AVIAN SPIROCHETOSIS IN NORTHERN NIGERIA

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An adequate and efficient veterinary department [in Nigeria] is not an expense; it is a sound investment - W.W. Henderson, 1943.

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ABSTRACT

PREFACE

"Generally the animal health position was good during the year [1952/53] with the exception of poultry disease which was high." -- Hon. K. O. Mbadiwe, 1953.

"...year after year disease takes a very heavy toll of poultry in each region, and in some areas poultry keeping is reported to have become impossible without vaccination. Poultry contributes no small measure to the diet of the population and it is unfortunate that in the past, staff shortage has precluded greater attention to this problem." -- R. S. Marshall, 1953.

These statements made over 2 decades ago return as echos and are just as true today as they were when they were made. In spite of the well recognized need for animal source protein in the diet and the ability of poultry and poultry products to readily fill this need, poultry losses continue to be enormous in Nigeria and the causes are still little known or little understood. This is particularly true in northern Nigeria where the major poultry resource is still the village fowl and small backyard flocks. The study of disease problems in these fowls is not as easy as periodically visiting neatly packaged flocks of several thousand or more birds managed by enlightened poultry men, who understand the value of studying and controlling poultry diseases. Intensively managed poultry in Nigeria have a set of disease problems, for the most part completely different from those of village fowls, which are just as unknown or poorly understood and deserve investigation. But in northern Nigeria intensive poultry production is only a small part of the poultry industry at this time. Therefore, it appeared that disease problems in those fowls constituting the major poultry resource in northern Nigeria deserved investigation and avian spirochaetosis which was found to be the most important bacterial disease of these fowls was selected and studied during the past 4

Although poultry losses are said to be "high", "great", "enormous", what is really meant by these terms? What is the magnitude of the economic loss that occurs annually as a result of poultry losses? It is, of course, impossible to give an accurate figure. Not only is it unknown how many birds die annually, it is not even known how many birds there are in northern Nigeria--there are too many gaps in our knowledge. It is possible, however, to put forth an educated guess on both points. From my studies at the Avian Diseases Laboratory, Ahmadu Bello University, mortality varied directly with management from nil to 100% but for the type of birds being investigated 40-60% was found to be typical. This figure was substanstiated and usually considered conservative when people were asked to estimate poultry mortality from day old to adult in their villages.

If we can use a figure of 50% for mortality, we still need to have some idea of the size of the "flock" in northern Nigeria. Previous estimates included 17.9 million fowls in 1960 (Northern Nigeria Statistical Handbook, 1965) and slightly over 41.7 million fowls in 1963 (FAO, Agriculture Development in Nigeria, 1965-1980).

From preliminary economic studies conducted at the Institute for Agricultural Research, Zaria, it was found that the ratio of fowls to inhabitants was about 1:1. This ratio may be higher in urban areas because a figure of 5:1 was reported by the animal husbandry division for Sokoto, but this included all domestic poultry. A figure of 1.3 fowls/person in Nigeria was used by FAO. The need to accurately know livestock numbers compared to population is obvious and could be a useful means of estimating livestock populations. If we accept the 1:1 or 1.3:1 ratio as reasonable estimates and using the value of 51,380,000 people from the current provisional 1973 census for the 6 northern states, we arrive at an estimated flock size of between 51.4 and 66.8 million fowls for northern Nigeria. With a 50% mortality this results in a loss of 25.7-33.4 million birds annually. At an average market value of ₦1.20 per bird (current post-Udoji price) the potential economic loss is between 30.8 and 40 million Naira/year. Using the prevalence rate of 3.7% which was

results can be usefully applied to reduce poultry mortality and thereby make poultry products more available to the populace.

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AVIAN SPRIOCHAETOSIS IN NIGERIA:
A REVIEW OF THE LITERATURE

SUMMARY

Avian spirochaetosis has been recognized in Nigeria as an important cause of mortality in indigenous and imported birds for over 60 years, however, there still is little known about the disease. It undoubtedly is much more common and widespread than the scattered reports would indicate although it is unquestionably more important in the northern parts of Nigeria.

REVIEW OF LITERATURE

Avian spirochaetosis in Nigeria was first definitively diagnosed in August, 1913, as the cause of a fatal disease occurring in chickens and Muscovy ducks at Iju, near Lagos (6). This first report of spirochaetosis in Nigeria also appears to be the only record of the disease in Western Nigeria except for mention of a single infected turkey at Ibadan over 40 years later (2).

The disease undoubtedly had been endemic in Nigeria for sometime since it was well known to indigenous poultry keepers. Also, lack of significant importations of exotic breeds prior to that time (7) minimized the chance that the disease had been recently introduced. The later observation that indigenous fowls were more resistant to spirochaetosis than imported breeds (15) further indicated a long host-spirochaetosis relationship. It would seem reasonable to speculate that spirochaetosis had been introduced into Nigeria centuries earlier by migrants who travelled with their livestock across the Sahara or by poultry carried for food purposes on ships that traded along the coast. Local trading would have resulted in the disease being spread throughout the area. Although it is not known how spirochaetosis came to Africa, Blaizot (1), after comparing Brazilian and African strains of Borrelia anserina was of the opinion that avian spirochaetosis probably spread from West Africa to Brazil by infected fowls carried on slave ships.

MacFie and Johnston (6) found the disease to be more prevalent

during the rainy season. The reported clinical signs were fever, diarrhoea, anaemia, pale comb, somnolence, paresis and staggering gait. These became progressively more severe and birds eventually became unable to stand, laying prone on the ground until they died. Appetite remained good and they ate until just before death. Local treatment consisted of smearing the bird's legs with shea butter--a treatment MacFie and Johnston found to be ineffective. Ticks were not found but fleas identified as Echidnophaga gallinaceus were present on the combs of a few affected birds. Splenomegaly and hepatic necrosis, occasionally massive, were typical pathological changes. These workers were unable to infect a rat with the avian spirochaete. Atoxyl and Salvarsan, especially the latter, were found to be effective in treating the disease.

During the next 30 years little attention could be given to diseases of poultry owing to limited staff and the pressure of other priorities. It was not until 1941 that spirochaetosis was again reported; this time as common among fowls, and to a lesser extent, turkeys in northern Nigeria (10). The following year, the importance of the disease was recognized, especially in the Northern Region, and it was stated, "Spirochaetosis, probably the most serious handicap to poultry keepers in this country, was very common in both Zaria and Ilorin" (11).

In 1948, spirochaetosis was reported from around Vom (Benue Plateau State) and common throughout the Northern and Eastern Regions (14). This was the first indication that the disease occurred on the Jos plateau and in the Eastern Region. Ikeme (4) subsequently confirmed the presence of spirochaetosis on the Jos plateau when he found spirochaetes in market fowls at Vom. Further reports of spirochaetosis in the Eastern region could not be found. However, a single, unreported outbreak was confirmed over 20 years later near Enugu (East Central State).* In that outbreak, ticks were found

*Dr. E. Udanochie, personal communication (1972). Veterinary Division, Engu, East Central State.

infesting the trees in which the chickens roosted. Removal of the trees resulted in control of the outbreak.

The disease in Nigeria was considered more serious in imported or crossbred fowls than indigenous fowls (13, 15). Introduction of the latter into flocks of exotic poultry was thought to be the principle source of spirochaetosis (13).

Acetylarsen or Soamin was effective in controllint the infection in Nigerian fowls. Initially, affected birds were given two subcutaneous injections of .05 gm Soamin on successive days which resulted in curing over 90% of treated birds (10). Later, relapses were found to be a problem in Soamin-treated birds and Acetylarsen was preferred (12). The optimum treatment was determined to be a single 1 ml injection of a 10% Acetylarsen solution (13).

In a report on diseases of poultry common in Nigeria (15), spirochaetosis was considered to be one of the most serious. Wild birds were suggested as possible carriers. Ability of the organism to survive in carcasses for up to 5 days in cool weather was reported. Transmission occurred chiefly through ticks but lice and biting flies were considered possible mechanical vectors. Ingestion of ticks or food contaminated by droppings was reported capable of infecting birds. Spread within a flock was facilitated by overcrowding and poor hygiene. An incubation period of 5 to 10 days was typical, with the disease lasting 3 to 5 days if acute or up to 3 weeks in the chronic form. Mortalities of 60 to 90% were reported as common. Affected birds huddled together and were characterized by ruffled feathers, loss of appetite, weakness, high fever, progressive anaemia, pale comb and wattles, greenish diarrhoea that gradually became worse, and various forms of paralysis. Postmortem changes included emaciation, rapid putrefaction, blood that failed to clot, enlarged, dark spleen, enlarged, congested greasy liver studded with pale foci of necrosis, and intense inflammation of the intestines with blood stained contents. Diagnosis was based on typical clinical signs, especially

the early appearance of paralysis, coupled with demonstration of the spirochaetes in stained blood films. The importance of taking smears during the height of the fever was stressed because as the fever fell prior to death, organisms tended to disappear from the blood. Arsenical drugs were reported to be very effective if treatment was started early. Methods of tick control were presented along with a brief account of the tick's life cycle. Eradication of ticks was considered as important as treatment in controlling spirochaetosis.

Attention was again diverted from spirochaetosis when Newcastle disease entered Nigeria from southern Cameroons across the Bamenda highlands in 1950 and swept acorss the country with devastating effect, decimating poultry flocks (3, 4). An all out effort was launched to bring the disease under control.

However, spirochaetosis continued to be a common cause of poultry mortality in northern Nigeria, although even here the disease was of greater importance in the more northerly parts of the region. Spirochaetosis was frequently reported between 1955-1972 (Table 1).

Studies on spirochaetosis were undertaken at the newly established Poultry Diagnostic Laboratory in Kaduna during the late 1950's and early 1960's. The disease was found to be common in local chickens and turkeys and to respond to penicillin (33). During clinical studies, spirochaetes were found in the blood 4 days after infection, usually associated with Aegyptianella pullorum (34). The spirochaete was cultivated successfully by inoculating the allantoic sac of 8-day old embryos with blood from infected birds (34). Mosquitos were shown to be capable of transmitting avian spirochaetosis experimentally in northern Nigeria (8), however, they were not believed to be important in field outbreaks (5).

During these studies, a colony of fowl ticks, Argas persicus, was established. Considerable work was carried out to devise a perch which would prevent ticks from reaching fowls at night breaking the life cycle even when houses were heavily infested (33). The tick was considered the most important external parasite of poultry

in Nigeria occurring more commonly and consequently of greater importance in the drier, more northerly parts of the country. It was reported that tick activity was higher in the hot dry periods before and after the rains than at other times of the year (5).

Spirochaetosis is a village problem (5) but often veterinary and diagnostic services are more available to commercial or government farms near urban centers where the standard of management precludes tick infestation and spirochaetosis. Therefore most reports of poultry diseases in Nigeria are slanted toward problems in these flocks and do not provide an accurate assessement of the importance of spirochaetosis. Occasionally such farms do become involved with devastating result. For example, at the government poultry farm in Sokoto a flock of layers brought from Mokwa was "wiped out" by spirochaetosis (36). The disease continued to be a problem and was finally controlled only by complete destruction of the flocks and houses (37). At the Avian Diseases Laboratory, Ahmadu Bello University, spirochaetosis was second only to Newcastle disease as a cause of mortality in free-range and back yard poultry of both indigenous and imported breeds between January, 1971, and March, 1975 (unpublished data).

TABLE 1

REPORTS OF AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA, 1955-1972

YEAR	LOCATION	COMMENTS	REFERENCE
1955/56*	Katsina		(25)
	Gumel	Response of infected fowls to 5 ml intramuscular injection of Acetylarsen was spectacular "...even in bringing half dead birds back to life."	(19)
	Zaria	Spirochaetosis reported from a number of ducks and chickens. Substantial reservoir of ticks found in railway compound which was thought to be the source of infection in transported birds.	(39)
1956/57	Katsina		(26,27)
	Kano	Spirochaetosis initially diagnosed as Newcastle disease.	(20)
	Kaduna	Common in chickens and turkeys. Responds to penicillin.	(33)
1957/58	Maiduguri	Ducks and fowls affected with spirochaetosis. Three of 58 smears positive for spirochaetes.	(16)

TABLE 1 (continued)

YEAR	LOCATION	COMMENTS	REFERENCE
	Dambatta	Husbandry a contributing factor to spirochaetosis	(21,22)
	Zaria	Three of 32 blood smears positive for spirochaetes.	(40)
1958/59	Maiduguri		(17)
	Zaria		(41,42)
1959/60	Katsina	Spirochaetosis controlled by 3 daily injections of pencillin and dusting birds with DDT powder.	(28,29)
	Maiduguri		(18)
1960/61	Kano	Six of 23 blood smears positive for spirochaetes. Spirochaetosis diagnosed in 5 of 7 birds examined by postmortem.	(23)
1961	Vom	Spirochaetosis found in village birds but not in birds at the Egg Production Unit, Federal Department of Veterinary Research.	(4)
1961/62	Kaduna	Forty-three of 3,852 birds examined positive for spirochaetosis. (Note: most birds in this series probably came from a single source, the Poultry Production Unit where spirochaetosis did not occur)	(35)

TABLE 1 (continued)

YEAR	LOCATION	COMMENTS	REFERENCE
	Kano	Several cases of spirochaetosis suspected and treated.	(24)
1968/69	Katsina	Spirochaetosis confirmed at a local poultry farm.	(30)
1969/70	Katsina	Spirochaetosis caused sporadic mortality. Controlled by treatment and destruction of ticks.	(31)
1970/71	Katsina	Tick infestation caused high mortality (spirochaetosis?)	(32)
	Sokoto	Layers brought from Mokwa "wiped out" by spirochaetosis. Brooder and layer houses destroyed.	(36,37)
1971/72	Zaria	Spirochaetosis common.	(43)
	Sokoto	Flock of Harco brown birds disposed of because of infection with spirochaetes.	(38)

*Year extends from April through March and is not a normal calendar year. The 1st quarter is April-June, 2nd quarter is July-September, 3rd quarter is October-December and 4th quarter is January through March. Therefore 1955/56 actually refers to April, 1955, through March, 1956.

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: DIAGNOSTIC METHODS WITH A DESCRIPTION OF AN IMPROVED TECHNIQUE USING BUFFY COAT SMEARS

SUMMARY

Wet and buffy coat smears examined by darkfield microscopy and stained blood and organ smears were compared during studies on fowls naturally and experimentally infected with Borrelia anserina in northern Nigeria. For routine diagnostic work, wet smears examined by darkfield microscopy, were found to be the method of choice because they were simple, rapid, reasonably accurate and could be used to detect spirochaetes in organs at postmortem. Stained smears were most valuable when larger amounts of blood could not be collected or there was a long period between collection and examination of samples.

Borrelia anserina was found to be concentrated in the buffy coat of centrifuged blood in microhaematocrit tubes when examined by darkfield microscopy. This technique was found to be more sensitive than wet smears and is recommended when low levels of spirochaetemia require detection.

INTRODUCTION

Several laboratory methods have been used to diagnose avian spirochaetosis including staining with aniline dyes (8), Romanowsky stains (8), heavy metal impregnation (8,10,11), immunofluorescent microscopy (7,9), darkfield or phase microscopy (6), and serological tests (1,2,12,13). All depend upon demonstrating the causative agent, Borrelia anserina, or its antigen.

In northern Nigeria avian spirochaetosis is a frequent cause of mortality in fowls, turkeys, and ducks (3). A rapid, accurate diagnosis is essential for controlling outbreaks, treating affected birds, and studying the disease. The purpose of this paper is to report observations on methods of diagnosis used in northern Nigeria and to describe a more sensitive technique for determining spirochaetemia by using buffy coat smears.

MATERIALS AND METHODS

Thin blood films were prepared on clean glass slides from either a drop of blood from a wing web capillary, wing vein, clipped toenail or blood samples collected in EDTA (4). Organ smears were prepared by blotting the cut surface on towelling until excess blood and tissue fluids had been absorbed and then touching the cut surface to a clean slide. Blood and organ smears were stained with Giemsa stain (1:10) at pH 7.2 for one hour. Some films were counterstained for 30 sec with 1% aqueous crystal violet (8). Carbol fuchsin stained films were prepared and examined as previously described (5). Stained blood and organ smears were examined for approximately 5 minutes (X1000) before being considered negative.

Wet smears were prepared by picking up a small drop of blood on the edge of a coverslip, mixing it with 6-10 times as much sterile physiological saline on a glass slide and covering the diluted blood with the coverslip. Wet smears were examined for 2-3 minutes (X300) by darkfield microscopy.

