* were prepared by growing selected immunogenic strains on suitable medium and suspending in formalinized saline solution. The killed organisms are usually incorporated with an adjuvant and injected subcutaneously. Losses in the field occurred due to improperly prepared or administered vaccine (Bairey, 1975).

2.8.12 Diagnosis of fowl cholera

Diagnosis of fowl cholera can be made from clinical observations, necropsy and isolation of *Pasteurella multocida multocida* from the viscera of birds that die and lesions of chronic cases. A tentative diagnosis of acute fowl cholera can be made by demonstrating bipolar organisms in liver imprints using Wrights stain. Bone marrow, heart blood, liver meninges or localized lesions are preferred for culturing using peptone broth or MacConkey agar or blood agar media. Colonies are transferred to dextrose starch agar slants, incubated at 18-24 h. Tubes of phenol red broth containing 1% glucose lactose, sucrose, mannitol and maltose respectively are then inoculated with the growth from the slant. *Pasteurella multocida multocida* ferments, glucose, sucrose and mannitol. Indole is produced by *Pasteurella multocida multocida* and there is no haemolysis on blood agar or growth on MacConkey agar (Marshall, 1963).

2.8.13 Treatment and prevention of fowl cholera

Treatment for fowl cholera is really very much a last resort. For treatment to be relatively effective, it must be carried out aggressively and very early in the course of the disease, before a significant number of birds in the flock become chronically infected. Treatment should be done on the basis of an antibiotic sensitivity screen done of the organism isolated from the outbreak. Treatment may reduce the effect of illness on the flock, by reducing mortality and allowing maintained production levels. However, once chronic infection is present, the benefits of treatment may be difficult to see. Furthermore, treatment will not necessarily remove the offending bacteria from the flock, so it will remain a reservoir both for neighbouring flocks and, potentially, for the next flock (Anon, 2008).

The best prevention against fowl cholera is biosecurity. All the basic steps of a solid biosecurity program are required to prevent entry of the disease. This include wearing clean coveralls, disposable boots, and head cover. A thorough hand washing with a disinfecting soap should also be required, as the hands are an important instrument of disease transmission (Anon, 2001).

Because infected birds are the most important source of the disease, birds from other farms should not be brought into a flock. At the very least, if males are needed to spike a flock, they should come from a farm on which the disease history is well documented and is demonstrated to be free of fowl cholera. Ideally, only one age of birds should be on a farm. If the farm is multiage, then biosecurity among barns becomes more difficult, and very strict biosecurity measures must be practiced (Anon, 2001).

Wild bird and rodent control is another important component of biosecurity, especially if faced with an infected flock. These animals can serve as reservoirs for new flocks entering the farm (USDA, 2001).

A thorough sanitation program for barns and equipment is also important especially to prepare a farm for a new, uninfected flock. Of greatest importance is cleaning and disinfection of the water and feed equipment (USDA, 2001).

A variety of vaccines are available for prevention of fowl cholera. Vaccination should serve only as a tool for flocks at high risk of infection, such as those close to a neighbouring infected flock. Vaccination against fowl cholera has its own inherent risks. For example, live vaccines, while giving broad-spectrum protection can also cause significant side effects. Killed bacterins, on the other hand, give good antibody levels, but the protection offered is specific only to the strains injected, and may be incomplete (Anon. 2001).

2.9 Quails

2.9.1 Biology of quails

Quail is a collective name for several genera of mid-sized birds in the pheasant family *Phasianidae.* Quails are small, plump terrestrial birds. They are seed eaters, but will also take insects and similar small prey. They nest on the ground and are capable of short, rapid bursts of flight. Quails have excellent full-colour vision. They can spot a food grain or small moving beetle under a leaf as well as sharply surveying their surroundings. They have a complex sensory and nervous system that includes a sense of smell, sensitive beaks, keen hearing, and a sophisticated memory capacity. Some species include the Japanese and common quail which are migratory and fly for long distance (Anon, 2001).

2.9.2 Japanese quails (*coturnix coturnix japonica)*

Japanese quails (*Coturnix coturnix japonica*) are native to East Asia and abundant across most of its range. They migrate to Manchuria, Southeastern Siberia, and Northern Japan and winter in Southern Japan, the Korean Peninsula, and Southern China. Their preferred habitats

are grasslands and cultivated fields. The plumage is a speckled yellow-brown, with a creamy white strip above the eyes. Adults are approximately 20 centimetres in length (Anon, 2001).

2.9.3 Nature and behaviour of japanese quails

Japanese quails are genetically designed to forage (to search over wide areas of fertile ground for their food). Japanese quails care for their young, mate for life, nest on the ground, dust bathe, and behave in many ways that characterize Galliforms (ground-dwelling and nesting birds). The Japanese quail have a powerful drive to migrate (Anon, 2001).

Like all quails, Japanese quails have a strong family life. Quails build sturdy nests on the ground, usually hidden under vegetation. While the female does most of the nest- building and egg incubation (21-25 days), the male often assists her and helps take charge of the young. Young quails run and are able to catch food for themselves within a few hours of hatching. In nature, quails remain in family groups. As the young birds mature, the families mingle with flocks of 100 or more birds that stay together and protect one another. They are hardy birds that thrive in small cages and are inexpensive to keep. They are affected by common poultry diseases but are fairly disease resistant. The Japanese quail mature in about 6 weeks and are usually in full egg production by 50 days of age. With proper care, hens will lay 200 eggs in their first year of lay. Life expectancy is only 2 to 2½ years. If the birds have not been subjected to genetic selection for bodyweight, the adult male quail will weigh about 100-140 g, while the females are slightly heavier, weighing from 120-160 g (Anon, 2008).

The females are characterized by light tan feathers with black speckling on the throat and upper breast while the males have rusty brown throat and breast feathers. Males also have a cloacal gland, a bulbous structure on the upper edge of the vent that secretes a white, foamy material. This unique gland can be used to assess the reproductive fitness of the males. Japanese quail eggs are mottled brown in colour and are often covered with a light blue,

chalky material. Each hen appears to lay eggs with a characteristic shell pattern or colour. Some strains lay only white eggs. The average egg weighs about 10 g, about 8% of the bodyweight of the quail hen. Young chicks weigh 6-7 g when hatched and are brownish with yellow stripes. The egg shells are very fragile (Kovach, 1975).

2.9.4 Breeding of quails

Research indicates that grouping a single male with two or three females will generally give high fertility. When quails are kept in colony pens, one male to three females is sufficient and this reduces fighting among males. Pair mating in individual cages also gives good fertility. Fertility decreases markedly in older birds. Avoid mating closely related individuals, because inbreeding increases the incidence of abnormalities and can greatly reduce reproductive performance (Kovach, 1975).

2.9.5 Nutrition of quails

A standard ration for either growing or breeding quail may not be available commercially, therefore, good quality, fresh, commercial turkey or game bird diets are recommended. In Nigeria, quails are fed commercial chicken feed, preferably fed as crumbles to minimize feed wastage (Haruna *et al.,* 1997). For the first 6 weeks quails should be fed a diet containing approximately 25% crude protein and about 12.6 mega joules (MJ) of metabolisable energy (ME) per kilogram, and 1.0% calcium. A good quality commercial starter ration for quail birds, game birds or turkeys contains about 25-28% crude protein. If this is not available, a chicken starter ration (20-21.67% crude protein) can be used, but the bird: will grow more slowly (Kovach, 1975).

The dietary requirements for quails nearing maturity are similar except that calcium and phosphorus levels must be increased. Shell grit or ground limestone can be added to the diets

after 5 weeks of age, or it may be provided separately as free choice. Laying diets should contain about 24% crude protein, 11.7 MJ of metabolisable energy per kilogram, and 2.5 - 3% calcium. The latter may need to be increased to 3.5% in hot weather when the birds eat less food but still require calcium to maintain egg production. Adult Japanese quail eat between 14 g and 18 g of feed per day (Kovach, 1975).

2.8.6 Husbandry of quails

Quails, like other species of poultry kept for commercial purposes, must be given proper care and attention. Environmental conditions should be adjusted according to the climatic conditions and the needs of stock of different ages.

Dry feed should be available at all times, and drinking water must be cool, clean and readily accessible. The birds are easily startled and will struggle vigorously when caught. Excessive or rough handling may kill them therefore sudden noises and disturbances should be avoided. Protection from cats, dogs, rats and predatory birds is essential (Kovach, 1975).

2.8.7 Uses of quails

Quail meat and eggs are renowned for their high quality protein; the high biological value and low caloric value makes it the choice of meat in hypertensive prone individuals. They are excellent materials for biological research because of their size and fast generation. Their feathers are colourful and soft making them good materials for mattresses and pillows. Eggs and tissues are used for the production of biological and the egg shells are a good source of calcium which could be treated and incooperated into feed, fertilizers and paints (Haruna *et al.*, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Quails

A total of one hundred (100), 4-week-old Japanese quails (*Coturnix coturnix japonica*) of both sexes were obtained from the National Veterinary Research Institute (NVRI) Vom, Nigeria. This quails were transported in a transport create viaa vehicle to Ahmadu Bello University research pen.

3.2 Feed

Commercially prepared Excel® feed, (Feedmasters Ltd) was used throughout the experiment. Grower mash and layers mash without antibiotics or coccidiostat were provided *adlibitum* and water from bore-hole was used and provided *adlibitum*. The layers mash contained 16.85% crude protein, 2700kcal/j of metabolizable energy 3.79% crude fat, 4.8% of crude fibre, 0.8% Lysine, 0.37% methionine, 0.68% Methionine + Cystine, 0.59% Phosphorus and 0.15% Sodium

3.3 Garlic

Garlic weight about 5 kg was obtained from a local market in Kaduna State in the month of December, 2012 and used for the treatment of fowl cholera in the experiment.

3.4 Ciprofloxacin

One hundred milligram of ciproflaxcin (ciprovet 10%) manufactured by Mobedco-Vet/The Arab Pesticides and Veterinary Drugs Mfg. Co. Jordan was purchased from a veterinary pharmaceutical shop in Kaduna and used for the treatment of fowl cholera in the experiment.

3.5 Source of *Pasteurella multocida multocida* serotype A:4

The bacterium, *Pasteurella multocida multocida* (serotype A:4) was obtained from Pasteurella Laboratory, National Veterinary Research Institute (NVRI), Vom, through the assistance of Mrs Sarah Ekundayo. The bacterium was stored in a refrigerator (+4^{0}C).

3.6 Housing and Experimental Design

The quails were housed at the Ahmadu Bello University Zaria Veterinary Teaching Hospital Research Pen of the faculty of Veterinary Medicine, Ahmadu Bello University Zaria. The facility was washed clean with detergent and then disinfectedwith diskol® (5% benzallkonium chloride, 7.5% gluteraldehyde, 7.5 formaldehde, stabilizers and antioxidants 9.5) at a recommended rate of 40 ml/10 litres of water before arrival of the quails, and subsequently cleaned every week. The cages were also disinfected with diskol® before the experiment commenced.