The ability to detect B. anserina at various intervals after

death in the liver and heart clot of a fowl that had died naturally of spirochaetosis (ADL Acc. No. P144/71) was evaluated by using Giemsa stained and wet smears. The carcass was kept at room temperature and a thermometer was placed next to it. Ambient temperatures ranged from 16.2-32.0°C. Small incisions were made in the body wall and pieces of liver and heart clot removed at intervals between 6 and 52 hours after death. The incisions were plugged with a paper towel moistened with sterile physiologic saline.

The location of B. anserina in centrifuged blood from 4 naturally infected fowls was determined by cutting microhaematocrit tubes at 5 levels: upper plasma, lower plasma, buffy coat, upper RBC and lower RBC. Smears were made at each level and examined by darkfield microscopy for spirochaetes.

Buffy coat smears were prepared by filling microhaematocrit tubes (plain or heparinized, depending upon type of sample) with blood and centrifuging for 5 minutes in a microhaematocrit centrifuge. Tubes were cut between the buffy coat and packed red cells (RBC). The buffy coat and plasma were placed on a clean glass slide by gently tapping the tube on the slide until the intact buffy coat was dislodged and plasma poured out. By gently placing a coverslip on the buffy coat, it spread out in a centrally-located circle under the coverslip. Buffy coat smears were examined in the same way as wet smears. Particular attention was given to the edges of the flattened buffy coat during examination.

The sensitivity of wet and buffy coat smears for detecting spirochatemia was compared by examining aliquots of blood by the two techniques. Blood samples were obtained from 518 market fowls (4) and from experimentally infected adult white leghorn cockerels at 12 hour intervals after inoculation.

RESULTS

During routine laboratory work, a number of spirochaete positive blood samples were compared using Giemsa stained thin

blood films and wet smears examined by darkfield microscopy. No difference was found in the results. However, when heart and liver smears from an infected carcass were compared by both methods wet smears were found to be superior to stained smears (Table 1).

Spirochaetes were well demonstrated with both 1% aqueous crystal violet and carbol fuchsin but these techniques did not appear to offer any particular advantages over Giemsa stained or wet smears.

When blood from 4 infected fowls was examined, spirochaetes were found to be concentrated in the buffy coat of centrifuged blood. They were not found in the plasma or lower part of packed RBC but were occasionally found in low numbers in the upper part of the packed RBC (Table 2).

Buffy coat smears were found to be more sensitive when wet and buffy coat smears were compared using blood samples from 518 market fowls. Spirochaetemia was detected in 36 (6.9%) of the 518 fowls examined by using buffy coat smears as compared with only 26 (5%) of the same fowls using wet smears (Table 3). In experimentally infected fowls, it was possible to detect spirochaetemia earlier by using buffy coat smears than wet smears of whole blood (Table 4).

DISCUSSION

Giemsa stained smears were found to have several advantages. Only a very small quantity of blood was required which could easily be obtained in the field. This was particularly important for experimental work because owners and traders were often suspicious of why blood was being taken or believed removing a larger quantity of blood would be injurious to the fowl. Slides could be stored indefinitely between collection and examination and could be retained as a permanent record for future reference. The haematological picture can be assessed and the presence of other

blood parasites ascertained in stained blood smears. Also, they could be examined by light microscopy which, in general, is more readily available. Blood films from suspected cases of avian spirochaetosis should be considered the preferred diagnostic specimen to be collected by veterinarians in the field for submission to a diagnostic laboratory.

Compared with wet smears, Giemsa stained smears had some disadvantages. They required more time for preparation and examination which delayed diagnosis and made examination of a large number of samples tedious. Stained smears could not be relied upon to detect spirochaetes in carcasses that had undergone some decomposition. In stained smears where postmortem decomposition had occurred, there was a uniform background staining. This apparently masked the presence of the spirochaetes for they were readily detected in wet smears. Furthermore, viability, which was easily determined by active motility of the spirochaetes in wet smears, obviously could not be determined in stained smears.

Wet smears examined by darkfield microscopy were found to be the method of choice for routine diagnosis of spirochaetosis in the laboratory. In general the advantages and disadvantages of wet smears were opposite to those of Giemsa stained smears. Wet smears were simple to prepare and examine, accurate--although not as sensitive as buffy coat smears, required a minimum of equipment, and were rapid. Using this technique, it was possible to have a diagnosis within a few minutes of when the bird was first seen. Wet smears were also the preferred technique for detecting spirochaetes in tissues of birds at postmortem. Because avian spirochaetosis is such a common cause of mortality in northern Nigeria, all clinically ill birds brought to the Avian Disease Laboratory, Ahmadu Bello University, were routinely checked for spirochaete infection by using wet smears. It would seem this same procedure could be adopted with advantages at all veterinary diagnostic laboratories in northern Nigeria or other areas where avian spirochaetosis is common.

Buffy coat smears have the same basic advantages and disadvantages as wet smears. Although they were the most sensitive method for detecting B. anserina in blood, they required a greater investment of equipment and time than wet smears. From a clinical or diagnostic viewpoint, this additional investment is probably not justified. Spirochaetemia is usually advanced when birds show clinical evidence of infection and B. anserina can be readily found in wet smears. The real value of buffy coat smears would be for spirochaetosis surveillance studies and in pathogenesis experiments when the onset and duration of spirochaetemia needed to be accurately determined. If done on a larger scale, the procedure could be useful in concentrating organisms for serological, biochemical and physical experiments. Buffy coat smears have been used in this laboratory to detect B. theileri spirochaetemia in sheep and goats (unpublished data) and the procedure may be useful for determining Borrelia infections in other species which are characterized by a low spirochaetemia.

TABLE 1

Comparison of Giemsa stained and wet smears of liver and heart clot for detection of Borrelia anserina in the carcass of a fowl that had died naturally of spirochaetosis.

Hours after Death	Giemsa Stained Smears		Wet Smears	
	Liver	Heart	Liver	Heart
6	Neg	+	+	+
24	Neg	+	+	+
28	Neg	+	Neg	+
46	Neg	Neg	+	+
52	Neg	Neg	+	+

TABLE 2

Location of Borrelia anserina in centrifuged blood from naturally infected fowls

Sample No.	Plasma		Buffy Coat	Red Cells		Whole Blood
	Upper	Lower		Upper	Lower	Wet Smears
1	Neg	Neg	+++	+	Neg	+++
2	Neg	Neg	++	Neg	Neg	+
3	Neg	Neg	+++	Neg	Neg	+++
4	Neg	Neg	+++	+	Neg	++

+, at least 1 spirochaete in 10 fields (X300)
++, at least 1 spirochaete/field
+++, 5-50 spirochaetes/field
+++, over 50 spirochaetes/field

TABLE 3

Comparison between whole blood and buffy coat smears in detection of Borrelia anserina in blood from market fowls in Nigeria.

	Buffy Coat Smears		
Wet Smears	+	-	Total
+	26	0	26
-	10	482	492
Total	36	482	

TABLE 4

Comparison between whole blood wet smears and buffy coat smears in detecting Borrelia anserina in blood of 6 experimentally infected adult White Leghorn roosters.

Method	Hours Post-inoculation				
	24	36	48	60	72
Whole Blood Wet Smears	0/6*	0/6	4/6	5/6	6/6
Buffy Coat Smears	0/6	2/6	6/6	6/6	6/6

*Number birds positive/number birds examined

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SUMMARY

The ability of Borrelia anserina to persist, remain viable, and remain infective at different ambient temperatures in carcasses of fowls that had died of spirochaetosis and were spirochaetemic at death was investigated. Tissues from two fowls that died of spirochaetosis but were no longer spirochaetemic were also examined.

In clotted blood from carcasses left at room temperature (19-26°C), B. anserina was found to remain infective for 2 days, motile for up to 7 days and was demonstrable for up to 12 days after death. Infective spirochaetes were found in the brain of these carcasses for 60 but not 67 hours. When carcasses were refrigerated (3-6°C), viable, infective spirochaetes were found for at least 15 days; the longest period tested. In carcasses that had been incubated (37-39°C), spirochaetes remained viable for only 1 day but could be demonstrated for 5 days. Freezing (-10°C) had a detrimental affect on the spirochaetes. From frozen carcasses, infective spirochaetes were found at 14 but not 32 hours.

The ability to demonstrate B. anserina in carcasses for long periods after death facilitates the diagnosis of avian spirochaetosis, even in carcasses that may have undergone considerable decomposition. The finding that B. anserina can remain infective for up to 2 days at room temperatures in carcases suggests that scavenging of carcasses may be an additional way birds could become infected when spirochaetosis occurs in a flock.

Spirochaetes could not be found in visceral organs or brains of fowls that died after spirochaetes had disappeared from the circulation. The absence of spirochaetes in these tissues further supports previous observations that B. anserina

disappears from the blood and tissues almost simultaneously and latent tissue infections are not a feature of avian spirochaetosis.

INTRODUCTION

The ability of Borrelia anserina to persist and remain viable in carcasses has important implications in the diagnosis and transmission of avian spirochaetosis. Conflicting views on this point are found in the literature. It has been reported that B. anserina disintegrates and can not be found even within a few hours after the fowl dies (13,15). Others have demonstrated the organisms in carcasses for several days (9,11,12). The finding of McNeil et al. (11) probably represents the longest reported interval between death and demonstration of B. anserina. They kept carcasses of poults and chicks that had died of avian spirochaetosis at 0°C and found viable spirochaetes for up to 31 days in spleen, liver, and heart. In northern Nigeria it has been stated that B. anserina may remain alive in a carcass for up to 5 days in cool weather (16).

This report describes studies undertaken to determine how long spirochaetes can persist, remain motile and are infective in fowl carcasses in northern Nigeria.

MATERIALS AND METHODS

Experiment 1. Eighteen 12 week-old Rhode Island red pullets from a tick and spirochaete free flock were experimentally infected with a field isolate of B. anserina. Fifteen died as a result of the infection. They were arbitrarily assigned to 3 groups of 5 carcasses each. Five carcasses were placed in a refrigerated cold room (3-6°C), 5 were left at room temperature (19-21°C) and 5 were placed in an incubator (37-39°C). A thermometer was placed with each group and the temperature recorded when each sample was collected. Wet smears were prepared and examined daily using clotted blood from a peripheral vessel or the heart (1). Presence and motility of B. anserina were evaluated.

Only one carcass was examined at a time because contamination and disappearance of B. anserina had been found by previous experience to occur more rapidly in opened carcasses even though covered with a moist towel. An opened carcass was examined until B. anserina could no longer be demonstrated at which time a new carcass was opened and examined. This procedure was followed until all carcasses in a group had been found negative for B. anserina.

Experiment 2. Twenty White Leghorn (WLH) fowls experimentally infected with B. anserina strain H (4) and one naturally infected WLH broiler that had died of spirochaetosis were examined for the presence of infective B. anserina periodically after death. The time of death of each of those birds was known and all were known to be spirochaetemic just prior to or after death. Fifteen carcasses were kept in the laboratory at ambient temperature (21-26°C). Of the remaining 6 three were refrigerated (3-6°C) and 3 were frozen (-10°C). Portions of the brain or heart clot were taken at various intervals following death, ground in sterile phosphate buffered saline (pH 7.2) containing 0.2% glucose (PBS/G) to make a 10% suspension and 1 ml inoculated subcutaneously immediately into each of 2 susceptible 1-3 week old WLH chicks. Only heart clot suspensions were prepared from frozen carcasses. Tissue suspensions were examined by darkfield microscopy for the presence and motility of spirochaetes. Wet smears (1) from inoculated chicks were examined daily. In two instances multiple samples of heart clot were taken from the same carcass through a small incision in the thoracic wall which was plugged with cotton moistened with PBS/G. Otherwise samples were collected only once. Multiple samples of brain tissue were never used.

Experiment 3. Two experimentally infected fowls that died 1 and 2 days after B. anserina (strain H) had last been detected in the blood were examined for tissue associated spirochaetes. They were not spirochaetemic at death. A part of the brain and a visceral

pool of approximately equal portions of heart, liver, spleen and kidney was prepared and inoculated into susceptible chicks as previously described for experiment 2. The chicks inoculated with tissue suspensions from one fowl were injected intramusclarly eleven days later with 0.2 ml infective serum containing the homologous strain of B. anserina.

RESULTS

Experiment 1. Borrelia anserina was found in wet smears prepared from clotted blood for up to 5 days after death when kept at incubator temperatures, 8 days at refrigerator temperatures and 12 days at room temperatures. Spirochaetes were still motile on days 1, 4 and 7 respectively.

Experiment 2. Spirochaetes capable of infecting susceptible chicks persisted for at least 48 hours in the heart clot and for at least 60 hours in the brain of fowls that had died of spirochaetosis and were kept at room temperature. Infective spirochaetes were not found in heart clot at 54, 60 and 72 hours or brain at intervals between 67 hours and 125 hours (Table 1). Non-specific deaths following inoculation of tissue suspensions from carcasses left at room temperature for over 60 hours were a problem. Of the susceptible chicks infected with heart clot specimens, 8/10 (80%) died whereas only 3/7 (43%) died when infected with spirochaetes from brain suspensions. Morphologically identifiable spirochaetes were found in heart clot suspensions for at least 72 hours; the longest time examined.

In refrigerated carcasses, motile spirochaetes were found in smears 15 days after the fowl had died. Both chicks inoculated with brain suspension and 1 of 2 chicks inoculated with heart clot suspension became infected and died. Periods longer than 15 days were not investigated.

Spirochaetes capable of causing infection in susceptible chickens were found in heart clot suspensions from carcasses frozen for 14 hours. All chicks inoculated with heart clot suspensions from carcasses frozen 32 hours and seven days died from non-specific causes. The morphology

of the spirochaetes had deteriorated at 14 hours and they were recognized only with difficulty at 32 hours and 7 days.

Experiment 3. Spirochaetes were not found in the brain or viscera of either of the birds in this experiment. The 4 chicks inoculated with brain and viscera suspensions of one fowl were found to be susceptible to B. anserina infection 11 days later.

DISCUSSION

In this series of experiments B. anserina was found to persist and remain infective in fowl carcasses for several days if the bird was spirochaetemic at the time of death. Spirochaetes could not be found in tissues of fowls that were not spirochaetemic at death. The length of time B. anserina remained in the carcass was related to the ambient temperature at which the carcass was kept. At room temperatures (19-26°C) B. anserina was infective for as long as 2 days, motile for up to 7 days and could be demonstrated for at least 12 days in the clotted blood from fowl carcasses. Infective spirochaetes in these carcasses persisted slightly longer in brain than heart clot.

When carcasses were kept refrigerated (3-6°), spirochaetes remained infective and could be demonstrated in the brain and heart clot for at least 15 days after death. Longer periods were not tested.

When kept at incubator temperatures (37-39°C) spirochaetes remained motile for only one day but could be demonstrated for as long as 5 days. Infectivity of these spirochaetes was not examined.

Freezing appeared to have a deleterious effect on the spirochaetes and they were only found to be infective for 14 hours in frozen carcasses. Even after this short period, the morphology of the spirochaetes had been considerably altered and motility

was not detected. It is not known if the carcass had been completely frozen throughout. From results of later periods it would seem likely that it was not. All chicks inoculated with suspensions of heart clot from carcasses frozen for 32 hours and 7 days died within 2-3 days of inoculation. It appeared the suspensions had become toxic but the reason for this in unknown. Spirochaetes from frozen carcasses were difficult to recognize having a beaded or ghost-like appearance. It is doubtful that they were infective.

The fact that B. anserina can be found in carcasses for long periods after a bird dies is most important in the diagnosis of spirochaetosis which depends upon demonstration of the spirochaetes (1). In this study, B. anserina was found in wet smears from carcasses that had undergone such a degree of decomposition that they probably would have been discarded as useless during routine diagnostic work. The climate in northern Nigeria causes rapid decomposition of fowls after death. However, if spirochaetosis is suggested from the history, even decomposed fowls should be examined for B. anserina by preparing wet smears of the heart clot and examining them by darkfield microscopy. Birds that die of spirochaetosis in northern Nigeria are usually, but not always, spirochaetmic when they die (2). If spirochaetes were present at death, it would be likely that they would still be present and could be demonstrated even if the carcass was not examined for 3 or 4 days. These results are consistent with clinical observations (2).

Direct transmission of spirochaetosis by experimentally feeding infective blood and cannibalism has been reported (3, 9, 11, 14). The ability of B. anserina to remain infective in carcasses suggests the likelihood that scavenging of carcasses may be yet another way in which fowls could directly contract spirochaetosis. In this study infectivity was determined by parenteral injection of brain or heart clot suspensions into susceptible chicks. Similar studies using the oral route to determine infectivity are indicated to

explore this possibility. It is also possible that natural scavengers such as crows, kites, and vultures could become infected in this way or mechanically transmit avian spirochaetosis to other susceptible birds.

The inability to find spirochaetes in suspensions of the brain or viscera of fowls that had died after B. anserina had disappeared from the blood stream is consistent with earlier reports that spirochaetes disappear from blood and tissues almost simultaneously and death occurring after spirochaetemia is the result of something other than activity of viable spirochaetes (10). From living fowls killed 24 and 48 hours after spirochaetes had disappeared from the blood, Djankov et al. (6) could not find spirochaetes in tissues using a biological test, and phase-contrast, darkfield, and immunofluorescent microscopy. In contrast Dickie and Barrera (5) were able to demonstrate spirochaetes in tissue sections and by sub-inoculation of the livers of fowls for up to 4 days after the cessation of spirochaetemia. In their study blood from the same fowl was not sub-inoculated simultaneously with the liver suspension. It would seem possible that spirochaetes may have been present in the blood although in numbers too low to detect in examination of stained films. Knowles et al. (10) found that with few exceptions once spirochaetes disappeared from the blood they could not be found in emulsions of viscera. They found viable spirochaetes to persist in kidney for 24 hours and brain for 24 to 48 hours in birds killed after spirochaetes had disappeared from the circulation. Unfortunately the number of birds examined was not stated but the impression given was that such an occurrence was uncommon.

The inability of B. anserina to remain in tissues is an important differentiating feature between avian and mammalian Borrelia infections. It has been well established that spirochaetes persist for long periods in the brain of infected mammals (7) and this, coupled with antigenic changes, is considered the basis for relapses which

characterize mammalian borrelioses. The inability of B. anserina to persist in avian tissues would explain the lack of relapses in avian spirochaetosis.

Some differences are apparent between the results of the first and second experiments. In experiment 1 motile spirochaetes were found for 7 days in carcasses that were kept at room temperature. Unfortunately it was not possible to test them for infectivity at the time. As such, it was surprising when infective spirochaetes could be demonstrated for only 48 hours in experiment 2. The experiments were conducted at different times of the year and the ambient temperatures were lower during the first experiment. It seems doubtful, however, that this alone could explain the difference found between persistence of motile and infective spirochaetes in the two experiments. It would appear likely that spirochaetes in carcasses can remain viable and motile for several days but lose the capability to infect a susceptible bird after a few days. In experiment 1 spirochaetes were not found in carcasses longer than 8 days after death when the carcasses were kept refrigerated whereas in experiment 2 spirochaetes were not only demonstrable for at least 15 days but were also viable and infective. No explanation for this difference is apparent unless by a fortuitous event the 5 carcasses selected for refrigeration in experiment 1 could have had a few spirochaetes remaining in the circulation when they died. A larger number of birds may have extended this time and provided more consistent results. It is clear, however, from the results of both experiments that spirochaetes can persist for long periods in carcasses kept refrigerated or at room temperatures although exact end-points were not determined. Certainly they can persist for the first 2 or 3 days after death when their presence would be most important to the diagnostician or as a possible source of infection.

The results of this study provide further evidence that B. anserina can persist for long periods in carcasses of fowls that were spirochaetemic when they died. There was no evidence that they

disintegrate shortly after death. The observation that in northern Nigeria spirochaetes may remain viable in fowls carcasses for up to 5 days in cool weather appears valid and is supported by these results. However, it is unliekly that they would remain infective for this period. Further evidence is provided by this study to indicate that spirochaetes disappear from tissues at approximately the same time they disappear from blood. Also, deaths that occur after spirochaetemia has ended are probably not the result of the activity of viable spirochaetes.

TABLE 1

Infectivity of Borrelia anserina in heart clot and brain of infected fowl carcasses kept at room temperature (21-26°C) for chicks.

Tissue	Intervals Between Death and Examination (Hours)																
	12	14	24	29	40	44	48	54	60	67	72	75	97	101	108	110	125
Heart	2*	2	2	2	2	2	4	2	2	NE	2	NE	NE	NE	NE	NE	NE
	2**	2	2	2	0	1	1	0	0	NE	0	NE	NE	NE	NE	NE	NE
	2***	2	0	2	0	1	1	0	0	NE	(2)	NE	NE	NE	NE	NE	NE
Brain	NE	NE	NE	NE	NE	2	2	2	2	2	NE	2	2	2	2	2	2
	NE	NE	NE	NE	NE	1	1	1	1	0	NE	0	0	0	0	0	0
	NE	NE	NE	NE	NE	2	2	1	1	(2)	NE	(2)	(2)	0	0	(2)	0

* chicks inoculated
** chicks infected
*** chicks dead
NE = not examined
() = non-specific deaths

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: SEPARATION OF *BORRELIA ANSERINA* FROM BLOOD BY DEAE-CELLULOSE

SUMMARY

The possibility of using DEAE-cellulose for the separation of Borrelia anserina from avian blood was investigated. Excellent separation was achieved when infected blood was mixed 1:3 with phosphate-buffered saline-glucose 6:4 buffer (Lanham and Godfrey, 1970), and passed through columns containing DEAE-cellulose in a 5:1 ratio with whole blood. Spirochaetes were associated with the haemoglobin containing fraction of the eluate. The technique was found to be useful in separating B. anserina from avian blood cells and may have wider application for other blood inhabiting organisms, e.g. trypanosomes, of birds.

INTRODUCTION

Mammalian trypanosmomes (2,3) and Borrelia (1) have been successfully separated from blood cells and platelets by using DEAE-cellulose columns. The more negatively charged blood cells and platelets are absorbed onto the DEAE-cellulose leaving the less negatively charged organisms to be eluted. The technique apparently has not been applied to avian blood. In the course of other studies on avian spirochaetosis, it became necessary to obtain B. anserina free of blood elements. The use of DEAE-cellulose columns for the separation of B. anserina from avian blood is described.

MATERIALS AND METHODS

Columns were prepared in medium-size (Diameter, 10 cm) sintered Buchner funnels (Fig. 1) with DEAE-cellulose (DE-52, Whatman) that had previously been equilibrated with several changes of phosphate-buffered saline-glucose (PSG) 6:4 buffer (3). The sintered glass base of the Buchner funnel was covered with a sheet of filter paper (Whatman #41) and buffered DEAE-cellulose placed in the column to a depth of 4-6 cm. The DEAE-cellulose was allowed to settle and the supernate containing "fines" removed by suction. An additional piece of filter paper was placed on top of the DE-52 and two changes of PSG put through the column. Blood containing B. anserina was collected in heparin or from heparinized birds, diluted 1:3 with chilled PSG, thoroughly mixed and gently layered on top of the column. Initially the eluate was collected by gravity but in later separations, it was collected under slight negative pressure with a vacuum pump. The collection flask was kept in an ice bath. Samples of eluate were examined at frequent intervals for B. anserina by darkfield microsopy. On 2 occasions small segments of the column were taken at various levels after separation had been completed. They were shaken thoroughly in normal saline, allowed to settle and the supernate examined for spirochaetes.

RESULTS

In properly prepared columns using adequate amounts of DEAE-cellulose for the amount of blood, the separation of B. anserina from blood elements was complete. As the buffer diluted blood seeped through the column, 2 distinct zones became apparent. The leading zone was a light reddish-brown which has previously been reported to contain haemoglobin (1) while the upper zone represented the blood cells. Spirochaetes were associated with the haemoglobin containing portion of the eluate.

Several variations in quantity of blood compared to quantity of DEAE-cellulose were done during the separations. It was found that a 5:1 ratio of DEAE-cellulose to blood gave consistently good separations. Blood cells generally penetrated 50-60% of the column using this ratio and eluates were free of blood cells.

A problem encountered during some separations was a prolonged elution time (12 hours or longer) in which case contaminating bacteria began to multiply in the eluate. For this reason a slight negative pressure was applied through the column by using a side-arm flask to collect the eluate (Fig. 1). The time required for separation was greatly reduced but slight contamination of the spirochaetes with blood cells occurred occasionally.

Spirochaetes were not found to be retained in the column although it was noted that only individual, motile B. anserina appeared in the eluate. When clumped or non-motile organisms were present in the whole blood these apparently were retained in the column and not eluted.

DISCUSSION

The use of DEAE-cellulose columns is a useful technique for separating B. anserina from avian blood. Ginger and Katz (1), who successfully separated B. duttoni and B. persica from mammalian blood by this technique, indicated that previous work had shown

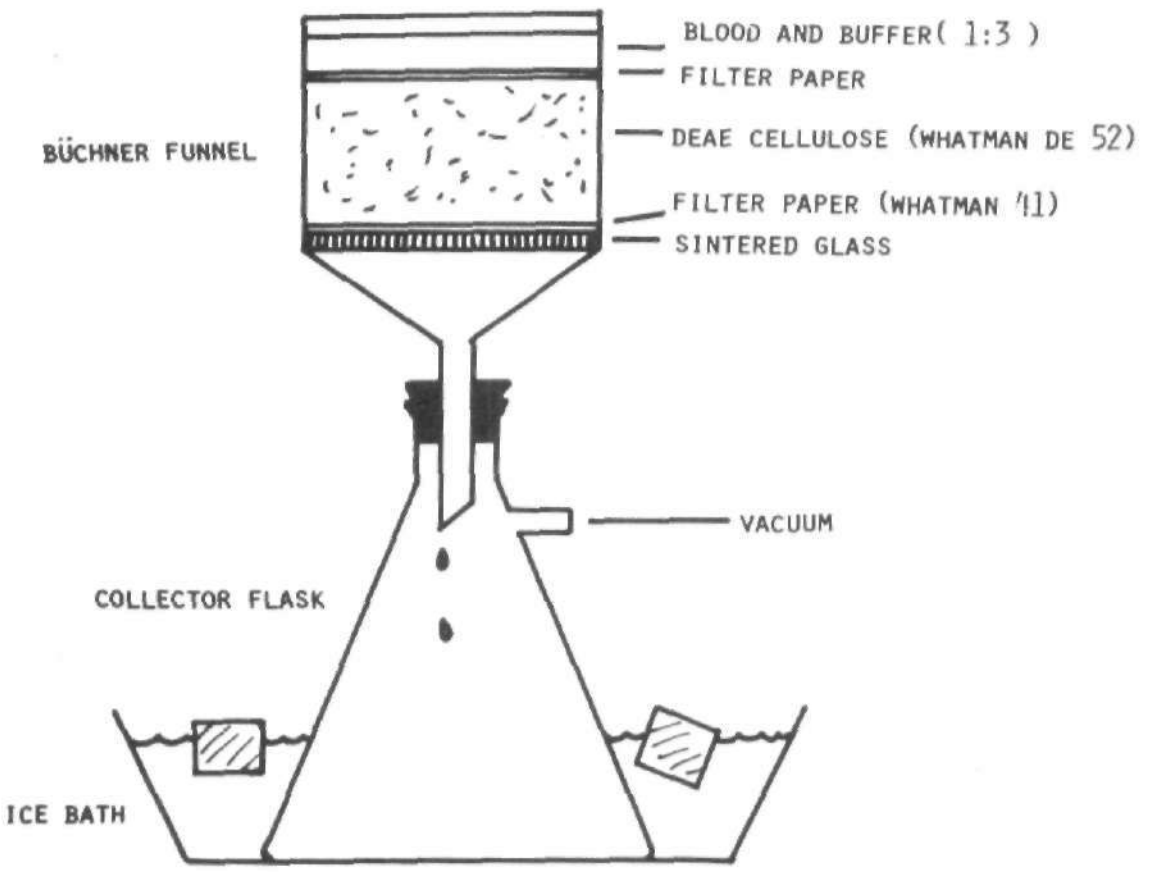
avian and mammalian Borrelia to have similar electronegative charges. However, the question of the electro-kinetic potential of Borrelia would not appear to be completely resolved (4). Results of this and the previous study indicate that Borrelia probably do possess a negative electrokinetic potential like that of most bacteria. The apparent inability of non-motile and clumped spirochaetes to pass through the column may indicate a change in electrokinetic potential of these organisms or may be merely physical retention.

The problem of prolonged elution times appeared to be related to technique since this did not occur with every separation. It has been pointed out that removal of the fine particulate matter in the supernate after the DEAE-cellulose has settled is important in maintaining high flow rates (1). Perhaps more diligent attention to this point might have adequately resolved the problem. The use of gentle suction by a vacuum pump or water tap suction device increased flow rates but also increased the likelihood of minor contamination with blood cells.

It has been reported previously (1) that spirochaetes are eluted with the haemoglobin containing portion of the eluate and the pink tinge characteristic of this fraction is a good indication of the point at which Borrelia are being eluted. Similar results were obtained in this study and provide additional support for this observation.

The findings of this study extend the application of this technique to include separation of B. anserina from avian blood. Based on this and previous studies, it would also seem probable that avian trypanosomes could be separated in similar manner.

DIAGRAMATIC REPRESENTATION OF APPARATUS USED FOR SEPARATING
BORRELIA ANSERINA FROM AVIAN BLOOD USING DEAE- CELLULOSE.



SCALE 5MM TO 10MM

FIG.1

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: A COMPLEMENT-FIXATION TEST TO DETECT *BORRELIA ANSERINA* ANTIBODIES

SUMMARY

A modified direct complement fixation (MDCF) test was utilized for the detection of Borrelia anserina antibodies in fowl serum. Antigen was prepared from the supernate of sonicated B. anserina. Anticomplementary activity did occur with the occasional serum sample particularly in low dilutions but this was not considered to be a significant problem. The test was found to be simple and specific in these preliminary studies. The application of the MDCF test to detect B. anserina antibodies further demonstrates the value of this test for avian serology.

INTRODUCTION

The complement-fixation (CF) test has found wide application in immunology. The advantages, disadvantages and modifications of the CF test for use in avian systems have been discussed (5). Use of CF test to detect Borrelia anserina antibody has been reported (9) but details of the test were not given. Earlier literature on the use of CF tests for avian spirochaetosis has been reviewed (8) as has the use of this procedure for relapsing fever in man (4). The purpose of this paper is to describe a modified direct CF (MDCF) test that has been developed to detect antibodies against B. anserina.

MATERIALS AND METHODS

Preparation of Antigen. Adult white leghorn (WLH) or Rhode Island red fowls were infected with B. anserina strain H (3). Spirochaetemia was monitored daily by examination of wet smears (1), until organisms were plentiful but intravascular clumping of the organisms had not begun to occur. Generally this was on the 3rd or 4th day following inoculation. At this point fowls were heparinized (1000 I.U./kg) and exsanguinated. Each lot of antigen was from the blood of one fowl except on a single occasion when blood from 2 fowls was pooled. Spirochaetes were separated from the blood by using DEAE-cellulose (2) or differential centrifugation following the procedure of Ginger.* Separated spirochaetes were centrifuged, triple washed in phasphate buffered saline (PBS) pH 7.2, resuspended in a volume of PBS approximately 10% that of the whole blood, and frozen (-10°C). They were alternately frozen and thawed 6 times and then sonicated while in an ice bath using an MSE sonicator set on high power 2 emitting 12 micron peak to peak waves for four 15 sec bursts at 1 minute intervals. Following this procedure no intact spirochaetes were found in wet or stained smears. The particulate matter was

*Personal communication (1974). C. D. Ginger, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, U.K.

sedimented in the high speed head of a MSE'Mistral 2L' centrifuge by centrifugation at 30,000 RPM for 20 minutes at 10°C. The clear supernate was aspirated, frozen (-10°C) and used as the antigen in the MDCF test. Protein concentration of the antigen was determined with a Unicam SP600 spectrophotmeter set at 280 mu by previously quantitated methods (Immunology Laboratory, Department of Medicine, Ahmadu Bello University) and found to be 40 mg/100 ml. A 1:4 dilution of antigen was found by titration to be optimum.