The one hundred (100) quails were divided into ten groups labeled A, B, C, D, E, F, G, H, I and J of ten (10) quails each in one hundred (100) wire mesh cages of 100 cm by 50 cm by 40 cm dimensions.Group A was housed in a separate room while groups B to J were housed in another room but in separate cages. In the same room, nine cages were raised to a height of 30 cm from the ground to allow bird droppings to fall unto the floor. To vaoid contamination of personnel a foot bath containg disinfectant (diskol) was provided at the entrance of the poultry pen and the windows were covered with poluythene to prevent contamination.

3.7 Feeding of Experimental Quails

Quails were fed for two weeks on commercial chick mash (Feedmasters Ltd Chick Mash) then subsequently on commercial layers mash from the same producer until the end of the experiment. The experiment lasted 17 days during which the quails were fed and water was provided, *ad libitum*.

3.8 Preparation of Garlic in Water and Feed for Treatment

Preparation in water: Dried, peeled and finely chopped, garlic were dissolved in distilled water at a concentration of 500 g of dried garlic per litre of water for 24 hours at room temperature as described by Cruz *et al.* (2004). The mixture was filtered using filtering paper into a conical flask and the extract (500 g/L) was used immediately in the experiment in different dilutions of 1%, 1.67% and 3.3%. The 500 grams of garlic in 1 litre of water will give 33.3% of the stock solution. From 100ml of water, 3 ml was withdrawn and replaced with 3 mil of the stock solution to get 100 ml of 1% garlic in water. Similarly, 5 ml and 10 ml were withdrawn from 100 ml of water and replaced with equivalent volumes respectively from the stock solution to obtain 100 ml each of 1.67% and 3% garlic in water respectively Cruz *et al.* (2004).

Preparation in feed:

Garlic was air dried peeled and pulverized using a blending machine. Different percentages of garlic at 3%, 5% and 10% were used in feed as medication. From 100 grams of feed, 3 grams of feed was removed and replaced with 3 grams of pulverized garlic to obtain 3% garlic in feed. Similarly, 5 grams and 10 grams were removed from two separate quantities of 100 grams feed and replaced with 5 grams and 10 grams each of pulverized garlic to obtain 5% and 10% respectively of garlic in feed.

3.9 Preparation of Ciprofloxacin

The dose of ciprofloxacin used in this experiment was 100 mg/2L of water as prescriped by the manufacturer and was administered orally 3 days adlibitum and for 14 more days of the experiment.

3.10 Culture and Determination of *Pasteurella Multocida Multocida* Serotype A:4 Inoculum

The bacterium was subcultured on blood agar at the bacteriology laboratory of the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria.

A 4 folds serial dilution in 10 test tbes was carried out, in which 0.1 ml each from test tube number 8, 9, 10 were inoculated on blood agar to observe for growth over 24 hours.

The original suspension was preserved in the refrigerator in order to inhibit proliferation of the bacteria. The average colony forming unit per 0.1 ml was counted from the test tubes 8,9 and 10, and the total colonies was determined per ml.

To obtain the number of organisms required for inoculation into the quails, the number of organisms in the colonies was multiplied by the dilution factor of 1:390625 (European Pharmacopeia, 2001).

The infective dose (of 10^6 cfu/birds) swas determined as decribed by Akpavi *et al.*, (2011). Infective dose of 10^6 and 10^4 cfu/birds were used for inoculation of the experimental quails with the *Pasteurella Multocida Multocida*

3.11 Inoculation of Quails with *Pasteurella Multocida Multocida* Serotype A4

Each of the quails used in the experiment was inoculated with 0.2 ml of *Pasteurella multocida multocida* inoculum. The methods of Chonchury et al (1987) and Assor (1963) were used for the inoculation of the quails. The experimental quails were inoculated with 0.1 ml intramuscularly and another 0.1 ml was administered by intranasal route to each quail using insulin syringe. All the quails in all the subgrous were challenged with 1×10^4 cfu/bird except subgroup J which received 1×10^6 cfu/bird.

3.12 Determination of Cloacal Temperature

Daily cloacal temperature in degree centrigrade ^{0}C of each bird was taken in all the groups (incoculated and uninoculated) using two different digital thermometer, (one each for inoculated and uninoculated) manufactured by Mabis Health Inc. Germany, (Model No. MT418-BDP). This was done between 7 am and 8 am daily.

3.13 Determination of Body Weight

All the ten (10) quails in each experimental group were placed in a basket and their combined weight taken with the aid of a weighing balance (Model DRONCO®, CAP 5 kg, Grad 2.5q England). The average weight of each quail in the group was estimated by dividing the value obtained by the number of quails in the group. This method was used to estimate the weight of the quails in all the groups.

3.14 Collection of Blood Sample

Blood sample used for the determination of packed cell volume (PCV) and total plasma protein (TP) were collected from 10 birds in each group from the wing vein using 1 ml sterile insulin syringe and needle into 5 ml sample bottles containing ethylene diamino tetra-acetic acid (EDTA). About 0.3 – 0.5 ml blood were collected once before and after inoculation, during and after medication to determine PCV.

3.15 Determination of Packed Cell Volume

Packed cell volume was determined in the Clinical Pathology Laboratory of the Department of Pathology,Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Packed cell volume (PCV) was determined using the microhaematocrit method (Sirois, 1995). Microhaemocrit capillary tubes were filled with blood by capillary action to about 75% of their lengths and the outside of the tubes were carefully dried with cotton wool after filling

each of the tubes with blood. The opposite ends were sealed with a flame from a Bunsen burner and the sealed tubes were packed in a haematocrit centrifuge with their sealed ends placed near the outside rim of the centrifuge (to touch the rim).

The microhaematocrit centrifuge was covered and then turned on for 5 minutes. The spunned tubes were then placed on a microhaemocrit tube reader one after the other to determine the PCV in percentage.

3.16 Determination of Total Plasma Protein

Total plasma protein was determined in the Clinical Pathology Laboratory as follows: Total plasma protein was determined using the hand refractometric method (Sirois, 1995). After reading the PCV, the haemotocrit capillary tube was taken by snapping the tube of the point immediately above the buffy coat. The end containing the buffy coat and packed red cells was discarded retaining the end containing the plasma. The plastic flap of the hand refractometer was reflected to expose "glass" sample surface which was thoroughly wipped with cotton wool, the broken end of the capillary tubes was then used to touch the sample surface of the rfractometer in order to place plasma sample unto the surface thereafter the capillary tube was then replaced, pressure was then applied on the flap against the plasmasample with the index finger and holding the refractometer against light, the concentration of plasma proteins was then read in grams/deciliter (g/dl).

3.17 Clinical Examination of Quails Inoculated with *Pasteurella multocida multocida*

Inoculated quails were monitored daily post infection for clinical signs, morbidity rate, mortality rate and any abnormal findings observed were recorded for the period of 17 days post inoculation.

3.18 Postmortem Examination of Quails Inoculated with *Pasteurella multocida multocida*

Dead quail birds from any of the groups were examined and postmortem examination conducted on them to determine the cause of death.

3.19 Histopathology

Samples of organs were collected for histopathology and these included the liver, spleen and heart. All organs were preserved in 10% formalin. H and E stain was done and the slides were examined under a light microscope. This was done in the histopathology laboratory in the Department of Veterinary Pathology, Ahmadu Bello University, Zaria.

3.20 Experimental Design

On arrival, to the experimental site (pen), the quails were allowed to acclimatize for two weeks, during which clinical signs of any infection were monitored. By day 14, they were randomly allotted into two groups (inoculated and non-inoculated groups). These groups were further subdivided into ten subgroups labelled A-J, (as shown in table 3.1). The ciprofloxacin was administered for 3 days in drinking water while garlic was administered as aqueous extract in water as 1%, 1.67% and 3.3% and as pulverized garlic as in-feed medication at concentrations of 3%, 5% and 10% for 3 days before inoculation with *Pasteurella multocida multocida* serotype A4.

3.21 Data Analysis

Data (weight, packed cell volume, total plasma protein, morbidity rate, and mortality rate) obtained were expressed as mean $\pm$ S.E.M. They were further analyzed using one way analysis of variance (ANOVA), followed by Tukey's post hoc test using graph pad prism version 4.0 for windows. Values of $p \leq 0.05$ were considered significant.

Table 3.1: Experimental design for the determination of the effect of *Allium sativum* in quails inoculated with *Pasteurella multocida multocida*

Group	Control			Treatment						
	A	B	C	D	E	F	G	H	I	J
	Negative	Positive	Cipro	Garlic						
				In-Feed			In-Water			
	0%	0%	100 mg/2L	3%	5%	10%	1%	1.67%	3.3%	3.3%
No of Quails	10	10	10	10	10	10	10	10	10	10
cfu/bird	0	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^4	10^6
Body weight	D1/17	D1/17	D1/17	D1/17	D1/17	D1/17	D1/17	D1/17	D1/17	D1/17
Cloacal temp.	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily
Morbidity	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17
Mortality	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17	D0-17
PCV	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17	D1/5/17
TP	D1/5	D1/5	D1/5	D1/5	D1/5	D1/5	D1/5	D1/5	D1/5	D1/5
Clinical sign	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily	Daily
Post mortem	Pm	Pm	Pm	Pm	Pm	Pm	Pm	Pm	Pm	Pm
Histopathology	Histo	Histo	Histo	Histo	Histo	Histo	Histo	Histo	Histo	Histo

KEY: Superscript with the same alphabet are significantly different ($p<0.05$), Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

CHAPTER FOUR

RESULTS

4.1 Changes in Body Weight of Quails Inoculated with *Pasteurella multocida multocida* under Medication of Ciprofloxacin and Garlic

The initial mean body weight recorded in each groups were group A (148.0±2.46g), B (140.0±2.75g), C (147.0±2.61g), D (145.0±2.37g), E (150.0±2.77g) and group F (139.0±3.00g). while groups G,H,I and J started with an initial weight of 130±2.58g, 136.0±2.86g, 146±1.37g and 140±2.04g respectively. However, at the end of the experiment groups A, B,C, D,E and F recorded an average increase of 155.0±2.45g, 150.0±0.00g, 193.8±2.27g, 150.0±3.4g, 162.5±1.66g and 150.0±5.0g respectively. Finally, groups G,H, I and J were having 150.0±2.04g, 166.7±2.40g, 158.3±3.4g and 164.3±2.97 respectively as shown in table 4.1. There was increased in body weights in both the inoculated and non-inoculated quails. There were significant difference in the body weights ($p \leq 0.05$) between the non inoculated and inoculated quails under medication. Also, there were differences ($p \leq 0.05$) in the body weight values among the inoculated quails under medication with either ciprofloxacin or garlic.