Hyperimmune serum. Eight week old WLH were experimentally infected with B. anserina strain H. All developed spirochaetemia and were treated with Terramycin (R) in the drinking water at recommended levels on the 3rd day of spirochaetemia. Three subsequent intramuscular injections of 0.2 ml virulent blood were given at 9, 10 and 11 weeks of age to each bird. No evidence of infection developed following the subsequent injections. Blood was collected 10 days after the last injection. Serum was separated, pooled and frozen (-10°C). At 26 weeks of age each bird was inoculated intramuscularly with 1 ml allantoic fluid containing an estimated 10^9 spirochaetes/ml. The flock experienced an acute outbreak of spirochaetosis in which 2 birds died. Two weeks later, blood was collected, and the serum prepared and stored as described above.

Normal Chicken Serum. Normal chickeu serum (NCS) for addition to the test was obtained from 3 month-old WLH from a B. anserina and tick free flock. Serum was separated from the clot, clarified by low-speed centrifugation and stored at -20°C. Optimal dilution of NCS was determined to be 1:200 in a series of checkerboard antigen-antiserum titrations (5).

Test Procedures. A micro-test was used according to previously reported methods (10). Interpretation, technique and controls followed this procedure.

Source of serum samples. Serum samples collected from fowls during experiments on avian spirochaetosis were used. In each case the complete history of each fowl relative to spirochaete exposure was known. Serum samples were inactivated at 56°C for 30 minutes before testing.

RESULTS

A total of 52 sera were tested from 32 fowls (Table 1). Seventeen sera collected prior to infection with B. anserina showed no specific complementary activity but six were anticomplementary at serum dilutions up to 1:4. Serum from four of 7 infected chickens taken 4 days after inoculation reacted at dilutions of 1:8-1:32. All sera from fowls that had been exposed to B. anserina more than 7 days previously reacted at dilutions of 1:64 to over 1:4096.

DISCUSSION

The MDCF test was found to be a simple test for detecting antibodies against B. anserina even in low concentrations. Although a much larger number of sera must be tested before the true specificity of the test can be determined, the results in this study indicate a high degree of accuracy in determining prior exposure to B. anserina. Anticomplementary activity occasionally occurred particularly in lower serum dilutions. The activity tended to occur in sera from specific birds. The degree of anticomplementary activity was considered minor and of little consequence compared with the overall performance of the test.

The CF test has not been widely used for avian serology because unheated sera are frequently anticomplementary while heated sera often will not fix complement (5). To overcome this problem, the standard CF test has been modified by adding normal chicken serum that has previously been determined not to be anticomplementary. Such a test has been termed a modified direct CF test (5). Modified direct CF tests have been used to detect antibodies against a number of avian diseases including mycoplasmosis (6), Marek's disease (7) and Newcastle disease (10). The procedure described in this report extends the applicability of this test to include avian spirochaetosis.

TABLE 1

Results of fowl serum samples examined for Borrelia anserina antibodies by a modified direct complement-fixation test.

Group	No. Birds	Spirochaete Exposure		Bird Number/Titre			
			1	2	3	4	5
A	15	Immunized at 8 wks. Booster at 9,10,11 and 26 weeks. Samples taken at 50 weeks.	0/4096*	0/256	0/512	0/4096	0/64
B	6	Adult fowls. Experimentally infected and recovered.	Pre-Inf.		0/0	4/4	0/0
			1-2 weeks Post-Inf.		0/256*	0/256*	0/256**
C	4	Chicks infected and recovered	Pre-Inf. 1 week		0/0	0/0	0/0
			Post-Inf.		16/256*	0/256*	8/256*
D	7	Infected chicks All died	Day - 2		2/2	0/0	2/2
			Day + 2		0/0	4/4	0/0
			Day + 4		0/0	0/0	0/16

*highest dilution tested
Numerator = anticomplementary activity/Denominator = specific complementary activity

TABLE 1 (Continued)

Group	6	7	8	9	10	11	12	13	14	15
A	0/4096*	0/1024	0/4096*	0/512	0/512	0/1024	0/2048	0/1024	0/1024	0/128
B	0/0 0/256*	0/0 4/256*	0/0 0/256*							
C	0/0 8/128									
D	0/0 0/0 2/8	2/2 4/16 4/32	2/2 0/0 0/0	4/4 8/4 4/8						

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: OBSERVATIONS ON FOWLS, TURKEYS AND DUCKS NATURALLY INFECTED WITH *BORRELIA ANSERINA*

SUMMARY

Observations were made on 191 fowls, 7 turkeys and 5 ducks from 84 outbreaks of avian spirochaetosis between January, 1971, and March, 1975, in northern Nigeria. The morphology of the spirochaete resembled previous descriptions, although thicker and thinner forms were recognized. Fowls that had recovered and been kept free of ticks were re-infected by another isolate 1-6 months later, suggesting multiple serotypes existed in northern Nigeria.

Natural infections were recognized in fowls, ducks and turkeys. A fowl isolate of B. anserina infected turkeys and ducks while a turkey isolate infected fowls. Ducks were not exposed to the turkey isolate. No isolations were attempted from ducks. Pigeons and guinea fowl were susceptible but only a few of the latter had spirochaetemias and they apparently have considerable resistance. Attempts to infect mice were unsuccssful.

The disease is more common in the northern parts of the country. It occurred most frequently in village and backyard poultry flocks. Mixing fowls was the most common way in which new outbreaks occurred. Mortality approached 100% in exotic breeds but appeared to be lower in indigenous breeds.

Clinical signs included anorexia, thirst, diarrhoea, dullness, paresis and pale or cyanotic combs. Signs in turkeys and ducks were similar but less severe. Paresis was common in ducks. Marked cyanosis of the head was typical for severely affected turkeys and was considered to indicate a poor prognosis.

The outstanding postmortem finding was a mottled, enlarged spleen. The mottling represented large proliferating nests of reticuloendothelial cells. Other changes could be found in the liver, which occasionally was speckled with foci of necrosis, plus the kidneys, muscles, ovary and heart. Catarrhal enteritis and bile staining, but not haemorrhage, were seen in the gastrointestinal tract. Avian spirochaetosis may resemble other septicaemias, leukosis complex and Newcastle disease in northern Nigeria.

Most outbreaks were associated with fowl ticks but other arthropods, ornithophilic mosquitoes or possibly dog ticks (Rhipicephalus Sp.) may have been vectors. Direct transmission via cannibalism was also suspected.

Water soluble oxytetracycline was effective if given for 3 days. Mixing 5% glucose with tetracycline and giving it into the crop gave spectacular results in severely affected fowls. Tick control was more important than treatment. Avian spirochaetosis is common in northern Nigeria causing economic loss.

INTRODUCTION

Avian spirochaetosis is an acute, subacute or chronic disease of birds caused by the spirochaete, Borrelia anserina. Natural infections have been reported from geese (31), fowls (19), turkeys (21), ducks (27), and canaries (20). A wide variety of other birds have been shown to be susceptible to experimental infection, however, guinea fowl and pigeons have considerable resistance (24). Mammals, reptiles, amphibians and fish have been shown to be refractory to infection except for transitory infections in white mice and rabbits which have been reported (14).

Avian spirochaetosis was first described by Sakharoff (31) in 1891 from affected geese in the Caucasus. In 1903, Marchoux and Salimbeni (19), first reported the disease in fowls from Rio de Janeiro, Brazil. The outbreak was acute and resulted in high mortality. Clinical signs were severe. They incriminated Argas miniatus ("persicus"), the fowl tick, as the vector and were successful in transmitting the disease from infected to susceptible birds by the tick. Ticks were found to remain infected for at least 5 months after feeding.

Subsequently, a host of investigators have studied various aspects of avian spirochaetosis. The disease has been found to be widespread occurring in most tropical, sub-tropical and warmer temperate areas of the world. Arsenicals and later antibiotics were found to be effective for treatment. Several modes of transmission and other arthropod vectors were discovered but the fowl tick remained the most important vector and reservoir of B. anserina. Considerable work was done on the epidemiology and pathogenesis of the disease and taxonomy of the causative agent. Often reports have been conflicting and a great deal remains to be clarified or determined concerning various aspects of the disease, biology of the tick vector, and causative agent. Several reviews on avian spirochaetosis have been published (9, 10, 11, 14, 21, 33).

In Nigeria, avian spirochaetosis was first reported in 1914 by MacFie and Johnston (17) from fowls and ducks near Lagos. Although it has subsequently been established that the disease is of considerable importance, particularly in the northern parts of Nigeria, no information on avian spirochaetosis in Nigeria exists outside of the initial description, a section on avian spirochaetosis in a general report on common diseases of poultry in Nigeria (38) and brief mention of the disease in several official reports. The available literature on avian spirochaetosis in Nigeria has been reviewed (2). In view of the scant information available on avian spirochaetosis in northern Nigeria it seemed worthwhile to present the field and clinical observations that have been made on the disease in this area during the past 4 years. The results of experimental studies on avian spirochaetosis in northern Nigeria will be presented elsewhere.

MATERIALS AND METHODS

Between January, 1971, and March, 1975, avian spirochaetosis was diagnosed in 191 fowls, 7 turkeys and 5 ducks submitted to the Avian Diseases Laboratory (ADL), Faculty of Veterinary Medicine, Ahmadu Bello University. The birds came from 84 outbreaks. Annual and monthly distribution of these outbreaks has been reported (6). Methods used for the diagnosis of avian spirochaetosis have been described previously (3). The known geographical distribution of avian spirochaetosis in Nigeria was determined from previously documented outbreaks (2) supplemented with observations made during the period of this study.

Observations On The Spirochaete. Previously, avian spirochaetes from various hosts and parts of the world had been considered separate species (for review see 14, 33). A number of serological, host susceptibility and cross-protection studies have been done with conflicting results. However, most studies tended to show only minor or no differences between the various named species and they

have all become synonyms of Borrelia anserina. This may be as erroneous as the early multi-species concept. In other parts of the world, different serotypes of B. anserina have been identified (8, 13, 22). There was some suggestion that more than one serotype of B. anserina may exist in northern Nigeria. On 3 occasions fowls that had recovered from infection with one isolate 1-6 months earlier were either accidentally or purposely exposed to another B. anserina isolate and the fowls became infected (Table 1). No attempt was made to compare strains and the homotypic immune status of the fowls at the second exposure was unknown so it remains possible that these findings could represent a loss of homotypic immunity rather than differences in antigenic structure of the spirochaete. Since the occurence of different serotypes of B. anserina has been reported, investigation of the possible occurrence of different serotypes in Nigeria is indicated. If a vaccination programme is to be implemented as an aid to controlling avian spirochaetosis in Nigeria as has been suggested (6), knowledge of different serotypes present in Nigeria would be a necessary prerequisite.

Detailed studies on the morphology of the spirochaetes were not done. They did not appear to differ noticeably from the numerous descriptions that have been published (Fig. 1), however, 2 types of B. anserina could be distinguished by darkfield microscopy. One type was shorter and thicker while the other type was longer and more slender. The former type was encountered most commonly and was the type used in experimental studies. The latter type was uncommonly found. As critical measurements were not done it is unknown whether this observation is actually valid, or, if so, whether the 2 types represent different strains or different stages in the life cycle. Thick and thin B. anserina have been described from India (35) and long and slender spirochaetes ("tenue" spirochaetes) have been described from ticks (14).

Species Susceptibility. Natural infections with B. anserina

were only found in fowls, turkeys and ducks. It would appear that avian spirochaetosis occurs most commonly in fowls but this may also be a reflection of populations since turkeys and ducks are less common in this area.

On two occasions subinoculations of a turkey origin isolate of B. anserina infected susceptible chickens and turkeys. Organisms isolated from fowls were found infective for turkeys and ducks. Spirochaetes were not isolated from ducks and therefore duck origin isolates were not tested in other avian species. However, one outbreak of avian spirochaetosis in 6 week old broilers occurred 4 weeks after 7 adult ducks obtained from a local village had been placed in an adjacent pen. Shortly after being introduced, 3 of the ducks died. One was examined at postmortem and lesions consistent with spirochaetosis were found but tissue smears were negative. Microbiologic examination of the duck was also negative. It seems likely that the duck died of spirochaetosis after spirochaetes had disappeared from the blood and the ducks were the source of infection for the broilers. In another outbreak 5 adult ducks were being kept with a flock of adult white leghorn (WLH) fowls in which avian spirochaetosis was occurring. The ducks remained perfectly healthy and blood smears examined on two occasions were negative for spirochaetes. As the house was heavily infested with ticks, the possibility that the ticks would not feed on the ducks was considered as a possible reason for their protection from infection. However, when the ducks were examined at night, numerous fowl ticks were found feeding on their legs. It seems likely that the ducks had recovered earlier and were then immune. This conclusion was supported by a later outbreak of spirochaetosis involving a mixed flock of fowls, ducks and guinea fowls. The ducks and fowls both became infected, however, no infections occurred in the guinea fowl.

Natural infections in guinea fowl or pigeons were not found. When 8 pigeons were inoculated with fowl blood containing B. anserina,

all became infected and 4 died. The inoculation of 6 guinea fowl with B. anserina of fowl origin resulted in a transient infection in 2 with no mortality. Although the previous history of these birds was unknown since they had been purchased in a local market, the observation that the guinea fowl were probably resistant is consistent with previous findings (14,24). Ticks have not been found on the few guinea fowl examined. In another study, Aegyptianella sp., also a fowl tick transmitted disease, has been frequently seen in blood smears from guinea fowl. It would appear then that guinea fowl may possess an innate resistance to Borrelia which is not related to tick activity.

In the literature, transient infections in mice (7) and young rabbits (14,15,24) have occasionally been reported. An attempt was made to infect 4 infant (3 day old) and 5 young (4 week old) mice with B. anserina. The infant mice were inoculated with 0.01 ml whole blood intracerebrally while the young mice received 0.1 ml intraperitoneally. Fifteen one day old chicks were also inoculated subcutaneously with 0.05 ml blood at the same time. No evidence of infection was found in daily blood smears from the mice while all of the chicks became infected and died.

Geographical Distribution. In addition to the review (2) of previous reports of avian spirochaetosis in Nigeria and the finding of the disease to be common in an area of northwestern Nigeria (6) a few additional observations have been made. In July, 1972, blood smears were taken from 23 indigenous fowls slaughtered at Kabba (Kwara State). Spirochaetes were not found. A collection of dead larval ticks from near Gumel that had been refrigerated were emulsified, examined by wet smears and inoculated into a susceptible fowl. Spirochaetes were found in the wet smears but they were apparently no longer viable because infection did not occur in the inoculated fowl. Two confirmed cases were reported from Yola* which is only the second location in NorthEast State where avian spirochaetosis is known to occur. Spirochaetes were not found or isolated from 30-40 ticks collected at Bambur, also in NorthEast State. The

*Personal communication (1974). Dr. J. B. Adeoye, Vet. Div., Yola, NorthEast State.

actual distribution of avian spirochaetosis and the fowl tick in Nigeria, particularly in the northeastern and southern areas is unknown and studies on this are required. The present known distribution of the disease in Nigeria is presented in figure 2.

Clinical Findings. Outbreaks of spirochaetosis generally did not occur in large, well-managed commercial flocks. They occurred most commonly in small backyard flocks or village birds that were allowed to roam about freely. Owner complaints were most often: a) chickens were dying or dead, b) birds were cold, c) birds were sleepy, d) birds had diarrhoea, e) birds did not eat, f) birds just sat and would not move, g) bird had "fleas" (larval ticks) which were making it sick. From the history one of 4 predisposing factors could usually be determined: recent addition of market fowls to the flock or, as in three outbreaks fowls from other flocks infested with ticks; a history of prior tick infestation, spirochaetosis or disease consistent with spirochaetosis characterized by high mortality; fowls have been allowed to roam free (or escaped) and mingled with other birds or new birds became sick after having been added to a flock. Acquisition of used poultry houses or equipment or the use of a poultry house that had been left on the property was the source of infection is at least 4 outbreaks. In one of these outbreaks, the house had been vacant for 1½ years.

The disease was more severe in exotic breeds than indigenous breeds. Of the exotic breeds layers and broilers appeared to be more susceptible and experienced higher mortality than dual purpose birds. Mortality was difficult to assess becasue treatment and control procedures were always instituted as soon as the disease was diagnosed. In two instances where the disease had probably run its course before birds were submitted 19 of 20 and 20 of 24 birds had died. Both of these flocks were WLH. In a flock of Rhode Island reds (RIR) where control procedures were instituted but probably not carried out diligently only 37 of 60 fowls remained 6 weeks after the outbreak began. The mortality in untreated

flocks of exotic fowls is undoubtedly high. Unfortunately little is known about the mortality avian spirochaetosis causes in indigenous fowls other than that they often die.

Avian spirochaetosis was a problem in older fowls. In only 2 outbreaks were the birds under 10 weeks of age. Some possible reasons for this might have been: younger birds are innately resistant to infection or mortality, fowl ticks do not feed on young birds, young birds may have passive immunity from the hen, owners place more value on older fowls and were more likely to submit them for diagnosis, young birds were generally raised in separate environments from older birds, or young birds were not as likely to move about and mix with birds of other flocks. Another factor which may have been important was that only older fowls can be readily purchased and these would only be added to flocks of older birds. Adding birds to a flock was the most common way that the disease was introduced into a new flock. Further studies are needed to determine the causes of this age distribution.

The disease rarely occurred as an explosive outbreak. Typical outbreaks were characterized by 1 or more new cases occurring daily for 2-4 weeks with infected birds being continuously present in the flock. In some outbreaks only a few birds were affected at any one time and mortality spread over a period of up to 3 months. This would seem to indicate that only a small percentage of ticks were actually infected or feeding on the fowls in the latter type of outbreak.

In flocks where the birds became infected with spirochaetes and recovered, problems with avian spirochaetosis became negligible unless new birds were introduced. In two outbreaks where the flock had stabilized following outbreaks of avian spirochaetosis subsequent mortality again began to occur. In each outbreak the cause of mortality was due to excessive blood loss from the enormous numbers of ticks that had multiplied in the environment; not spirochaetosis.

Clinical signs in affected fowls presented for diagnosis varied from anorexia to a comatose state depending upon the stage of

infection. Typical clinical signs included anorexia, although the crop was often distended with a foul smelling, grayish fluid that was regurgitated when the crop was roughly palpated or bird held upside down. This was believed to have resulted from excessive thirst. Another indication of increased water consumption was wet feathers along the ventral part of the neck seen in some birds. Diarrhoea was a common finding. Faeces were green to yellow-white and mucoid or watery in consistency. Bright yellow, green or white faeces could be found dried on the feathers below the vent. The cypical attitude of affected birds was characterized by a general lack of awareness or disregard for external stimuli. Affected birds tended to huddle up with neck withdrawn or head drooped, feathers ruffled, and yeys closed; the impression being that the bird was cold and sleepy (Fig. 3). Occasionally, they would lie flat appearing weak or paralyzed and stretch out the neck on the ground. Close examination revealed paresis but true paralysis was not found in this study except for fowl tick paralysis which was seen twice. In neither instance was the paralysis associated with spirochaetosis. Comb and wattles were generally shrunken, dry, scaly and varied in colour from normal to pale or cyanotic. Infected fowls were often associated with larval tick infestation or ticks could be found in the poultry house. Abnormal respiration was not a feature. Infected birds often felt hot especially when palpated in the inguinal areas. Body temperatures were elevated (over 108^{o}F) when spirochaetes appeared in the bloodstream but fell terminally when intravascular clumping of the spirochaetes began or after they disappeared from the circulation. Moderate to marked weight loss characterized subacute and chronic forms of avian spirochaetosis.

Spirochaetes could always be demonstrated in wet blood smears (3) when affected fowls were showing marked clinical signs. A few birds were observed that had been ill for long periods and were characterized by weakness and weight loss. Borrelia anserina could not be demon- at this stage. Apparently these fowls had overcome the spirochaete

infection but were suffering from residual effects of the disease. This period has been termed the after-phase (14) of avian spirochaetosis.

Clinical signs of infection in turkeys and ducks were similar but not as severe as those of fowls. Three of the 4 ducks examined clinically in this series had been presented because of paresis. Affected ducks sat in one place and did not move. Even when supported they made only feeble attempts to walk. Two of these were sacrificed for another study and the third recovered after treatment although it had lost a good deal of weight and was still weak for nearly a month after recovery. Cyanosis of the head was a feature common to severely affected turkeys and was considered to be a sign indicative of a poor prognosis since all turkeys found with this died in spite of treatment. Most turkeys were less severely affected. In common with fowls, diarrhoea and anorexia were prominent features of infection in turkeys and ducks. Larval ticks were found on infected turkeys but not on infected ducks although one duck was known to have come from a tick infested mixed flock of fowls, turkeys and guinea fowls.

Haematological Changes. The anaemia that has been described for avian spirochaetosis (34) was evident from blood smears of fowls in the later stages of acute infection. Immature erythrocytes were numerous. In addition granulocytic leukocytes which had basophilic granules, rods or ring inclusions in the cytoplasm appeared. They resembled those described from fowls in southern Nigeria that had been tentatively identified as "chlamydozoa" (Chlamydia) (16). The precise nature of these cells was not determined but they were believed to represent immature heterophils indicative of a "left shift" in the leukocyte distribution.

In wet smears, it was observed that blood cells probably platelets, were often intimately associated with the tangled masses of spirochaetes occuring at crisis (Fig. 4). From the size of these agglomerations, thrombosis of vessels would undoubtedly occur and may be part of the

mechanism causing death. It has been reported that the focal areas of necrosis in the liver are only found in those birds in which the aggregated masses of spirochaetes occur and that they are probably the result of vascular thrombosis (30).

The profound haematological changes that have been reported to occur in spirochaetosis (34) brought up the question as to whether a simple procedure such as measuring buffy coat or packed cell volumes could be used for screening large numbers of potentially infected birds. If infected birds had consistently different values then only birds with values in that range would need to be examined and the rest could be discarded. Blood samples from 175 market fowls were examined with this in mind. Only minor differences were found between the buffy coat and packed cell volumes of B. anserina infected and non-infected fowls (Table 2). It seems doubtful if measuring buffy coat or packed cell colums could be used as a reliable screening method for spirochaete infection even if larger numbers of birds had been examined.

Postmortem Findings. Changes observed during postmortem examination of fowls that had died of spirochaetosis resembled those of any septicaemia and were not diagnostic. The most consistent finding was splenomegaly. Typically the spleen was enlarged 3 or 4 times normal size, varied from dark purple to pale brown or gray in colour and had small pale foci often arranged in a rosette-like pattern scattered over the surface (Figs. 5, 6) giving the spleen an appearance of being speckled or mottled. Occasionally deep red blotches representing heamorrhage were also seen. The basic shape of the speleen was unaltered and the surface remained smooth without elevations or projections.

The liver was typically enlarged, yellowish-brown and friable, with a barely discernable reticulated pattern over the surface. This pattern was most obvious along the posteriorventral margins of the liver (Figs. 6, 7). In about 5% of the birds examined, scattered pale foci studded the surface of the liver (Fig. 7) and

could also be seen throughout the parenchyma on cut surface. The gall bladder was usually enlarged containing thick, viscous bile.

Kidneys were generally swollen and pale. Instead of the normal rather flat surface of the kidneys, those of infected fowls were rounded, raised and had a pebbly-textured surface. The latter represented swollen lobules which were thought to be the result of tubular swelling. Often collecting tubules and ureters were prominent as a result of their being impacted with urates.

The blood was thin and watery. Clots distinctly separated into clear and dark red portions. Fibrin in the pericardial sac or adhesions between the pericardium and epicardium were found in a few birds. Lesions from tick bites could often be found between and underneath the leg scales, particularly in heavy infestations.

Gastro-intestinal tract changes were not pronounced. The crop was generally filled with fluid. The mucosa of the proventriculus, ventriculus and upper intestinal tract was heavily bile-stained or in some cases the latter was hyperaemic. The small intestine was often empty except for bile and mucus. Bile-stained ingesta mixed with increasing quantities of urates was found in the distal small intestine and large intestine, particularly in the cloaca. Gastro-intestinal hemorrhage was not a finding in this study although a few petechial or ecchymotic haemorrhages in the caecal tonsils occurred uncommonly.

Other changes included large, irregular pale areas in the pectoral and leg muscles, particularly of heavily muscled birds (Fig. 8). Muscle atrophy or emaciation was not a finding in birds that died during the acute stage but was found during the chronic stage. Often muscles were congested and dark. In layers, abnormally shaped, discoloured ovarian follicles and follicular haemorrhage were observed.

Posmortem findings in turkeys and ducks were essentially the same but a few differences were noted. In 3 turkeys that died all had extensive, linear, swollen, pale yellow areas in myocardium with visceral congestion and pulmonary oedema. This finding was

believed to represent myocardial necrosis and subsequent cardiac failure. Mottling of the spleen was not as pronounced in turkeys as in fowls. Both ducks examined at postmortem had massive white foci (up to 6 mm) in the spleen. Unfortunately naturally infected turkeys and ducks were so uncommon that it was impossible to draw any definite conclusions about the gross pathology outside of these limited observations.

Spirochaetes were demonstrated in the fluid from the heart clot in all but 3 of the birds in this series. These 3 were known to have come from flocks where spirochaetosis was occurring and had typical postmortem findings. It was the general impression that birds dead of spirochaetosis were almost invariably spirochaetemic at the time of death. Whether this assumption was completely true or not remains to be determined. Birds were occasionally examined that had lesions suggesting septicaemia but were negative when examined for spirochaetes and cultured for bacteria, and a diagnosis of spirochaetosis could not be made from other birds in the flock. It is possible that they may have recovered from spirochetemia but died afterwards. The difficulty of establishing a diagnosis of spirochaetosis in these birds where the spirochaetes have disappeared is obvious.

The gross pathology of infected birds in this series differs somewhat from previous descriptions of infected fowls in Nigeria. MacFie and Johnston (17) reported the most conspicuous feature in their study to be the condition of the liver which was reported to be greatly enlarged with necrotic patches sometimes of considerable size. They also reported a large blood clot in the stomach of one fowl. In this series of fowls, only mild liver changes were consistently seen and small, pale, foci in the liver found uncommonly. Gastro-intestinal haemorrhage was not found. In a later report (38), emaciation, rapid putrefaction, blood which failed to clot and marked inflammed intestines with blood stained contents were

described. These findings do not correlate with the findings in this series. The reasons for these different observations is not clear.

Histopathology Findings. Detailed studies on the microscopic pathology of naturally infected fowls were not done but a few observations are presented. The pale foci seen grossly in the spleen were found microscopically to be large nests of reticuloendothelial (RE) cells that apparently had proliferated from the mass of reticulum cells surrounding the sheathed arteries (the Schweigger-Seidel or ellipsoid sheath). In some instances the cytoplasm of these cells was vacuolated. Hyaline necrosis and an apparent depletion of lymphocytes were also infrequently observed.

In the liver, hepatocytes were granular and swollen causing a general disruption of the normal architecture. In some cases there were dense periportal infiltrations of heterophils while in other birds the infiltration was composed of round cells. In one liver, focal areas of necrosis were found which had been surrounded and infiltrated by RE cells.

In other tissues, an acute nephrosis of the proximal tubules characterized renal changes. Increased granularity and degenerative changes of the myocardial fibers, and slight cellular infiltrations of the epicardium were found in the heart. Lungs were often congested and sometimes oedematous. There was an increase in goblet cells and mucus in the jejunum indicative of catarrhal enteritis. No changes were observed in the brain.

Few detailed studies on the histopathology of avian spirochaetosis have been done. McNeil et al. (21) gave a detailed account of the tissue changes occurring in turkeys in the southwestern U.S. Studies have also been done on the histopathology of infected fowls in India (18, 28, 36), Egypt (1) and Sudan (32). Detailed studies on tissue changes that occur in poultry infected with Nigerian isolates of B. anserina are needed.

Differential Diagnosis. Avian spirochaetosis should be suspected whenever an obviously ill fowl, turkey or duck is presented for diagnosis in northern Nigeria which is not showing respiratory or marked nervous signs. The disease occurs most commonly in adults, during March - August (6) and is often associated with the presence of larval ticks (5). Clinical signs and postmortem findings are not diagnostic but an enlarged, mottled spleen indicates the likelihood of avian spirochaetosis. It has been reported (2) that spirochaetosis has been misdiagnosed as Newcastle disease. Newcastle disease in northern Nigeria is characterized clinically by respiratory signs, mild conjunctivitis appearing as a slight cloudiness of one or both eyes, nervous signs, and haemorrhage or ulceration of the cloaca and vent (Barnes, unpublished data). At postmortem haemorrhages in the proventriculus, intestine and caecal tonsils are characteristic. A slight enlargement of the spleen occurs early in Newcastle disease (12) but the change is considerably less than in spirochaetosis and it does not present the characteristic mottled appearance. Fowl typhoid, avian leukosis and colisepticaemia can at times resemble spirochaetosis. The differential characteristics of these disease compared with avian spirochaetosis are presented (Table 3).

Vectors and Transmission. In virtually all outbreaks of avian spirochaetosis investigated, fowl ticks were found either on the infected birds or in the poultry house. In one outbreak where fowl ticks could not be found, cannibalism had been a chronic problem. In another outbreak that occurred during April, experimentally infected chickens were placed in one pen while normal, susceptible fowls were located in another pen about 6 feet away from the first pen. The pens were separated by two other pens and 4 feet high concrete walls. No contact between birds in the two pens occurred. Two fowls in the normal flock became infected. A thorough search of the birds and pens failed to reveal any fowls ticks, mites, or lice, but numerous dog ticks (Rhipicephalus Sp) were found. It was subsequently determined

that dogs had been kept in the pens about 6 months earlier. Both pens were also open to flying insects and mosquitoes were observed to feed on the birds at night. Attempts to procure some of these for identification failed. The exact means of transmission was never established but it was through some means other than fowls ticks or cohabitation--probably by ornithophilic mosquitoes or perhaps the starved dog ticks. Mosquitoes have been reported to be potential vectors of B. anserina (29, 39) where hard ticks have not, although the latter are known vectors for B. theileri of ruminants (9).

When a tick infested fowl was introduced into a susceptible flock mortality began 2-4 weeks later. However, when susceptible birds were introduced into a tick infested environment infected birds were found 5-7 days later.

Fowl ticks transmit B. anserina and Aegyptianella pullorum, can cause tick paralysis and if in sufficient numbers, exsanguination of fowls. They are known to survive for several years without feeding (37) under a variety of climatic conditions. Their importance in northern Nigeria can probably best be illusted by a few cases.

Case 1. A flock of 300 adult WLH became infested with ticks from an unknown source. Spirchaetosis broke out and 90% died. Egg production in the survivors was reduced approximately 30% of their pre-infection levels.

Case 2. Seven improved breed pullets were placed into a poultry house where all birds had died 6 months earlier of an undiagnosed illness. Within 2 weeks all birds had contracted spirochaetosis and 3 died in spite of therapy.

Case 3. A mature RIR hen was placed in a pen with birds that had survived an undiagnosed illness 3 weeks earlier. She died acutely 9 days later. Ticks and spirochaetes were found.

Case 4. Two indigenous fowls were purchased in a local market for slaughter. They were placed overnight with a flock of RIR hens and removed the following morning. Three weeks later, one hen died. A week later, 2 more died. Avian spriochaetosis was diagnosed and ticks were found. During the next 4 months all

birds died or were slaughtered.

Case 5. Forty-one day old chicks had been obtained three years earlier and the flock maintained by natural brooding without any significant health problems. Additional birds were obtained from a friend in May and added to the flock. In June spirochaetosis broke out in the flock. In spite of treatment deaths continued even when spirochaetes were no longer found in the dead birds. Emaciation and severe anemia were the only findings at post-mortem examination. When the house was examined in July, thousands of ticks were collected.

Case 6. A poultry house was obtained that had been vacant for 1½ years. When 6 previously healthy WLH hens were placed in it, they became ill 1-2 weeks later and 4 died of spirochaetosis. Ticks were found in the house.

The fowl tick is not only the most important vector of avian spirochaetosis, it is also the reservoir of B. anserina. Birds do not remain carriers (4) and usually die of the infection. Outside the host the spirochaete dies rapidly. If it were not for the tick and trans-ovarian transmission from adult to larval ticks, B. anserina would soon die out with its avian hosts.

Treatment and Control. A wide variety of antibiotics have been reported useful in treating birds infected with B. anserina (25, 26). Terramycin (TM) and penicillin were used successfully in treating avian spirochaetosis in northern Nigeria. A soluable TM powder was readily available and easily administered. Smears taken from infected fowls after TM medication had been started showed spirochaetes to still be present on the second but not the third day. For this reason treatment for 3 days was considered a minimum course. In one outbreak where treatment with TM was given for only one day and discontinued because of the marked improvement in the flock on the next day, a severe relapse occurred on the 3rd day and all affected birds died within 8 hours. Blood smears showed numerous massive aggregations of spirochaetes in the circulation. In severely affected fowls, 5 ml

of TM prepared at 4 times the recommended concentration was mixed with 5 ml of 5% glucose and given orally by tube directly into the crop. Sometimes this treatment had spectacular results; virtually comatose fowls would be walking around and eating the next day. Results with injectable TM were discouraging and it was not used extensively.