Table 4.1: Mean changes in body weight (g) of quails inoculated with *Pasteurella multocidamultocida* serotype A4 under medications of ciprofloxacin and garlic

Group	A	B	C	D	E	F	G	H	I	J
Initial weight	148.0±2.46^{ab}	140.0±2.75	147.0±2.61^{cd}	145.0±2.37	150.0±2.77	139.0±3.00	130±2.58^{bde}	136.0±286^{ac}	146±1.37	140±2.04^{e}
Final weight	155.0±2.45	150.0±00	193.8±2.27^{abcd}	150.0±3.42^{c}	162.5±1.66^{d}	150.0±5.0^{b}	150.0±2.04^{bef}	166.7±2.40^{ae}	158.3±3.4^{c}	164.3±2.97^{fd}
Weight change	7	10	46	5	12	11	20	30	12	24

KEY: Superscript with the same alphabet are significantly different ($p<0.05$), Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

4.2 Changes in Packed Cell Volume of Quails Inoculated with *Pasteurella multocida multocida* Serotype A4 Under Medication of Ciprofloxacin and Garlic

On day one prior to medication and inoculation the mean packed cell volume for group A was 46.75±1.1% and group B was 33.25±3.66%. Groups C to,D,E and F were 34.0±2.49%, 36.67±2.12%, 31.50±3.90%, and 29.25±4.09% respectively; while groups G, H,I and J were 29.63±5.77%, 39.0±5.52%, 32.44±3.88% and 31.0±3.87% respectively. However, at day five of the experiment group B had a mean PCVof 37.0±3.51% while groups C, D, E and F were 30.0±2.66%, 31.67±0.88%, 30.25±2.07% and 26.0±3.51% respectively. Also, groups G, H, I and J were 30.60±2.5%, 25.0±3.62%, 35.74±3.86% and 34.25±6.4% respectively (Table 4.2).

Finally, at day 17, the mean PCV of non-inoculated group A was 47.63±7.64% while that of inoculated (group C) 34.64±3.43%, (group D) 45.57±3.74%, (group H) 45.57±3.74% and (group J) 44.67±4.28%. The PCV in the inoculated group B was found to be as low as 19%.

On day 5 post inoculation there were significant decreases ($P<0.05$) in PCV in all the inoculated groups (C, D, E, F, H and J) as compared to the non-inoculated group A except groups B, G and J. On day 14 post inoculation, there were significant difference in PCV ($p<0.05$) between non-inoculated group A and inoculated control (group B) without garlic or ciprofloxacin medication. There were no significant different between non-inoculated group and those inoculated, and with gartlic or ciprofloxacin medication in PCV values. Finally, there was significant increase in PCV in day 5 and day 17 in group D only under 3% garlic in-feed medication.

Table 4.2: Mean changes in packed cell volume of quails inoculated with *Pasteurella multocida multocida*serotype A4 under medication of ciprofloxacin and garlic.

Group	A	B	C	D	E	F	G	H	I	J
Day 1	46.75±.1[ab]	33.25±66	34.0±2.49	38.67±2.12	31.50±3.90[b]	29.25±4.09[a]	29.63±5.77	39.0±5.52	32.44±3.88	31.0±3.87
Day 5	47.67±0.88[abcd]	37.0±3.51	30.0±2.66[a]	31.67±0.88[c*]	30.25±2.07[d]	26.0±3.51[b]	30.60±2.5	25.0±3.62[b]	35.74±3.86	34.25±6.4
Day17	47.63±7.64	19.0±00	34.63±3.43	45.57±3.74[*]	32.0±6.15	25.50±8.50	31.60±4.78	45.57±3.74	37.0±3.44	44.67±4.28

KEY: Superscript with the same alphabet are significantly different ($p<0.05$), Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

4.3 Changes in Total Plasma Protein of Quails Inoculated with *P*asteurella *multocida multocida* under Medication of Ciprofloxacin and Garlic

The initial values of mean total plasma protein on day one of occelation for non-inoculated group A was 7.45 ± 0.49 g/dl. For inoculated groups B, C, D, E, F were 7.40 ± 0.54g/dl, 7.72 ± 0.68 g/dl, 5.50 ± 0.59 g/dl, 7.83 ± 0.75 g/dl, 8.40 ± 0.68 g/dl respectively. For inoculated groups G, H, I, and J were 6.28 ± 0.57 g/dl, 6.5 ± 72 g/dl 6.21 ± 0.46 and 7.49 ± 0.51 g/dl respectively. On day five (5) postinoculation, group A had 7.93 ± 0.67 g/dl while b, C, D, E, F were 6.87 ± 0.58 g/dl, 8.24 ± 44 g/dl, 8.81 ± 0.61 g/dl7.10 ± 0.57 g/dl, 8.13 ± 0.24 g/dl, respectively. Finally, for groups G, H, I, and J were 7.6 ± 1.12 g/dl, 8.76 ± 0.52 g/dl, 7.25 ± 0.38 g/dl and 7.03 ± 0.46 g/dl respectively, as shown in Table 4.3.

Table 4.3: Mean changes in total plasma protein of quails inoculated with *Pasteurella multocida multocida* serotype A4 under medication of ciprofloxacin and garlic.

Group	A	B	C	D	E	F	G	H	I	J
Day 1	7.455±0.49	7.40±0.54	7.72±0.68	5.50±0.59	7.83±0.75	8.40±0.68	6.28±0.57	6.5±0.72	6.2±0.46	7.49±0.51
Day 5	7.93±0.67	6.87±0.58	8.24±0.44	8.81±0.61	7.10±0.57	8.13±0.24	7.6±1.12	8.76±0.52	7.25±0.38	7.03±0.46
Deference	+0.48	-0.53	+0.52	+3.3	-0.73	-0.27	+1.32	+2.26	+1.05	-0.46

KEY: Superscript with the same alphabet are significantly different ($p<0.05$), Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

4.4 Changes in cloacal Temperature of Quails Inoculated with *P*asteurella *multocida multocida* under Medication of Ciprofloxacin and Garlic

Mean cloacal temperature on day one prior to inoculation for non-inoculated group A was 41.71±0.12^{0}C, while for inoculated groups B,C,D, E and F, were 41.69±0.12^{0}C, 41,76±0.11^{0}C, 41.96±0.14^{0}C, 40.80±1.06^{0}C, 41.8±0.09^{0}C respectively. For groups G, H, I and J, had 42.06±0.17^{0}C, 41.99±0.11^{0}C, 41.97±0.15^{0}C and 42.10±0.14^{0}C respectively, (Table 4.3). On day five, the mean cloacal temperature for group A was 41.38±0.30^{0}C while for groups B,C,D,E and F, it was 42.50±0.26^{0}C, 42.0±0.15^{0}C, 41.56±0.05^{0}C, 42.64±0.19^{0}C and 42.48±0.18^{0}C respectively. For groups G,H,I and J, it was 42.64±0.24^{0}C, 42.16±0.28^{0}C, 41.98±0.32^{0}C and 42.56±0.27^{0}C respectively (Table 4.3). There were significant differences ($p \leq 0.05$) in cloacal temperature on day five between non inoculated group A and the inoculated groups (E-J). Also, P-values were significant ($p \leq 0.05$) between the non inoculated group A and the inoculated groups under medication. However, on day 17, the P-values of the non-inoculated group A and inoculated group B without medication were different from the other groups.

Table 4.4: Mean changes in daily cloacal temperature (^{o}C) of quails inoculated with *Pasteurella multocida multocida* serotype A4.

Group	A	B	C	D	E	F	G	H	I	J
Day 1	41.71±0.12	41.69±0.12	41.76±0.11	41.96±0.14	40.80±1.06	41.80±0.09	42.06±0.17	41.99±0.11	41.97±0.15	42.10±0.14
Day 2	41.70±0.16	41.77±0.18	41.67±0.11	41.77±0.11	41.84±0.16	41.87±0.14	41.41±0.18	41.93±0.07	41.90±0.17	41.94±0.15
Day 3	41.75±0.18	41.64±0.20	41.94±0.18	41.98±0.15	41.94±0.14	41.88±0.08	41.71±0.14	41.91±0.15	41.96±0.14	42.06±0.21
Day 4	41.71±0.16	41.62±0.25	41.78±0.15	41.71±0.19	41.93±0.20	41.88±0.20	41.67±0.12	41.62±0.22	41.71±0.19	42.06±0.15
Day 5	41.38±0.30^{abc}	41.40±0.26^{ad}	42.10±0.15	41.56±0.05^{def}	42.64±0.19^{be}	42.48±0.18^{cf}	42.64±0.24^{a}	42.16±0.28	41.98±0.32	42.56±0.27
Day 6	41.50±0.17	42.20±0.26	41.78±0.24	42.18±0.28	42.34±0.28	42.48±0.16	41.58±0.22	42.20±0.24	41.96±0.19	41.78±0.07
Day 7	41.68±0.18^{a}	42.90±0.10^{a}	42.38±0.24	42.42±0.20	42.70±0.30	42.50±0.31	42.10±0.30	41.80±0.44	41.92±0.28	41.86±0.20

KEY: Superscript with the same alphabet are significantly different ($p<0.05$), Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

4.5 Clinical Signs, Morbidity and Mortality of Quails Inoculated with *Pasteurella multocida multocida* Serotype A:4 Under Medication of Ciprofloxacin and Garlic

No obvious clinical signs were observed within the first few hours, but a day post inoculation there were clinical signs of illness manifested by sudden death, 1 in group D. and 2 in group B. Generally, quails in groups B,F,G and I shoed somnolence and loss of appetites.

Within two days post inoculation some of the quails in all the groups that were inoculated with *Pasteurella multocida multocida* showed clinical signs of illness (somnolence, loss of appetite, decrease water consumption, ruffled feathers, whitish diarrhoea and mortality). Morbidity was higher in group E (50%) followed by groups B,D,F,G and J (40%). The least, H (20%) and I (30%) and non in A and C (Table 4.5).

All the signs observed on day 2 post inoculation were more severe on day 3 and 4 with the highest mortality on day 4. Mortality was highest in group B inoculated without medication (90%) groups E (60%), G (60%) and H (70^). Mortality was least in groups C (20%) J (30%) and I (40%) (Table 4.6).

In this research, mortality started a day post inoculation and continue in group B upto the end of the experiement. However, mortality ended after two days post inoculation in group C and in D, I and J, it ended after three days. However, in groups G and H mortality ended after 5 to 6 days respectively.

Table 4.5: Morbidity rates of quails inoculated with *Pasteurella multocida multocida serotype* A:4 under medication of ciprofloxacin and garlic.