In a recent outbreak birds continued to die of spirochaetosis although they reportedly were being treated with soluable TM as recommended. This suggested possible resistance to TM. The organism was isolated and inoculated into 16 twelve week old WLH. When the first bird in this group died from spirochaetosis, they were started on soluble TM. Four more deaths occurred during the night and next day. Spirochaetes were found in the blood of all 11 remaining birds but there were no more deaths in this group. Five were positive on the next day and by the third day all were negative without further deaths. It would seem doubtful that the organism causing this outbreak was TM resistant. It was more likely that the owner was negligent in treating the fowls.

A problem with treatment in some flocks is that all the birds are usually not infected at one time. Infected birds may continue to occur over a period of 2 or 3 months requiring separate treatment each time. Although it was not tried and there are risks involved, it would seem better to infect all the fowls at once from an infected bird, allow the disease to develop and then treat them at one time. Following recovery they would all be actively immune. Such a procedure was used to successfully control outbreaks of avian spirochaetosis in Tanzania (23).

Crystalline penicillin was effective when given intramuscularly in doses of 20-50,000 units per bird but this did require individual handling. Procaine penicillin was less satisfactory and was believed to have caused some mortality from infection alone. For these reasons it was only used for a few birds.

Control or eradication of the fowl tick is more important than treating infected fowls and is considerably more difficult. Complete eradication of the ticks was only accomplished in three outbreaks and in each instance this was done by completely depopulating the flock and burning the poultry house. Since outbreaks of avian spirochaetosis are most common in small backyard flocks, burning of the houses which were simple wood frame or mud-walled structures did not represent a great financial loss. When mud-walled houses were burned, it was necessary to put considerable quantities of inflammable material, e.g. grass, straw, leaves, paper, wood, inside the house to ensure that the thick walls were heated sufficiently to destroy the ticks within them.

A wide variety of control procedures were used with varying degrees of success. The use of a readily available insecticide powder (Flea and Louse Powder, Pfizer) containing a carbamate compound, carbaryl, was effective in killing larval ticks on the host when used as a dust. Spraying fowls with 0.5% malthion or dipping them in organophosphate (Asuntol, Bayer; Pfizona, Pfizer) solutions prepared at half the recommended concentration for mammals was more effective than dusting for killing larvae on the host. Stronger concentrations of organophosphate solutions must not be used. In one outbreak, directions were not followed and a stronger solution prepared. All fowls died within ½ to 2 hours after dipping. Commonly available spray insecticides should not be used on fowls. In a flock of birds being treated for lice, the owner sprayed them "lightly" with a commonly available insecticide containing vapona (R) (Shelltox). The majority of the flock died within 8 hours. On another occasion a group of fowls were dusted with the carbaryl containing insecticide and then placed in a poorly ventilated house in which several "pest-strips" containing vapona (R) had been hung. The following morning 14 out of 16 RIR but none of 12 WLH in the same house were dead. Insecticides, particularly the more potent organophosphates must be used as recommended only as these examples illustrate.

Three percent malthion applied at monthly intervals to the house, perch and equipment with a pressurized hand sprayer was found to be highly effective in controlling ticks in the environment. The only unfortunate event that occurred with its use happened when the hose of the sprayer slipped off and the attendent tried to replace it without first releasing the pressure. He was drenched with the insecticide and became quite ill requiring medical attention which again demonstrates the need for caution when handling insecticides.

The simplest method of tick control was to isolate the perch in such a way that ticks could not reach the fowls to feed on them at night. Two techniques for isolating the perch were tried. In one the perch was placed away from the walls with legs resting in the center of large tins filled with oil. In the other, the perch was suspended from the roof by wires and the upper parts of the wires heavily coated with grease. The first required daily removal of any debris and dead ticks that might have fallen into the oil and the legs had to be kept so that they did not contact the side of the tin. In the second, fresh grease had to applied as frequently as needed. With both techniques, the birds had to use the perch and not roost on nest boxes, feeders, or window ledges, etc. These methods, although the simplest and most effective, were not highly successful because they required constant attention of the flock owner.

TABLE 1

Re-infection of fowls with Borrelia anserina following recovery from avian spirochaetosis.

Fowls		Exposure to B. anserina			Challenge Results of Exposure	
No./Type	Age	First	Challenge	Interval	%Infected	% Mortality
14 WLH	16 wks.	Strain H* Series of inoc.	Strain L Natural Infection	1 month	100	50
4 RIR	Adults	Field Isolate	Strain 6* Experimental Infection	6 months	75	50
6 WLH (Broilers)	Adults	Field Isolate	Strain 6 Experimental Infection	3 months	100	67

*Isolated from market fowl. Kept as laboratory strain.
WLH = White leghorn
RIR = Rhode Island red

TABLE 2

Comparison between packed cell and buffy coat volumes of blood from Borrelia anserina infected and non-infected market fowls in northern Nigeria.

Date Blood Collected	Borrelia No. Fowls	Anserina PCV*	Negative B/C**	Borrelia No. Fowls	Anserina PCV	Positive B/C
14/2/74	40	30.4(4.0)	1.3(.6)	3	27.2(.8)	1.5(.5)
22/3/74	38	30.3(3.4)	1.2(.7)	None		
30/3/74	50	30.7(2.8)	1.3(.5)	7	28.9(2.7)	1.2(.5)
1/4/74	47	31.5(3.8)	1.5(.6)	8	31.6(5.4)	1.3(.5)
Total/Mean	175	30.8(3.5)	1.3(.6)	18	29.8(3.6)	1.3(.5)

* PCV = Mean Packed Cell Volume
** B/C = Mean Buffy Coat Volume
( ) = Standard Deviation of the Mean

TABLE 3

Differential diagnosis of diseases of Nigerian poultry that may resemble spirochaetosis.

Disease	Clinical Signs	Postmortem Findings	Other
Spirochaetosis	May occur as outbreak or sporadically. Acute illness with diarrhoes, depression, anorexia and thirst.	Marked splenomegaly with distinctly mottled appearance swollen, discoloured liver and kidneys.	Spirochaetes in blood smears (rarely absent). Larval ticks often present. Most common prior to and during rains in northern Nigeria and indigenous fowls.
Newcastle disease	Occurs as outbreak. Acute illness, nervous signs, ocular involvement, respiratory signs, inflammed cloaca.	Moderate splenomegaly (early). Discoloured, swollen liver, gastrointestinal haemorrhages and ulcers, tracheitis, pneumonia.	Blood semars and bacterial cultures negative. Marked nervous signs in recovered birds. Inadequate vaccination.
Fowl typhoid	Clinical signs often indistinguishable from spirochaetosis.	Marked splenomegaly. Liver-extensive foci of necrosis resulting in spotted or mottled appearance.	Blood smears negative. Salmonella gallinarum readily isolated. Positive plate agglutination test in survivors.

TABLE 3 (continued)

Coli-septicaemia	Birds often found dead or die after brief illness.	Marked splenomegaly. Discoloured liver often associated with peritonitis or fibrinous lesions of pericardium or air sacs.	Blood smears negative. Escherichia coli readily isolated. Most common in exotic, intensively managed flocks.
Fowl cholera	Varies from sudden death to clinical signs identical to spirochaetosis. Signs in Nigerian birds unknown.	Inflammation or haemorrhage in intestines may be pronounced. Findings in Nigerian birds unknown.	Bipolar bacteria in smears. Pasteurella multocida readily isolated. Rare or absent in Nigeria.
Marek's disease	Varies from lameness, paralysis to sudden death. Emaciation common. Irregular pupil and affected iris may be found.	Enlarged peripheral nerves in nervous form. Tumours of the spleen, liver, kidney and other organs in acute visceral form. More pronounced splenomegaly.	Blood smears negative. Bacterial cultures negative. Most common in intensive management systems. At present not known to occur in indigenous breeds.

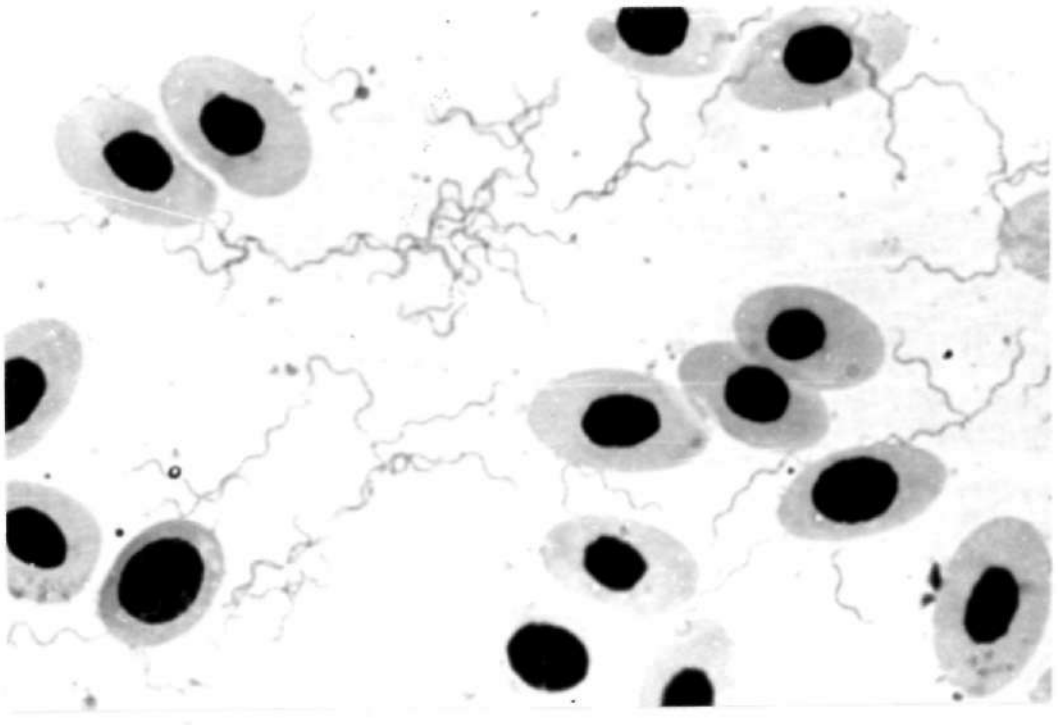


Figure 1

Borrelia anserina in the blood of a fowl with avian spirochaetosis. Immature erythrocytes as seen here are commonly found in the later stages of the disease. (Giemsa, x 500)


SOKOTO
KATSINA
KAZAURE
GUMEL
BIRNIN KEBBI
DAMBATTA
KANO
MAIDUGURI
ZARIA
KADUNA
VOM
YOLA
ILORIN
IBADAN
IJU
ENUGU
● Confirmed cases of Avian spirochaetosis
— —Area of present study and high prevalence

FIG. 2


SOKOTO
KATSINA
KAZAURE
DAMBATTA
GUMEL
BIRNIN KEBBI
KANO
MAIDUGURI
ZARIA
KADUNA
VOM
YOLA
ILORIN
IBADAN
IJU
ENUGU
Confirmed cases of Avian spirochaetosis
Area of present study and high prevalence

FIG. 2



Figure 3

Mature Rhode Island red hen with avian spirochaetosis. The fowl could not stand. Note the characteristic "sleepy" attitude and watery, greenish-white diarrhoea.

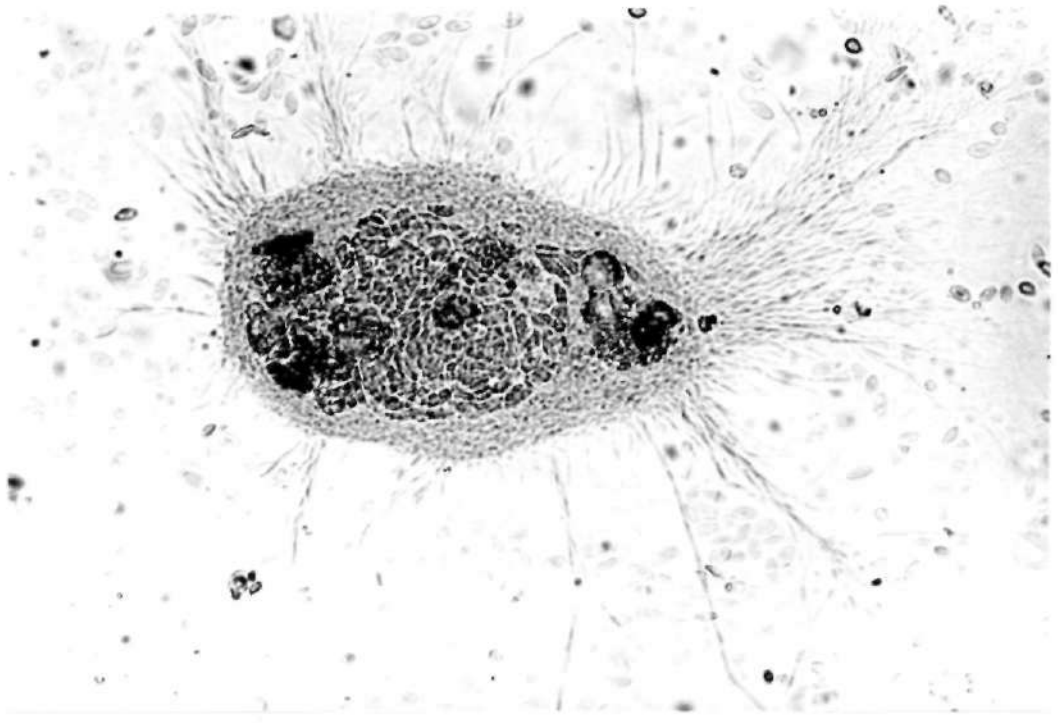


Figure 4

Agglomeration of Borrelia anserina and blood cells found in a terminally ill fowl that had previously received tetracycline for only one day (see text). The blood cells are mostly platelets and a few erythrocytes. This mass is larger than normally found in untreated, infected fowls but even in these fowls, blood cells are often found in the spirochaete masses. (wet smear, x 80)



Figure 5

Splenomegaly in a fowl that died of avian spirochaetosis. The mottled appearance is characteristic. Note how the foci are arranged in a flower-like, rosette pattern.

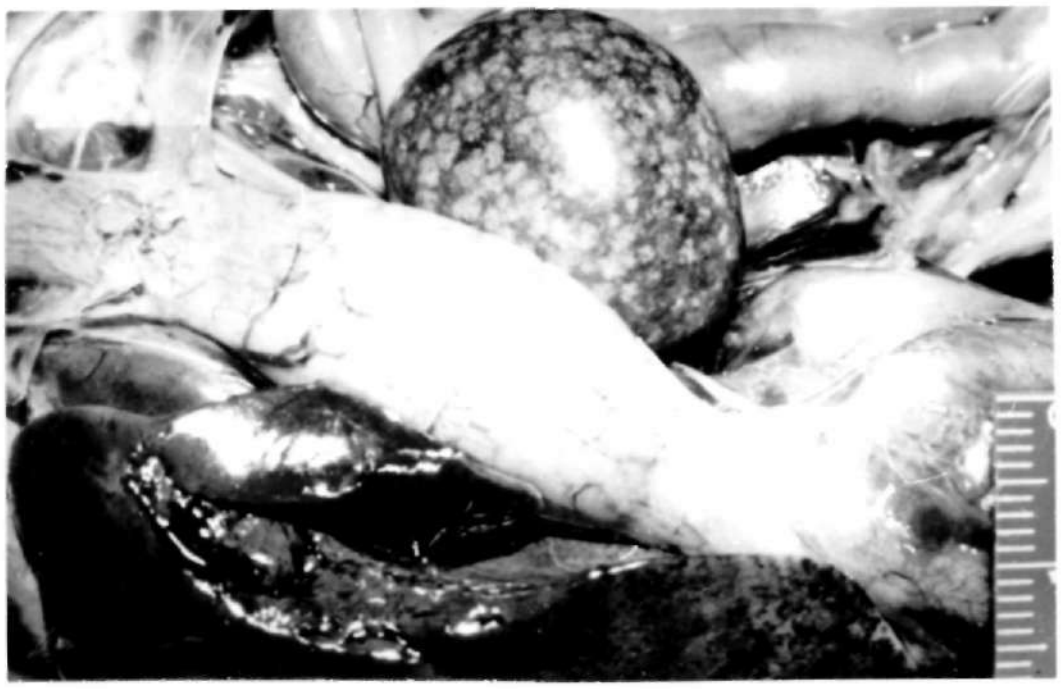


Figure 6

Splenomegaly in a fowl that died of avian spirochaetosis. Liver is friable with a fine reticulated pattern that became more prominent along the posterior border (lower right).