Group											
Days Post Inoculation	A	B	C	D	E	F	G	H	I	J	Total
Number of quails sick											
1	0	2	0	0	0	1	1	0	1	0	5
2	0	3	0	4	5	4	4	2	3	4	29
3	0	0	0	1	0	1	0	0	0	0	2
4	0	4	0	0	0	0	0	0	0	0	4
5	0	4	0	0	0	0	0	1	0	0	5
6	0	3	0	0	0	0	0	0	0	0	3
7	0	2	0	0	0	0	0	0	0	0	2
8	0	2	0	0	0	0	0	0	0	0	2
9	0	2	0	0	0	0	0	0	0	0	2
10	0	2	0	0	0	0	0	0	0	0	2
11	0	2	0	0	0	0	0	0	0	0	2
12	0	2	0	0	0	0	0	0	0	0	2
13	0	0	0	0	0	0	0	0	0	0	0
	0	40%	0	40%	50%	40%	40%	20%	30%	40%	

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

Table 4.6: Mortality rate of quails inoculated with *Pasteurella multocida multocida* Serotype A:4 under ciprofloxacin and garlic medication

Day post inoculation	A	B	C	D	E	F	G	H	I	J	Total (%)
1	0	2	0	1	0	0	0	0	0	0	3
2	0	2	2	1	2	2	0	2	1	2	14
3	0	2	0	2	1	2	5	4	3	1	20
4	0	0	0	0	2	1	1	0	0	0	4
5	0	1	0	0	0	1	0	1	0	0	3
6	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	0	0	0	0	0	0	1
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	1	0	0	0	0	0	0	0	0	1
Rate	0%	90%	20%	40%	60%	50%	60%	70%	40%	30%	

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

4.6 Postmortem findings in quails inoculated with *Pasteurella multocida multocida* Serotype A:4 Under Medication with Ciprofloxacin and Garlic

Postmortem examination conducted on dead quails a day post inoculation showed no visible gross lesions in any organ. At 2 days post inoculation dead quails from all the inoculated groups had organs with visible gross lesions. The following lesions were observed in all the inoculated groups, congestion of the breast muscles (Plates I and II), congestion and hyperemic duodenum (Plate III), congested and haemorrhagic spleen and misshapen ovaries (Plate IV).

4.7 Histopathological Findings in organs of Quails Inoculated with *Pasteurella multocida multocida* Serotype A:4 Under Medication of Ciprofloxacin and Garlic

Plates V showed the normal photomicrograph of the heart of non-inoculated quail (group A) on day 2 post inoculation. No cellular infiltration into the heart muscle. Plate VI is a photomicrograph of the heart of inoculated quails with mononuclear cellular infiltration. Group C on day 2. Plate VII is a photomicrograph of the heart of quails from group C on day 17 without pathological findings.

Plate VIII is a normal photomicrograph of the liver of quail from group A on day 2 post inoculation no sinusoids. Plate IX, showed mild fatty degeneration, congested sinusoids and distorted hepatic architecture of quails inoculated with *Pasteurella multocida multocida* under garlic in-feed medication (group D) on day 2 post inoculation. Plate X is a photomicrograph of inoculated quails (group D) on day 17 post inoculation without pathological findings.

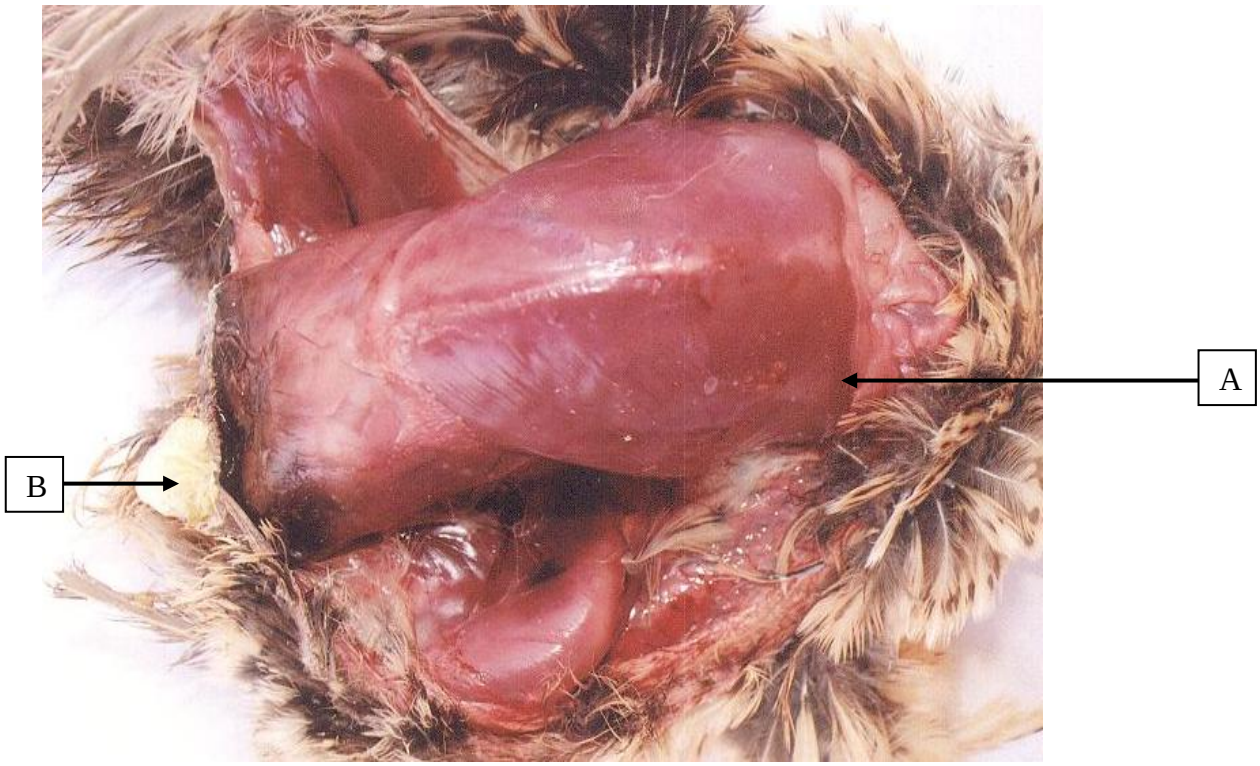


Plate I: Congestion on breast muscles (arrow A) and whitish diarrhea (arrow B) in quail that died on day 2 post inoculation with *Pasteurella multocida multocida* quail (Group B).



Plate II: **Congestion of the breast muscles of quail that died on day 2 post inoculation with *Pasteurella multicida multicida* (group H).**

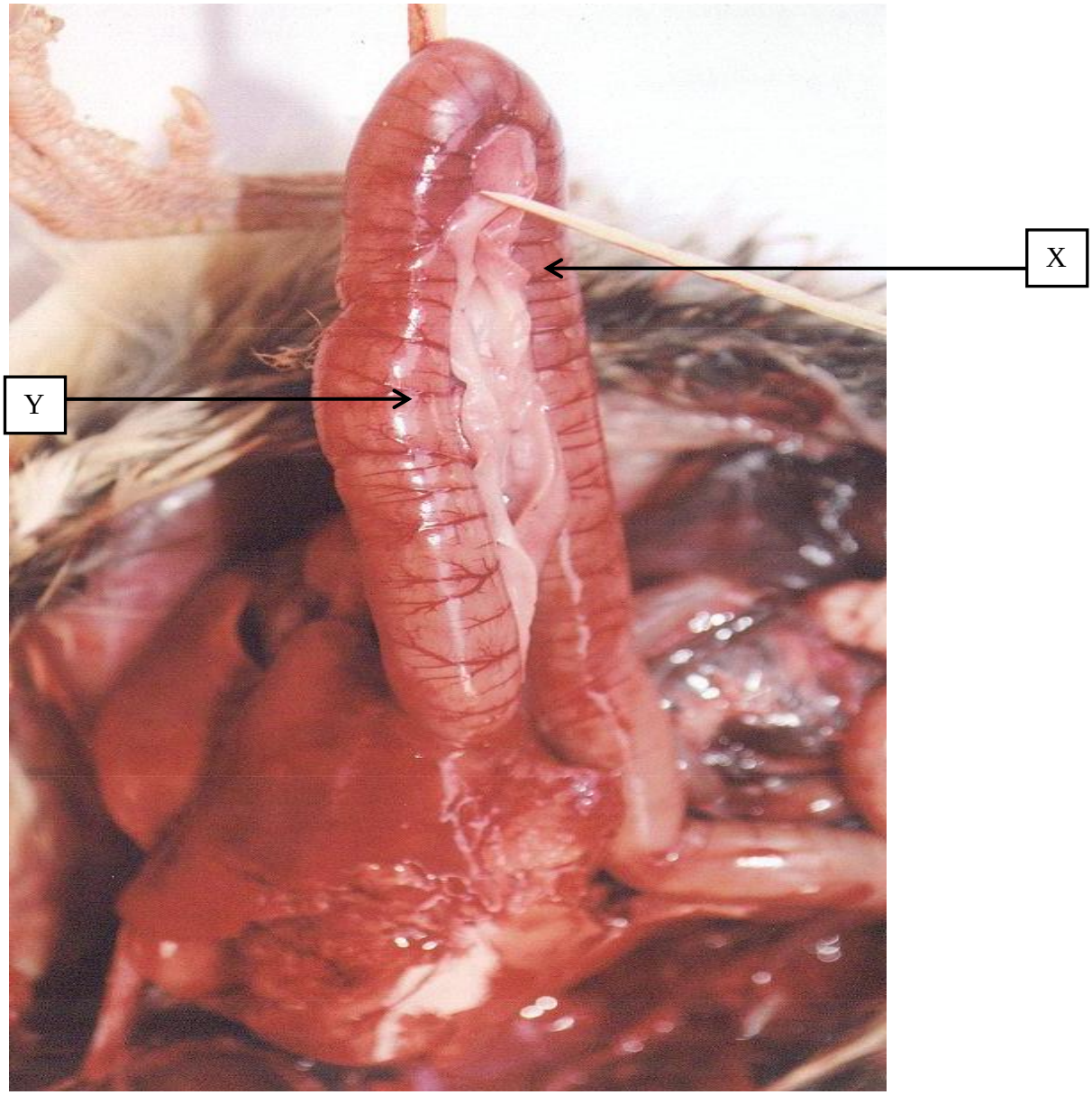


Plate III: **Congested and haemorrhagic duodenum of quails on day 2 post inoculation *Pasteurella multocida multicida* quail, (group D), congestion (arrow X) and haemorrhagic (arrow Y)**

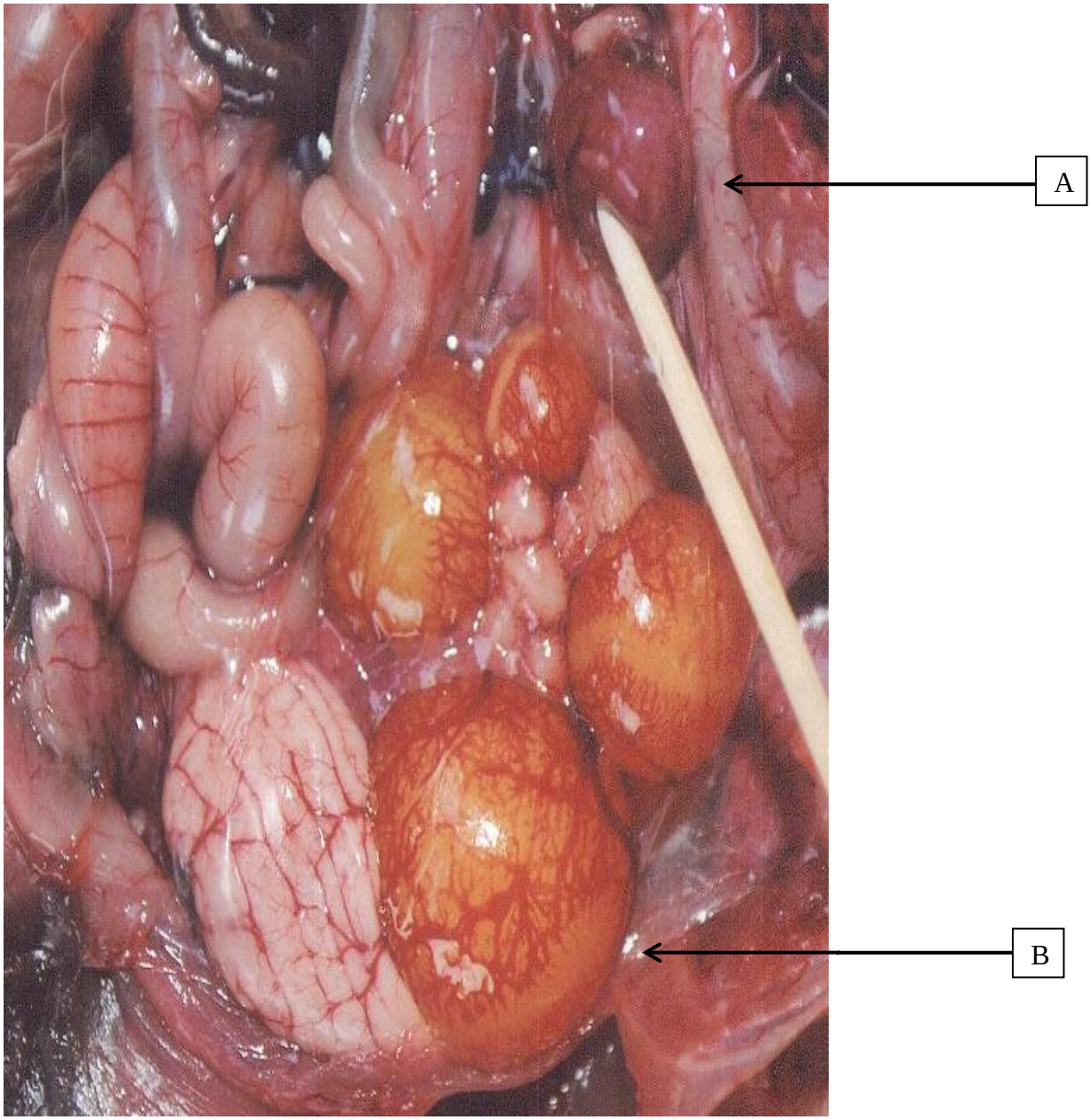


Plate IV: **Enlarged, congested and haemorrhagic spleen (arrow A) and mishapen ovarian follicle (arrow B) of quail that died on day 2 post inoculation with *Pasteurella multocida multocida* (group D).**

Plate XI is a normal photomicrograph of the spleen of non inoculated quail (group A) on day 2 post inoculation with normal lymphocyte. In plate XII, there is depletion of lymphocyte in the germinal centre of the spleen of quail (group J) on day 2 post inoculation. In Plate XIII showed a photomicrograph of spleen of quail inoculated with *Pasteurella multocida multocida* under garlic in-water medication (group J) on day 17 post inoculation without any histopathological finding.

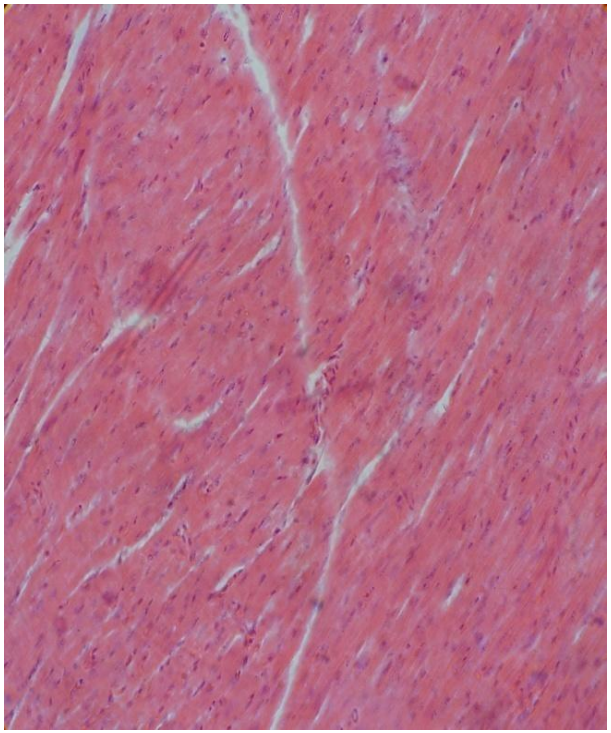


Plate V: Photomicrograph of a section of the heart of non-inoculated quails group A day 2 post inoculation (H and E x 400)

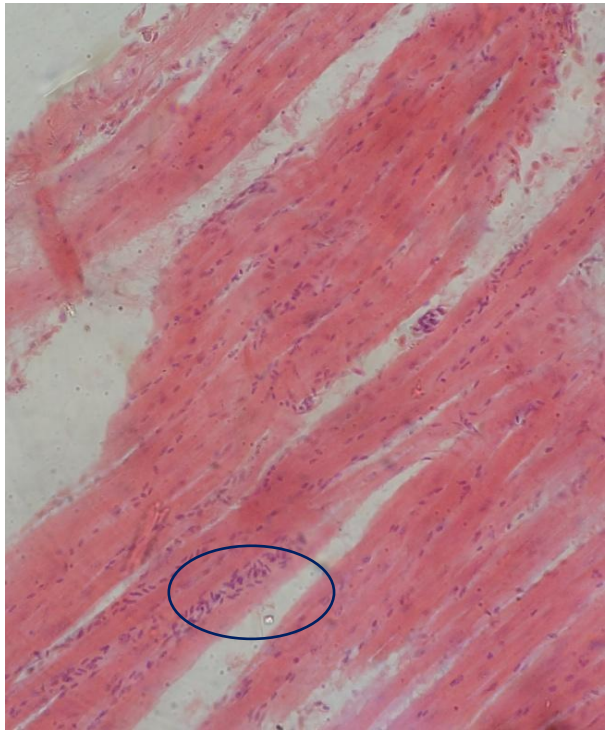


Plate VI: Photomicrograph of a section of the heart muscle of quail inoculated with *Pasteurella multocida multocida* circle showed mononuclear cellular infiltration (groyup C) on day 2 post inoculation (H andE x 400).

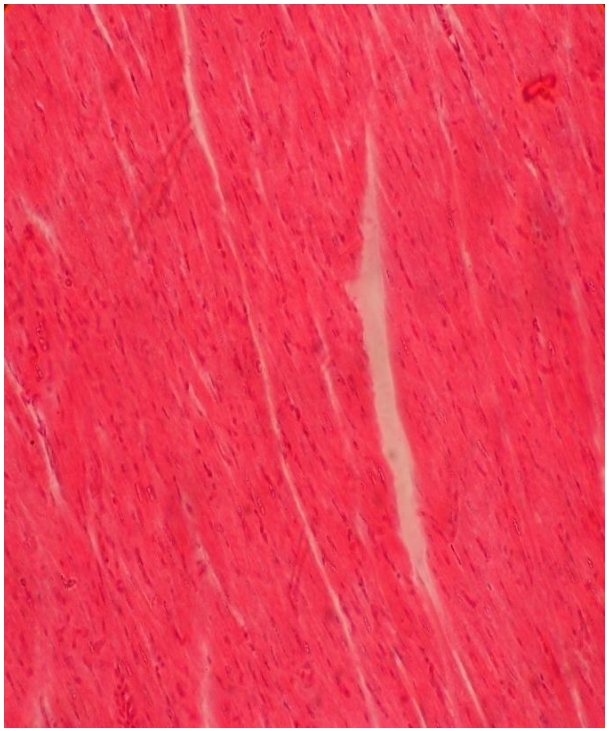


Plate VII: Photomicrograph of a section of the Heart Muscle of quail inoculated with *Pasteurella multocida multocida* with no significant leision (H and E x 400) group C day 17 post inoculation.

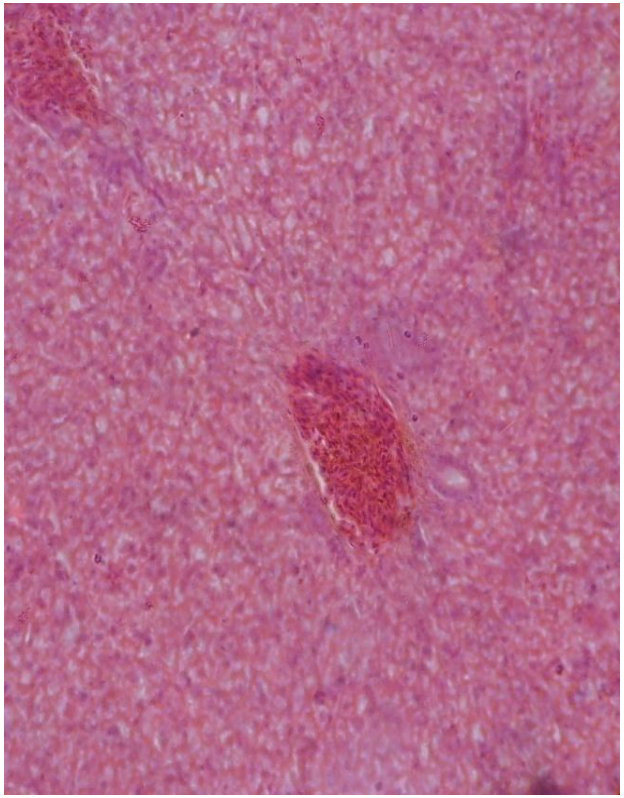
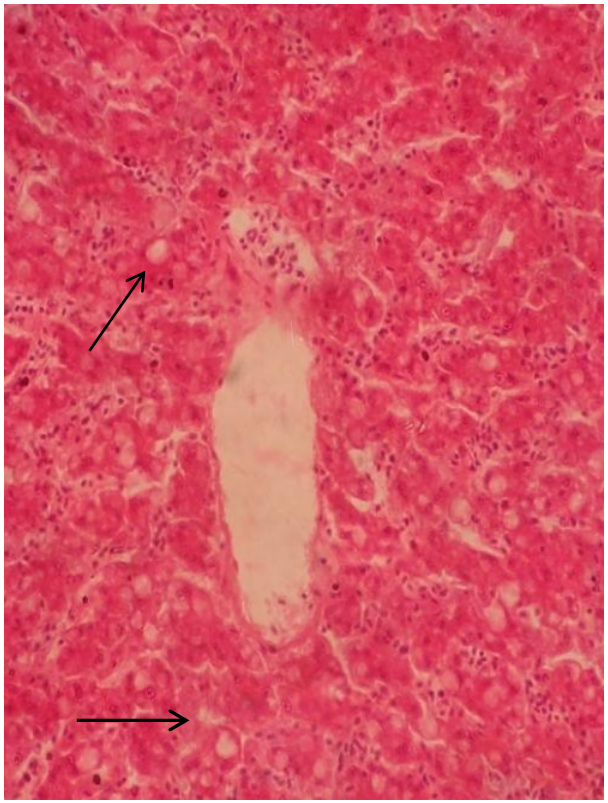