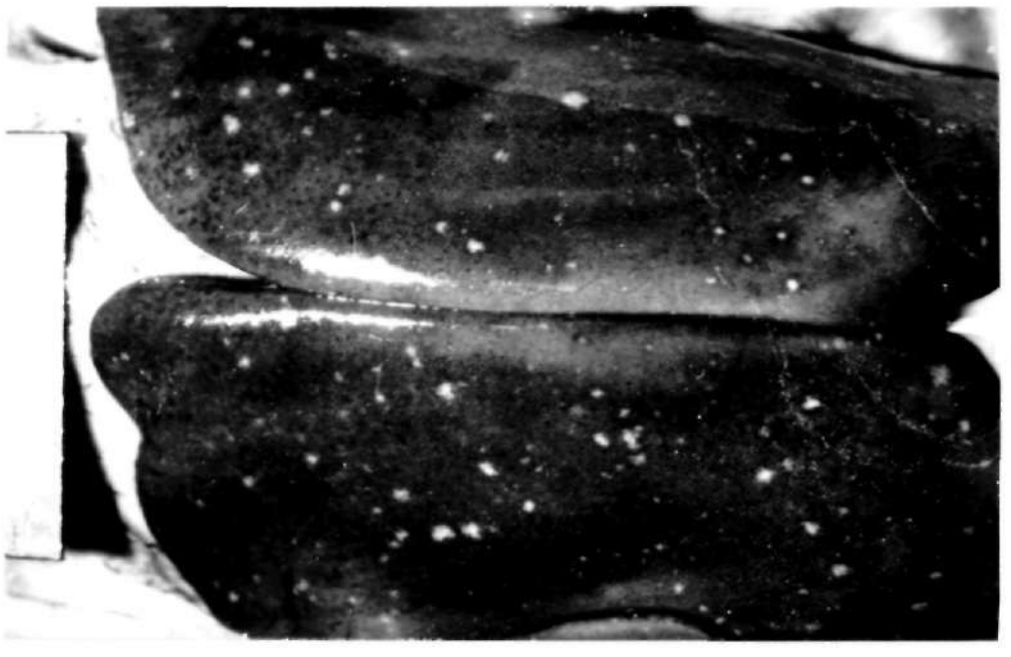


Figure 7

Liver from a fowl that died of avian spirochaetosis. Focal areas of necrosis and a fine reticulated pattern can be seen. The latter is a characteristic change seen in avian spirochaetosis. Foci of necrosis were found in about 5% of fowls examined. They are particularly pronounced in this liver.



Figure 8

A large pale area in the pectoral muscle of an adult broiler that had died of avian spirochaetosis. This was a common finding in heavily muscled fowls. The remaining muscles show the dark, congested appearance that was characteristic of fowls that had died of avian spirochaetosis.

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: RELATIONSHIP BETWEEN LARVAL TICK INFESTATION OF FOWLS AND INFECTION WITH *BORRELIA ANSERINA*

SUMMARY

The study was conducted during July-August, 1971, and March-April, 1972. Of 487 indigenous fowls examined, 36 (7.4%) were infested with larval ticks. Of these, 18 (50%) were infected with Borrelia anserina. By contrast only 20 (4.4%) of the remaining 451 tick-free birds were infected. It was concluded that larval tick infestation increased the likelihood of fowls also being infected with B. anserina and that this, in conjunction with the high mortality resulting from spirochaetosis, forms the basis for local discriminatory buying practices. When examining sick birds infested with larval ticks there should be a strong suspicion of spirochaetosis.

INTRODUCTION

A common practice in some markets of northern Nigeria is to discriminate against fowls purchased for flock replacements if they are infested with larvae of the fowl tick, Argas persicus. Infested birds are generally sold at a reduced price because it has been recognized they often die soon after purchase. Considering the larval tick's role in transmitting spirochaetosis and high mortality characteristic of this disease, it was thought spirochaetosis might be responsible for many of the deaths. Also, if a close correlation existed between infection with Borrelia anserina and infestation with larval ticks it was felt their presence could be a useful diagnostic indicator of spirochaetosis.

MATERIALS AND METHODS

During July and August 1971, and March and April 1972, indigenous fowls slaughtered at the student hostel kitchens, Ahmadu Bello University, Zaria, were examined for larval ticks and blood samples collected. Source of the fowls and methods for collecting and examining blood samples have been described (1). Because of the slaughtering procedure it was not possible to examine all birds for larval ticks or even all birds from which samples were taken. However, blood samples from birds which had been examined for larval ticks were kept separate and those from tick infested birds marked on the specimen bottle.

All samples from each group were processed and examined identically. Some blood samples were evaluated by two individuals independently. No discrepancies between their results were found. Examiners could have been aware of the tick status of the blood donors.

Although not a specific objective of this study, some idea of the distribution and number of larval ticks on the fowls was obtained.

RESULTS

Of 487 fowls examined, 36 (7.4%) were found to be infested with larval ticks. Of these, 18 (50%) were found to also have B. anserina septicaemia. By contrast, of the 451 tick-free fowls only 20 (4.4%) were infected (Table 1).

Larval ticks were found most frequently attached to the skin of the wing web, less commonly on the skin of the inguinal, flank, and sparsely feathered areas of the legs and rarely elsewhere. Most commonly only 1-10 larvae were found but a few birds were infested with large numbers.

DISCUSSION

From this study it would seem that larval ticks are not commonly found on indigenous fowls. The presence of larval ticks should, however, indicate a greater likelihood of B. anserina infection and tick infested birds should always be examined for spirochaetes, especially if showing signs of illness. When the high mortality caused by B. anserina infection and the greater chance of infection in tick infested birds are taken into account, local discriminatory practices against the purchase of such birds would appear justified.

TABLE 1

A comparison between larval tick infestation and infection with Borrelia anserina in indigenous fowls.

Date Slaughtered	Tick Free Fowls	Tick Infested Fowls
7/7/71	1/71*	0/2
30/7/71	2/81	2/2
6/8/71	0/19	0/1
16/8/71	1/86	1/5
19/8/71	0/61	4/5
28/3/72	3/32	0/1
1/4/72	1/27	0/1
4/4/72	6/42	0/2
8/4/72	6/32	11/17
Total	20/451 (4.4%)	18/36 (50%)

*Numerator = number fowls with B. anserina
Denominator = Number fowls examined.

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA:
TRANSMISSION OF AVIAN SPIROCHAETOSIS IN FOWLS

SUMMARY

Avian spirochaetosis developed in susceptible fowls following exposure to infective blood by intravenous, intraperitoneal, intranasal, intraocular, oral and subcutaneous routes. Infection was not established in 24 susceptible chicks given faecal samples orally or parenterally from infected fowls in various stages of avian spirochaetosis. Of 96 fowl ticks, (Argus persicus) from 3 outbreaks examined either microscopically or by inoculation into susceptible chicks, 12 (12.6%)were found infected with Borrelia anserina. It is concluded that any husbandry procedure or activity of the fowls which could result in blood from one fowl contacting another could result in transmission of avian spirochaetosis. From this study it would appear unlikely that faeces from infected birds is an important source of infective spirochaetes in northern Nigeria. The finding of a 12.6% infection rate in fowl ticks from outbreaks of avian spirochaetosis was sufficient to cause continual new cases of the disease in affected flocks.

INTRODUCTION

The importance of fowl ticks of the genus Argas in the transmission of avian spirochaetosis has been well established since the original discovery of Argas miniatus ("persicus") as a vector by Marchoux and Salimbeni (16). There appear to have been few studies to determine what proportion of the tick population of an affected flock are actually infected with Borrelia anserina. Experimentally, Marchoux and Couvy (15) produced infection in fowl embryos with 8 of 16 ova from infected female Argas. Knowles et al. (13) found 40.7% of male ticks and 59.1% of female ticks infected after having fed on a heavily infected experimental fowl. Other arthropods including the argasid tick Ornithodorus moubata (8), Culex (24) and Aedes (19) mosquitos, and the fowl mite Dermanyssus avium ("gallinae") (12) have been found to be experimental or natural mechanical vectors. Fowl lice have been incriminated (1,2,11) but this observation has not been confirmed.

In addition to arthropod vectors several methods of parenteral, direct and indirect transmission not requiring arthropod vectors have been reported (9,13,18). In the southwestern United States ingestion of bile stained faeces was concluded to be the primary means of transmission in outbreaks of avian spirochaetosis in turkey flocks (14,18). This conclusion was based on either the inability to find known vectors associated with infected flocks (18) or the inability to demonstrate spirochaetes from fowl ticks collected from infected flocks (13) and the experimental infection of poults by feeding them bile-stained faeces (18). Details of the latter infections were not given, however, other workers have also reported faecal transmission (6,16,17). Cannibalism has been reported as yet another possible means of non-arthropod transmission (18,22). Infection has been reported to occur by cohabitation with infected birds (10,21) but the mechanism for this was not established.

In Nigeria it has been reported that poor hygiene and overcrowded conditions facilitate the spread of avian spirochaetosis and birds could be infected by ingesting food soiled with infective droppings (23). In a clinical and field study on avian spirochaetosis in northern Nigeria (4) occasional outbreaks were reported where fowl ticks or other known vectors could not be found. This suggested that some means other than arthropod-borne transmission may have been occurring in these outbreaks. It was further reported that during some outbreaks birds were infected sporadically over a period of several weeks. A low level of spirochaete infection in the tick population might have been responsible for this observation. The purpose of this paper is to report results of experimental studies on the transmission of avian spirochaetosis and observations on the percentage of ticks infected with B. anserina from outbreaks of avian spirochaetosis in northern Nigeria.

MATERIALS AND METHODS

Preliminary Study. A flock of 18 Rhode Island red (RIR) pullets from a tick and spirochaete free flock was divided into groups of 3 and exposed to infective blood from a clinical case of avian spirochaetosis by intravenous (IV), intraperitoneal (IP), intranasal (IN), intraocular (IO), oral (PO), or subcutaneous (SQ) routes. One-half ml of whole blood was given to each of the birds in the IV, IP and PO groups while those in the IN and IO groups were given 2 drops (approximately 0.1 ml) each. The SQ group was inoculated by stabbing the wing web with a grooved vaccinating needle (Salsbury's Laboratories) previously dipped in the infective blood. Blood samples were taken daily and examined for B. anserina by wet smears (3).

Faeces as a Source of Infection. Trial 1. Faeces containing bile voided naturally into sterile Petri dishes by each of 2 adult RIR roosters that had been inoculated 4 days earlier with B. anserina, strain H (5) and had 1+ and 2+ spirochaetemias* was used. Each

*Spirochaetemia was evaluated as: 1+ = ≥ 1 spirochaete/1-10 high power fields (HPF,X300), 2+ = 1-5/HPF, 3+ = 5-50/HPF, +4 = > 50/HPF.

RECEIVED

sample was centrifuged lightly to sediment particulate matter. One-half ml of supernate from each sample was inoculated IN into a susceptible white leghorn chick (WLH). Chicks were examined daily for spirochaetemia. Trial 2. Faeces containing bile was collected in the same way from each of 2 adult WLH roosters that had been inoculated 4 days earlier with B. anserina, strain H and had 4+ spirochaetaemia. Both samples were pooled and mixed with an equal quantity of sterile phosphate buffered saline containing 0.2% glucose (PBS/G) and allowed to settle. The supernate was aspirated and given to 2 susceptible WLH chicks. One chick received 1 ml IP while the other was given the remaining 4 ml PO. Trial 3. Four RIR hens and 4 WLH roosters were inoculated IN with 0.2 ml of a 1:100 dilution of infective blood in PBS/G containing B. anserina, strain H. A total of 20 faecal samples containing bile were collected on post-inoculation days (PI) 4,5 and 6. All birds had 2+ to 4+ infections during the period. Two faecal samples were collected just before or just after 2 fowls died.

Each sample was naturally voided into sterile Petri dishes then diluted 1:2 with PBS/G. The total suspension was aspirated into a sterile disposable syringe and given PO to a susceptible WLH chick. Blood from exposed chicks was examined daily for 11-13 days for B. anserina as in previous trials. Seventeen to 19 days after exposure, all chicks were inoculated IN with 0.2 ml of a 1:10 dilution of blood containing the homologous strain.

Infection of Fowl Ticks with Spirochaetes. Fowl ticks, Argas persicus, were collected from 3 separate poultry houses where outbreaks of avian spirochaetosis were occurring. In each instance the stage of tick, whether larva, nymph or adult was determined and in the third study ticks were also evaluated as being fully, partially or not engorged. From the first 2 collections, ticks were examined by placing them in a drop of physiological saline, crushing them thoroughly under a coverslip, and examining the resulting preparation by darkfield microscopy. From the 3rd collection, ticks were examined by grinding each tick in a sterile TenBroek tissue grinder with a small quantity

found infected on PI 7. The overall total of infected and noninfected ticks was 12/95 (12.6%)

DISCUSSION

From the results of this study, it was found that blood containing viable spirochaetes was infective by several routes but faeces from infected fowls was not capable of inducing infection or providing immunity in susceptible birds. It was further shown that of the ticks examined for B. anserina infection from 3 separate outbreaks of avian spirochaetosis approximately 1 in every 8 was infected.

Infection following exposure to infective blood by virtually any route even smearing blood on the skin or comb (18,20) has been reported. The spirochaete appears to have considerable capacity to penetrate intact, healthy tissues. The finding that direct transmission of B. anserina to susceptible birds via blood from infected fowls has important implications in both field and experimental situations. In the field, any husbandry procedure or activity of the birds that would enable blood from one fowl to contact another fowl could be a means of transmission. Thus vaccinations, treatments, blood sampling, surgical procedures, traumatic injuries, and cannibalism could be important. When blood samples are taken during experiments, disinfected or preferably, sterile equipment must be used, any excess bleeding has to be controlled, and blood on the body removed before birds are placed together again. Hoffman and Jackson (10) indicated this may have been the reason for an uninoculated chick becoming infected when caged with 2 infected chicks in their study.

The ability to initate infection by instilling infective blood in the eye has been used in this laboratory as a simple means of serially transferring infection from one chick to another. A toenail of an infected chick is clipped short enough so that a drop of blood can be expressed which is directly instilled into the eye of the susceptible chick. The latter becomes spirochaetemic 3-4 days later.

The inability to infect chicks with 24 different samples of faeces containing bile taken at various stages during the infection is in contrast with previous reports. Marchoux and Salimbeni (16) successfully transmitted spirochaetosis to fowls in Brazil by feeding them blood or faeces from infected birds. Similar results were reported by Denev in Bulfaria (6). The presence of a few B. anserina in the cloaca of fowls during spirochaetemia has been reported but infection in susceptible birds with cloacal contents was apparently unsuccessful (7). Red blood cells were also noted to be present. Spirochaetes were found in the cloacal fluid of a duck that had been infected with B. anserina initially isolated from a pheasant in the United States and passaged through a fowl. A chicken that had been inoculated intravenously with the fluid developed spirochaetosis a week later. Two uninoculated ducks in the same cage as the infected ducks also contracted spirochaetosis. The source of infection in these two ducks was believed to be infective faeces (17). Faecal transmission of avian spirochaetosis in turkey flocks in the United States has been reported. Poults were apparently successfully infected by feeding them bile stained faeces collected from infected flocks, however, details on this aspect of the study were not given (18). The difference in species or characteristics between African and American types of B. anserina may account for the different results. Gastrointestinal haemorrhage has been reported by some workers as a feature of avian spirochaetosis in some areas of the world. In a recent report (4) it was stated that gastrointestinal haemorrhage was not a feature of avian spirochaetosis in Nigeria which could also be an explanation for the differences. Although further studies are needed, it would appear that the importance of faeces as a source of infection in fowls may have been overemphasized. From these studies, there was no indication that ingestion of food soiled with droppings from infected fowls would be important in the transmission of avian spirochaetosis in northern Nigeria as has been previously reported (23).