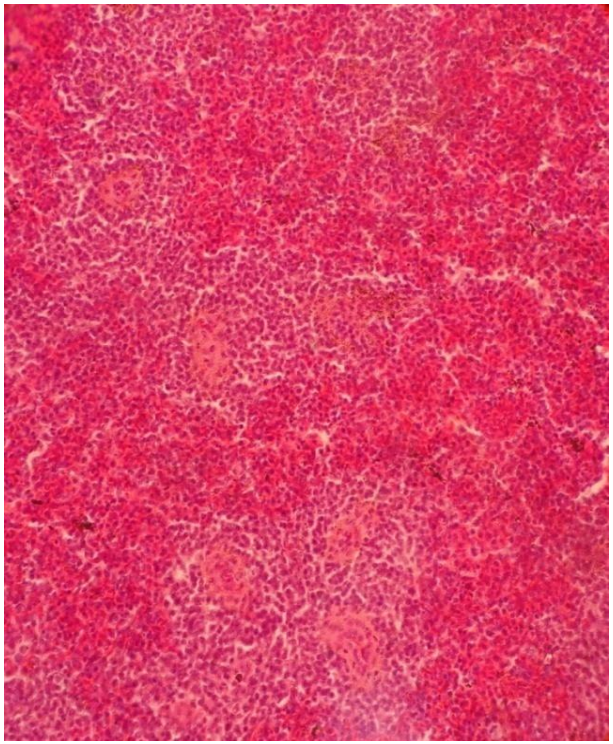
Plate VIII: Photomicrograph of a section of the liver of non-inoculated quail (H and E x 400) group A, on day 2 post inoculation



Plates IX: Photomicrograph of a section of the liver of quail inoculated with *Pasteurella multocida multocida* (arrow showed mild mild fatty degeneration, congested sinusoids and distorted hepatic architecture (ring)(H and E x 400) group D, day 2 post inoculation.



Plate X: Photomicrograph of asection of the Liver of quail inoculatedc *Pasteurella multocida multocida* with no significant leision (H and E x 400) group D on day 17 post inoculation.



PlateXI: Photomicrograph of a section of the spleen of *non*-inoculated quail (H and E x 400) (group A) on day 2 post inoculation.

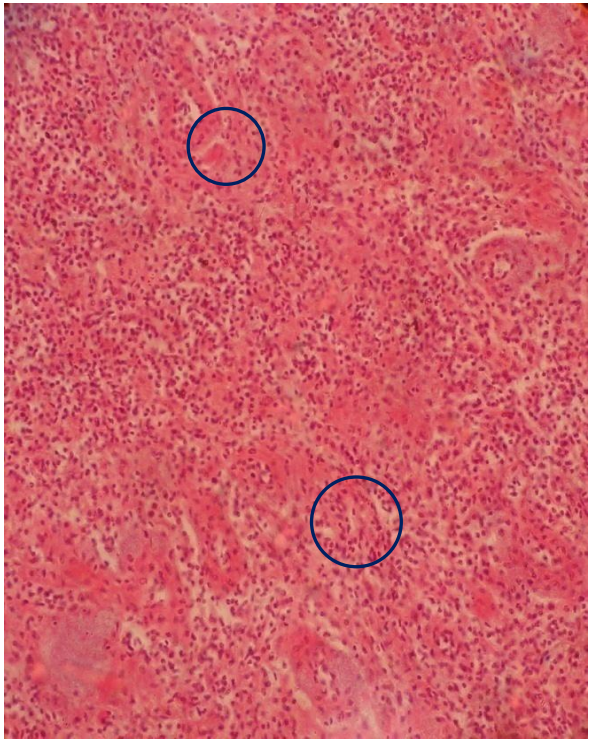


Plate XII: Photomicrograph of a section of the spleen of quail inoculated with *Pasteurella multocida multocida* (ring) showed depletion of lymphocytes at the germinal centres (H and E x 400) group J on day 2 post inoculation.

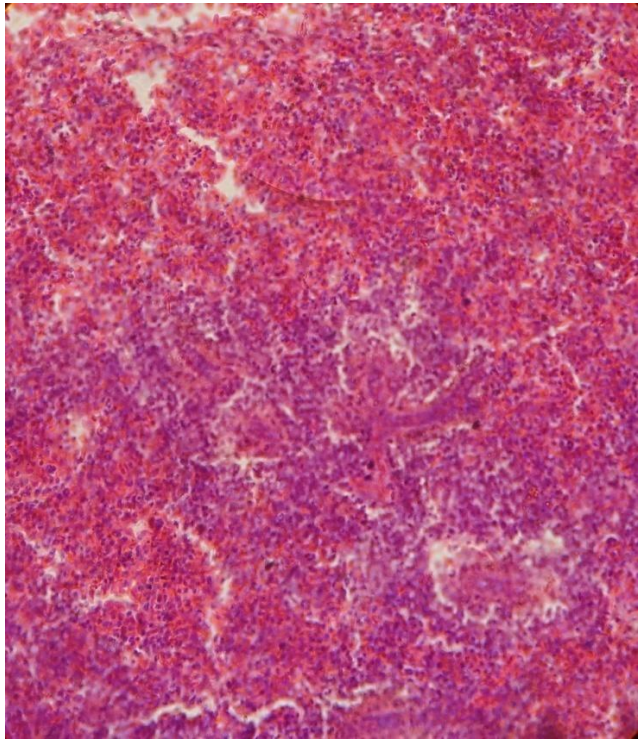


Plate XIII: Photomicrograph of a section of the spleen of quail inoculated with *Pasteurella multocida multocida* with no significant leision (H and E x 400) (group J) on day 17 post inoculation.

CHAPTER FIVE

DISCUSSION

In this study, there were significant increase ($P<0.05$) in temperature value a day post inoculation. Any value aboe 41^0C indicate pyrexia. There were fever in groups B, C, D, E, F, G, H and J. This fever persisted for 2 day in group B, 3 days in group C. No fever in A and I in all the days post inoculation. This is because group A is negative control and group I is treated with 3.3% garlic in water. Furthermore, increase in temperature is similar to the report of Akpavi *et al* (2011) where temperature increased a day post inoculation. There were also reports of fever in quails by Odugbo *et al.* (2004) and in chickens by Glisson *et al.* (1989), and Rimler and Glisson (1997) where they were inoculated with *Pasteurella multocida multicida.*

Clinical signs set in a day post inoculation in groups B,F,G and I the clinical signs progress in groups D, E, F, G, H, I and J under 3.3%. Garlic in water 2 days post inoculation. This mean that quails in these groups I and J recovered promptly. Clinical signs persisted for 12 days post inoculation in group B, 2 days in D and I, 4 days in group H, 3 days in group F and H, a day in group E and J. The highest morbidity rate recorded were in groups B, D, E, F, G and J and lowest in H and I. Groups A, and C show no evidence of morbidity. The highest morbility rate was recorded 2 days post inoculation. Thgese clinical signs were of acute forms of the disease as reported by Glisson *et al.* (1989), Rimler and Glisson (1997) in quails and commercial laying hens, Odugbo *et al.* (2004) and Akpavi *et al.* (2011).

There was general increase in weight in all the groups. Highest weight gain was in group C and lowest in group D. Statistically, there was increase in weight gain in groups G, H, I and J as compare to group A. There was increase in group B because pasteurellosis normally occur in peracute and acute forms were the body conditions remain intact. There were significant increase in body weight gain in groups inoculated with *Pasteurella multocida multicida* and

under medication (C-J) as compared to inoculated group B medication. This result in weight gain is in agreement with report of Akpavi *et al.* (2011), Rimler and Glisson (1997) were pasteurellosis occur in acute form. The results of groups E, G, H and J are in conformity with reports of Levis *et al.* (2013), Raeesi *et al.* (2010) and Fadialla *et al.* (2010) where garlic was used in broilers for improvement in performance and immunity.

Mortality started a day post inoculation in group B and D. mortality persisted a day in group C, 2 days in groups G, I and J, 3 days in groups B (90%), (60%), (60%) and (70%) and lowest in group C (20%), (30%) and I (40%). Most of the quails dies 3 days post inoculation that is garlic in water 3.3% was able to reduce mortality rate. This high mortality (90%, 70% and 605) agreed with the report of Odugbo (2004) and Akpavi *et al.* (2011) were they recorded 80 and 90% respectively. Garlic in water at 3.3% (group I and J) reduce mortality.

The PCV on day 5 post inoculation for inoculated groups with medication decreased. For group C, there was decease from 34% to 30%, group D from 38% to 30%, group E from 31.5% to 30%, group H from 39% to 25%. However, in groups B, G and I there were no decrease in PCV. The effect of disease process like acute dehydration, loss of appetite and impaired absorption can make a drop in PCV a gradual process. This drop in PCV is in agreement with report of Akpavi *et al.* (2011) where PCV decreases from 49.6% to 38.16%. On day 17 there were increase in PCV due to garlic (D to J) and ciprofloxacin (C) as compared to inoculated group B without medication.

The gross lesions seen on dead quail inoculated with *Pasteurella multocida multicida* day two post inoculation included congested breast muscle, congested and hyperemic duodenum congested and haemorrhagic spleen and misshapen ovaries. These gross reasons are in conformity with the findings of Rimler and Glisson (1997), Odugbo *et al.* (2004) and Akpavi *et al.* (2011) were pasteurellosis occur in acute form.

The heart muscles of quails inoculated with medication of ciprofloxacin day 2 post inoculation showed that there was monocuclear cellular infiltration lesion. This is similar to reports of Akpavi *et al.* (2011) where focal haemorrhagic lesioins were observed in quails inoculated with *Pasteurella multocida multicida*, however, due to medication, the lesion was not seen.

The liver of quails inoculated with medication of garlic on day 2 post inoculation showed fatty degeneration, congested sinusoids and distorted hepatic architecture. This is similar to the report of Akpavi *et al.* (2011), Rhoade (1964) and Rimler and Glisson (1997) where they reported areas of necrosis and heterophilic infiltration in the liver to be a common findings in birds inoculated with *Pasteurella multocida multicida*. However, this lesion was not seen at the end of the experiment due to the presence of garlic.

The spleen of quails inoculated with garlic medication on day 2 post inoculation revealed depletion of lumphocyte at the germinal centre. This is similar to the reports of Glison *et al.* (1989) who earlier reported multifocal splenic lesion in a natural outbreak of wol cholera involving three flock of Japanese quails. However, the lesion in this study was not seen at the end of the experiment due to galic.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

Garlic reduced the effect of pasteurellosis against quails at 3.3% in water.

Garlic in water at 3.3% administered to quail inoculated with *Pasteurella mutlocida multicida* serotype A:4 increased PVC from 31.0± 3.87% at day one post inoculation to 44.67± 4.28% at day thirteen post inoculation.

There were no significant histopathological lesion in the spleen in quails inoculated with *Pasteurella mutlocida multicida* serotype A:4 and treated with 3.3% garlic in water.

6.2 Recommendations

Further study using higher number of quails to confirm the efficacy of garlic at 3.3% in water should be conducted.

The minimum inhibitory concentration (mic) of garlic that is effective against *Pasteurella mutlocida multicida* should be estatablished.

Acute and chronic toxicity test of garlic should be conducted on quails.

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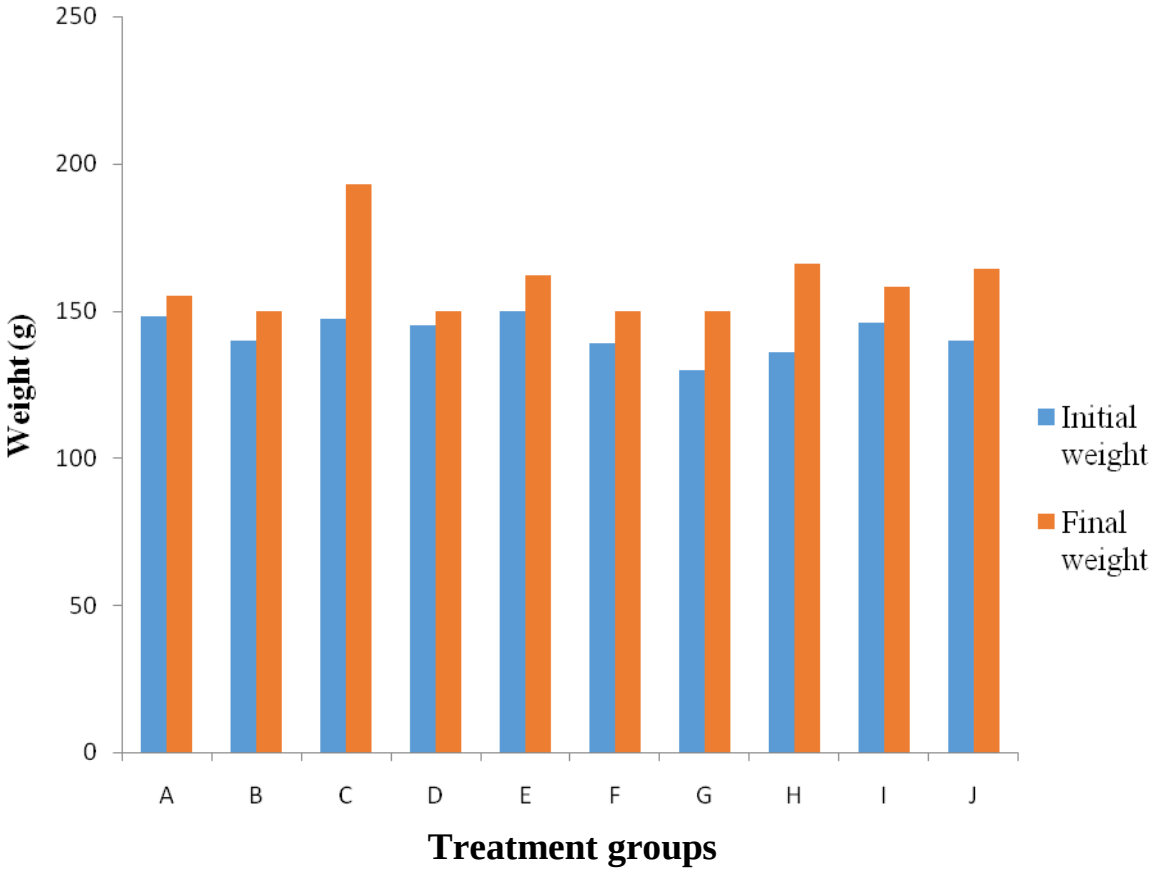
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Appendix 1: Preparation of 10% formalin saline

One hundred milliliters (100ml) of 40% formalin was measured using a measuring cylinder, 900ml of distilled water was also measured, and both were mixed by adding the formalin into the water and then stirred, nine grams of laboratory sodium chloride was then added into the solution and also stirred. This was then stored until use.



Appendix 2: Mean changes in body weight (g) of quails inoculated with *Pasteurella Multocida multocida* serotype A4 under medications of ciprofloxacin and garlic

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

Appendix 3: Culture and Determination of *Pasteurella Multocida Multocida* Scrorype A:4 Inoculum

The bacterium was subcultured on blood agar at the bacteriology laboratory of the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria. The resulting colonies were examined for their features, colour, morphology (non-haemolytic, moderate sized round colonies) and tested for gram reaction (Gram negative). Microscopically bipolar staining organisms with Gram staining were observed.

Three colonies were scooped and inoculated into 20 ml of 0.5% normal saline. After a 4-fold serial dilution in 10 test tubes, 0.1 ml each from test tube number 8, 9, 10 were inoculated on blood agar to observe for growth over 24 hours.

The original suspension was preserved in the refrigerator in order to inhibit proliferation of the bacteria. The average colony count from test tubes 8, 9, 10 was 280 cfu/0.1 ml. Therefore for 1 ml there were 2,800 cfu/ml.

To obtain the number of organisms that will be inoculated into the quails, the number of organisms was multiplied by the dilution factor 1:390625 (EP, 2001). Therefore 1 ml x 2,800 x 390,625= 1,093,750,000.

1ml of stock was estimated to contain approximately 1 x 10^9cfu.

At 10^6 colony forming unit (cfu/bird, 10^4cfu/bird and 10^2 cfu/bird quails were found to.be experimentally infected (Akpavi *et al.*, 2011). Hence, infective doses of 10^6 cfu/birds and

10^4cfu/bi/d were used.

At 10^4cfu/bird, 80 quails required 8 x 10^5 organisms.

If I ml contain 1 x 10^9cfu

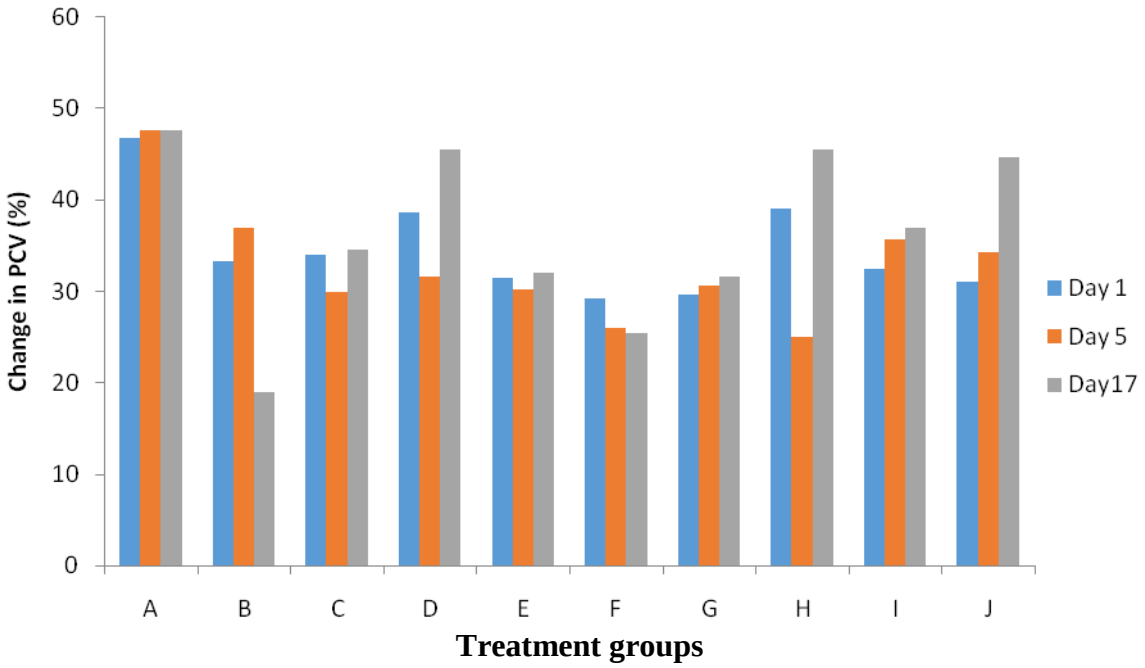
X ml contain 800,000

X ml $= \frac{800,000}{1 \times 10^9} = \frac{8}{10^4} = 0.001$ ml

0.01 ml of stock constitute into 80 x 0.2 = 16 ml total quantity group will required.
Similarly, at 10^6 cfu, ten (10) quails will require
10^6x 10= 10^7
1 ml contains 10^9
Y ml-10^7

Y ml = $= \frac{10^7}{10^9} = 0.001$ ml

Therefore, with 0.2 ml of diluents, 10 quails received 2 ml. This microbial estimation was done following a modified European Pharmacopeia" Ep method of determination of inoculation (Ep, 2001).



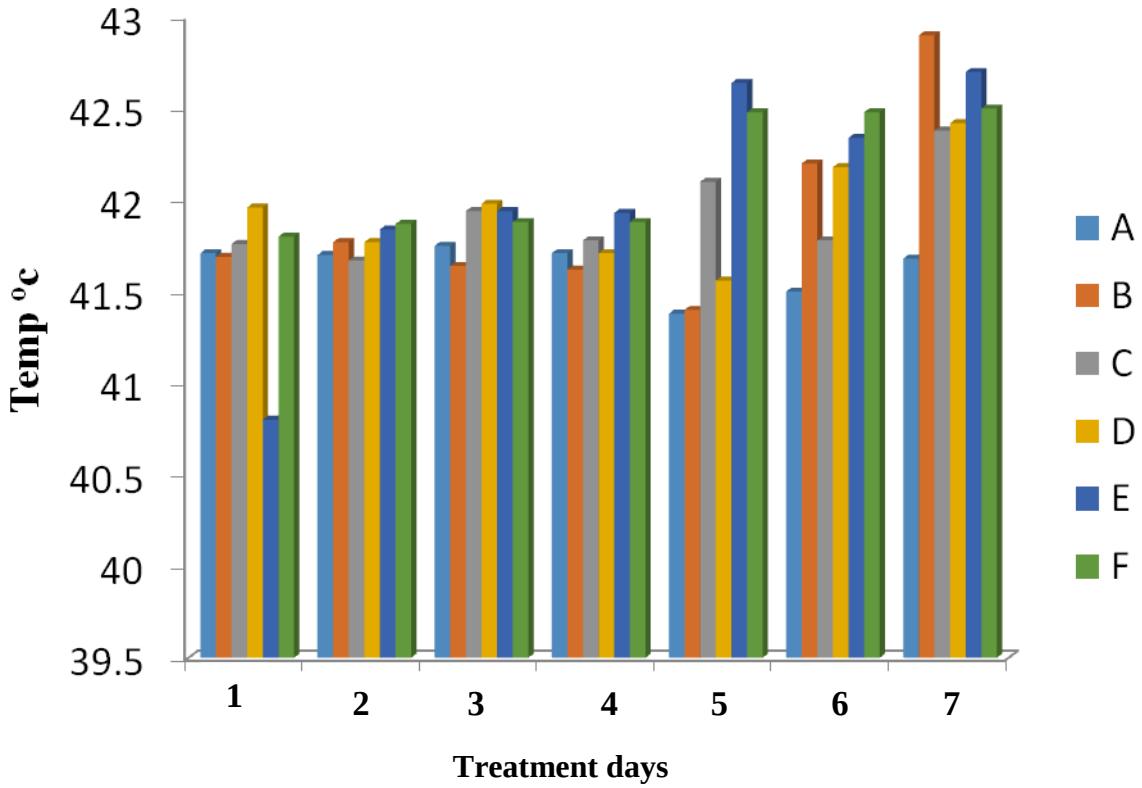
Appendix 4: Mean change in packed cell volume of quails inoculated with *Pasteuralla multicida multicida* with garlic and ciprofloxacin medication

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water

Appendix 5: Mortality rate of quail inoculated with *Pasteuralla multocida multocida* setotype A:4 under ciprofloxacin and garlic medication

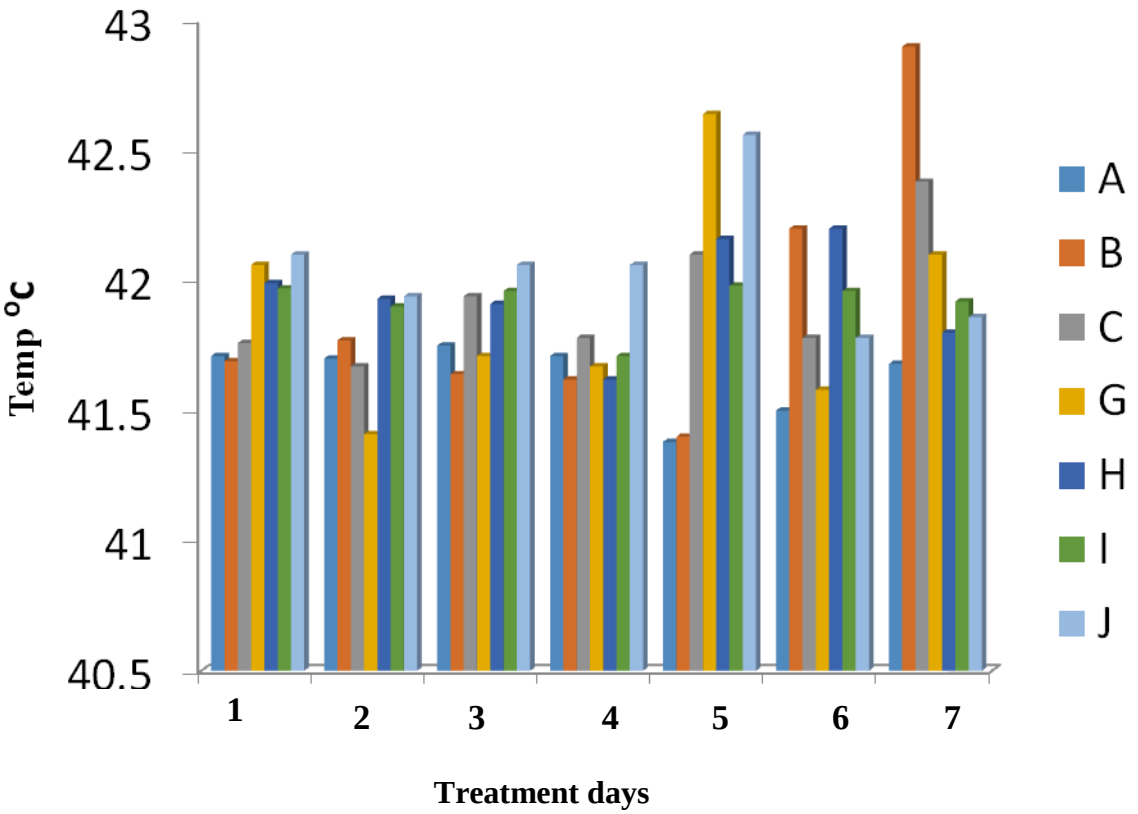
Days post inoculation	A	B	C	D	E	F	G	H	I	J
0										
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	Ma-0			Ma-1						
6	Ma-0	Ma-2	Ma-2	Ma-1	Ma-2	Ma-2		Ma-2	Ma-1	Ma-2
7	Ma-0	Ma-2	Ma-0	Ma-2	Ma-1	Ma-2	Ma-5	Ma-4	Ma-3	Ma-1
8	Ma-0	Ma-0	Ma-0	Ma-0	Ma-2	Ma-1	Ma-1	Ma-0	Ma-0	Ma-0
9	Ma-0	Ma-1	Ma-0	Ma-0	Ma-0	Ma-1	Ma-0	Ma-1	Ma-0	Ma-0
10	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
11	Ma-0	Ma-1	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
12	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
13	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
14	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
15	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
16	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
17	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0	Ma-0
Rate	0%	-60%	-20%	-40%	-60%	-50%	-60%	-70%	-40%	-30%

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water



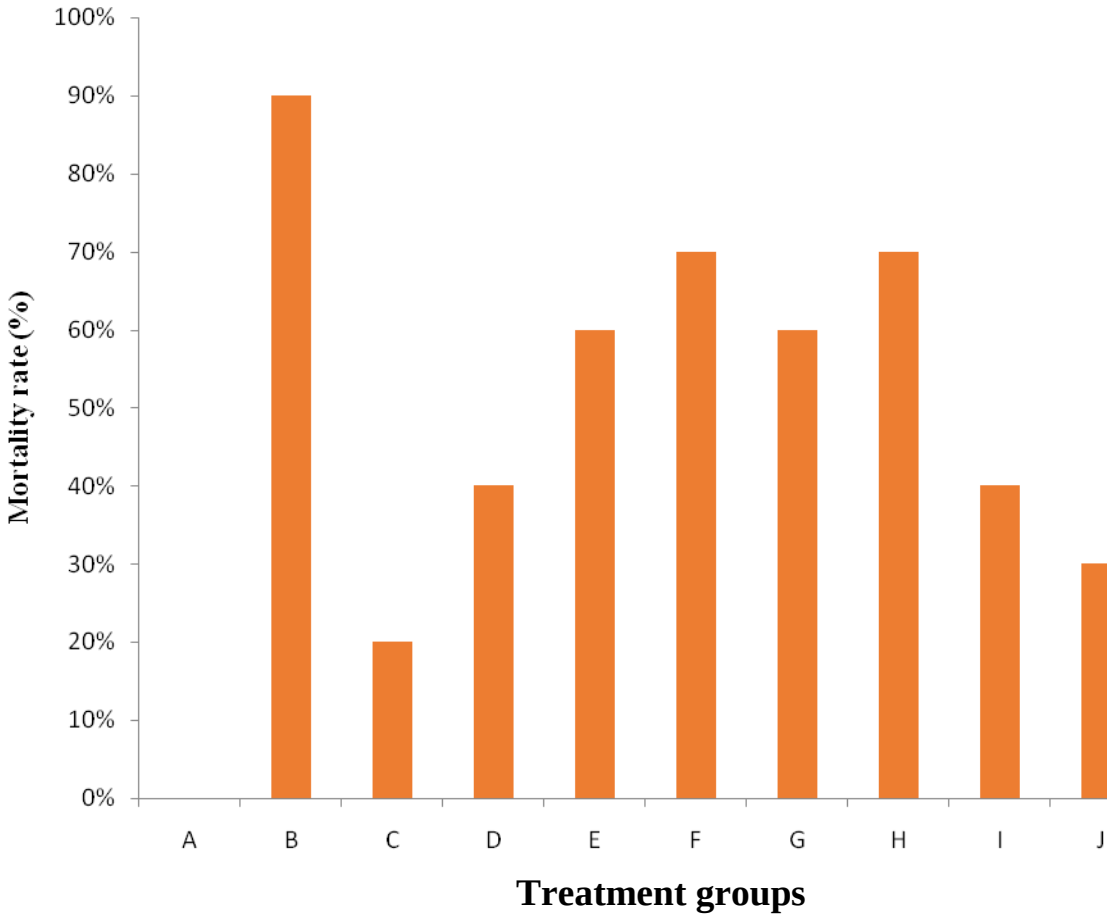
Appendix 6: Mean changes in daily cloacal temperature (^{o}c) – Controls Vs Garlic in-feed

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water



Appendix 7: Mean changes in daily rectal temperature (^{o}c) –Controls Vs Garlic in-water

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water



Appendix 8: Mortality rates of quails inoculated with *Pasteuralla multocida multocida* serotype A:4 under medication of ciprofloxacin and garlic.

KEY: Group A represent = negative control (not inoculated/not treated); B = positive control (inoculated/not treated), C= inoculate/treated with Cirprofloxacin; D = Inoculated/treated with garlic (3%) in feed; E = Inoculatedc/treated with garlic (5%) infeedc; F = Inoculated/treated with garlic (10%) infeed; G = Inoculated/trated with garlic (1%) in water; H = Inoculated/treated with garlic (1.67%) in water; I = Inoculated/treated with garlic (3.3%) in water; J = Inoculated/treated with garlic (3.3%) in water