The finding that slightly over 12% of the ticks examined from

3 outbreaks were infected with B. anserina is difficult to interpret because little previous information concerning natural infections in tick populations appears to be available. It was, however, somewhat higher than expected. Loomis (14) examined approximately 900 fowl ticks by several methods from outbreaks of spirochaetosis in turkeys with negative results. Knowles et al. (13) found a high percentage of ticks to be infected but these had been experimentally fed on a known infected fowl. In the outbreaks from which these ticks were collected, infection was occurring at a continuous rate. Further studies on the biology of the fowl tick and the host spirochaete relationship are indicated.

The mortality resulting from spirochaetosis following inoculation of tick emulsions was extremely low compared with previous experimental studies in young chicks (5). The reason for this was not apparent. It would seem most likely that only a few spirochaetes were present in the infected ticks or some technical factor in the preparation of ticks for injection may have been responsible.

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: SEASONAL VARIATIONS IN PREVALENCE

SUMMARY

Blood samples were collected from 7096 indigenous fowls between March, 1972, and February, 1975. Borrelia anserina was found in 265 of these resulting in an overall prevalence rate of 3.7%. Mean monthly prevalence rates varied from a low of 0.7% in December to a high of 13.9% in July. Highest prevalence rates occurred during March, June and July. Climatic factors during these months should favour tick hatching, tick activity and development of the spirochaete in the tick. Lowest prevalence rates were found during December and January, which in northern Nigeria is the coolest period of the year and one of low relative humidity. Outbreaks of avian spirochaetosis occurred in a similar pattern to that of prevalence rates in indigenous fowls although there were some minor differences. It is concluded that a definite seasonal pattern of B. anserina infection in fowls occurs in northern Nigeria which is related to climatic conditions. These in turn probably influence activity of the vector, Argas persicus.

INTRODUCTION

Seasonal variations in the prevalence of avian spirochaetosis have been reported from several parts of the world. In general, the disease occurred most commonly during warmer spring or summer periods and was uncommon or absent when temperatures were low. Increased tick activity or hatching of larval ticks associated with warmer temperatures was observed to coincide with increases in Borrelia anserina infection.

In Southern Rhodesia, avian spirochaetosis was most prevalent during the spring (November - December). Ticks were more plentiful during this time and conditions favoured larval hatching (4). Avian spirochaetosis occurred only during the summer (June and August) in Cyprus (19). Avian spirochaetosis did not occur during the winter in Roumania but reappeared after a few warm spring days (16). Leger and Le Gallen (13) found avian spirochaetosis to be common during the hot season (December - June) at Daker, Senegal, but to diminish or disappear during the rainy season. Avian spirochaetosis was reported to occur most frequently in the hotter areas and seasons (November - April) in Australia (6,8). Gorrie (6) reported that an unusually mild summer and autumn in Victoria during the previous 2 years corresponded with a significant decrease in the number of reported outbreaks of avian spirochaetosis. At Mukteswar, India, birds kept in tick infested runs developed the disease during the summer but rarely during the winter. Presence of larval ticks during the summer was considered primarily responsible for the greater incidence of avian spirochaetosis (18).

In Nigeria, the seasonal prevalence of avian spirochaetosis has not been studied although such information would be useful in diagnosis and control programs. Kaschula (10) suggested a seasonal pattern of activity for the fowl tick in northern Nigeria which should correlate with a seasonal pattern of spirochaete infection.

This study was undertaken to determine seasonal variations in the prevalence of B. anserina infection in northern Nigeria.

MATERIALS AND METHODS

Collection of Blood Samples. Blood samples were collected between March, 1972, and February, 1975, from indigenous fowls and examined for B. anserina. Virtually all samples came from chickens that were slaughtered for food purposes at the Student Hostel Kitchens, Ahmadu Bello University, Zaria, or Sabon Gari Market, Zaria. Occasionally blood samples were collected from other groups of indigenous fowls being slaughtered or offered for sale elsewhere. Birds were slaughted by severing the neck and 2-4 ml of blood was collected in a bottle containing 2 drops 10% EDTA as an anticoagulant. It was not possible to examine all birds slaughtered because of the procedures used, however, at least 10% of the birds in each group were sampled. Only thin smears from a wing web capillary were taken from birds offered for sale. Blood samples were collected once or twice weekly whenever possible but this was subject to several variables e.g. number of students on campus, cooperation of vendors, transportation, and market prices. Blood samples were refrigerated within 2 hours after collection.

Source and Description of Fowls. Birds brought to the University for slaughter were supplied by vendors who obtained them from markets and villages in an area of northwestern Nigeria bounded roughly by Sokoto, Katsina, Kano and Zaria (Fig. 1). Those slaughtered in the market generally came from within a 20 mile radius of Zaria. Only fowls of indigenous breeds were slaughtered. They represented both sexes and a variety of breeds and ages. Prices generally dictated the type of birds and most of those examined were either pullets or young hens as these were less expensive and most often traded or sold.

Examination of Blood Samples. Blood samples were usually examined within 48 hours of collection. Methods used have been described elsewhere (2) and included wet or buffy coat smears examined by darkfield microscopy and Giemsa stained blood films.

Outbreaks. Information on outbreaks of avian spirochaetosis between January, 1971, and February, 1975, was taken from the annual records of the Avian Disease Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Flocks were located within the Samaru-Zaria area. Outbreaks were recorded on the basis of the initial date diagnosis was made even though birds may have been examined over a period of several weeks. If the disease reoccurred later on the same property this was considered a new outbreak and not a continuation of the previous outbreak. Only confirmed cases were tabulated.
Meteorological Data. Meteorological data was obtained from published material (12) and records of the Meteorological Office, Department of Agronomy, Institute for Agricultural Research, Zaria (Fig. 2).

RESULTS

Prevalence of Spirochaetes in Indigenous Fowls. Of 7096 fowl blood samples examined, 265 were positive for B. anserina; an overall prevalence rate of 3.7%. Prevalence rates were higher for 1972 (4.4%) and 1974 (5.7%) than for 1973 (1.7%). Mean monthly prevalence rates for the 3 year period were highest during June (6.5%) and July (13.9%) and lowest during December (.7%) and January 1.5% (Fig. 3). Mean monthly prevalence rates were above average during March, April, June, July, and August. Below average mean monthly prevalence rates occurred during January, February, May, September, October, November and December.
Outbreaks. Between January, 1971 and February, 1975, 84 outbreaks of avian spirochaetosis were diagnosed; a monthly mean of 1.6. Outbreaks occurred most frequently during February, June and July and least frequently in October, December and January (Fig. 4).

DISCUSSION

Although avian spirochaetosis was first reported from Nigeria over 60 years ago, and only sporadic references to its occurrence and importance have been made since then (1), infection of indigenous

fowls with B. anserina is still common. If the high mortality that is characteristic of B. anserina infection is taken into account along with the overall prevalence rate of 3.7%, avian spirochaetosis must still be considered one of the most important diseases affecting indigenous poultry in this area and the cause of considerable economic loss. It is possible, however, that this figure may be higher than would be found if birds were examined in the villages. Marketing of sick birds is a common practice in the area which could result in a higher proportion of B. anserina infected birds being found in this study. Also, if the birds remained in the market for several days, it would be possible for them to become infected there. By contrast, spirochaetemia usually lasts for only a short period, 3-5 days in adults (3), and it is only during this period that spirochaetes would have been found. A higher overall prevalence rate may have resulted if serial samples could have been collected from the same birds over a 2 or 3 week period.

A definite seasonal pattern for B. anserina infection occurs in northern Nigeria as evidenced by the monthly distribution of outbreaks and changes in the prevalence of spirochaete infection in indigenous fowls. The prevalence of B. anserina infection in indigenous fowls increased in relationship to air temperature increases early in the year (Fig. 2). As temperatures reached maximum levels prior to the onset of the rainy season, a decline occurred. But as temperatures moderated and the rains began, the prevalence of spirochaete infection again increased reaching a peak in July. The prevalence of B. anserina infection continued to fall as did temperatures until November when maximum air temperatures and the prevalence of spirochaete infection again increased. Prevalence rates reached their lowest levels during December and January, which was the coolest period of the year and also one of low relative humidity.

Spirochaete infection would appear to be influenced most by temperature with moisture, as humidity and rainfall, having a secondary but important influence. Infection occurred most frequently during the

warmer periods of the year and less commonly during extremely hot or cool periods. Peak infection levels occurred during the rainy season (July). MacFie and Johnston (14) who studied avian spirochaetosis in southern Nigeria also found the disease to occur most commonly during the rainy season. In a review of avian spirochaetosis in Nigeria (1) outbreaks of spirochaetosis in northern Nigeria were reported most frequently in the fourth quarter reports of the veterinary divisions which represents the period April - June. Outbreaks were less commonly reported during the second quarter (July - September) and first quarter (January - March). No outbreaks were reported during the third quarter (October - December). That distribution of reported outbreaks correlates well with the findings in this study. Since these reports cover a period between 1955-1972 they provide additional evidence for a predictable seasonal variation in prevalence of avian spirochaetosis in northern Nigeria.

With few exceptions, patterns of mean monthly disease outbreaks and percent infected birds correlated well. However, changes in mean monthly disease outbreaks tended to precede changes in percent infected birds. A possible explanation for this difference may lie in the fact that information on disease outbreaks was based on the date when the first bird in a flock was found infected, and is therefore qualitative in nature while data concerning percent infected birds is quantitative.

Another difference between the patterns of monthly disease outbreaks and percent infected birds is the prominent July peak observed in percent infected birds but not reflected in the number of outbreaks. Part of this difference may be attributable to the habits of the flock owners; many of whom were university employees and went on leave during the summer months. It was not uncommon to get a history of an owner returning from leave to find either his flock had been reduced considerably because of illness or the birds were sick.

The duration of this study is relatively short and the number of outbreaks is small compared with the number of market birds examined. If information were available for a longer period a greater correlation

between the number of outbreaks and infected fowls might be achieved. There is no doubt that the data on market birds is the more accurate but the two parameters used to measure spirochaete infection do complement each other and support the fact that a marked seasonal variation in the prevalence of B. anserina infection occurs in northern Nigeria.

Occurrence of any vector-borne disease is dependent upon activity of the vector and extrinsic incubation period of the pathogen. Both of which are directly influenced by climate. Argasids are typically xerophilic, thriving under dry conditions, however, Argas persicus appears less so than other argasids (9). It can withstand wide temperature and humidity ranges although warmer temperatures favour increased activity and rapid development. It has been shown that infected ticks kept at 15-20°C could not infect susceptible birds until the temperature was increased to 35°C when they were once again capable of transmitting spirochaetes (5). Temperatures below 15°C inhibit A. persicus egg hatching while a high humidity (over 60%) is required for eggs to hatch when temperatures exceed 33°C (9).

In northern Nigeria periods when climatic factors should be favourable to tick activity, larval hatching and development of B. anserina within the tick, coincide with those periods when outbreaks of avian spirochaetosis are most likely and B. anserina infection in indigenous birds highest. Conversely, climatic factors adverse to tick activity, spirochaete development and larval emergence prevail when B. anserina infection is lowest. Kaschula (10) postulated fowl tick activity in northern Nigeria to be highest just prior to and at the end of the rainy season in which case spirochaete infection should occur most commonly in April and September. The results of the current study suggests tick activity may be greatest in February and March and again from mid-May to July.

Larval ticks may be particularly important in B. anserina transmission in northern Nigeria because periods favourable to larval tick

hatching correlate closely with those in which spirochaete infection is highest. The importance of larvae in the transmission of spirochaetosis has been reported (4,19). In India, it was found adult ticks do not transmit spirochaetosis as readily as larvae (18).

Critical studies are needed on the fowl tick in Nigeria, particularly distribution, effect of temperature and humidity on development, activity, larval hatching, and transmission of B. anserina. In addition, the time of night that ticks are active as influenced by the microclimate inside infested poultry houses needs investigation.

Spirochaetosis outbreaks in northern Nigeria are currently controlled by antibiotic treatment and eradication of fowl ticks. Vaccination has been used successfully in control programs elsewhere (6,7,8,11 15,17) and would seem to be indicated in northern Nigeria. In a control program for avian spirochaetosis it would be best to vaccinate birds between September and January, preferably toward the end of the period. In this way birds would have a high immunity as they entered the peak season of March through July. Veterinary pharmacies should be well stocked with antibiotics and insecticides during the peak season of March through July in order to be able to handle outbreaks quickly and efficiently to minimize losses. Education programs in villages on recognition and control of avian spirochaetosis and the tick vector A. persicus would be a necessary adjunct to other measures.

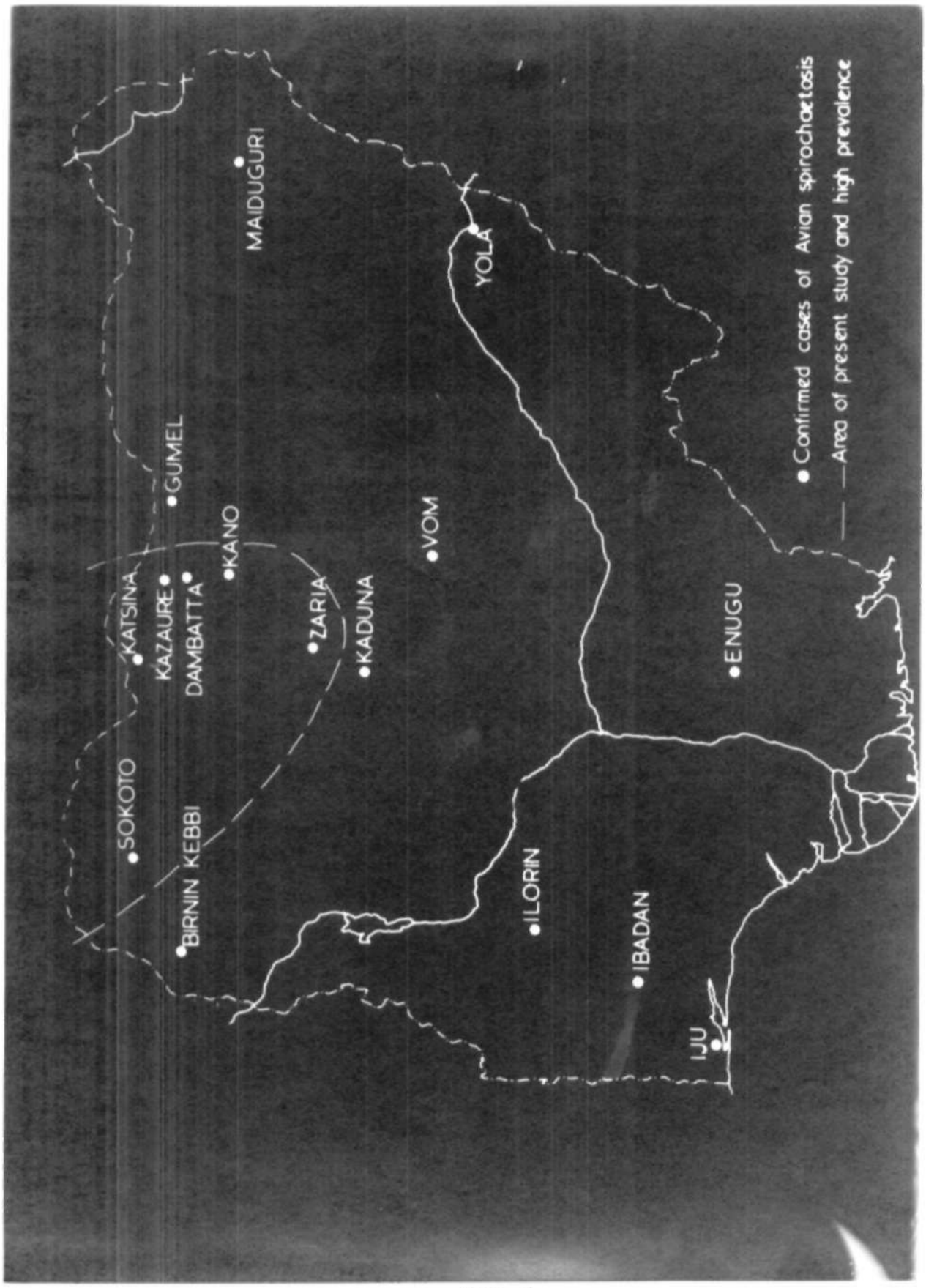

● Confirmed cases of Avian Spirochaetosis
—— Area of present study and high prevalence
SOKOTO
KATSINA
KAZAURE
DAMBATTA
BIRNIN KEBBI
ZARIA
KADUNA
GUMEL
KANO
MAIDUGURI
YOLA
VOM
ILORIN
IBADAN
IJU
ENUGU

FIG. 1

Average meteorological data — Samaru

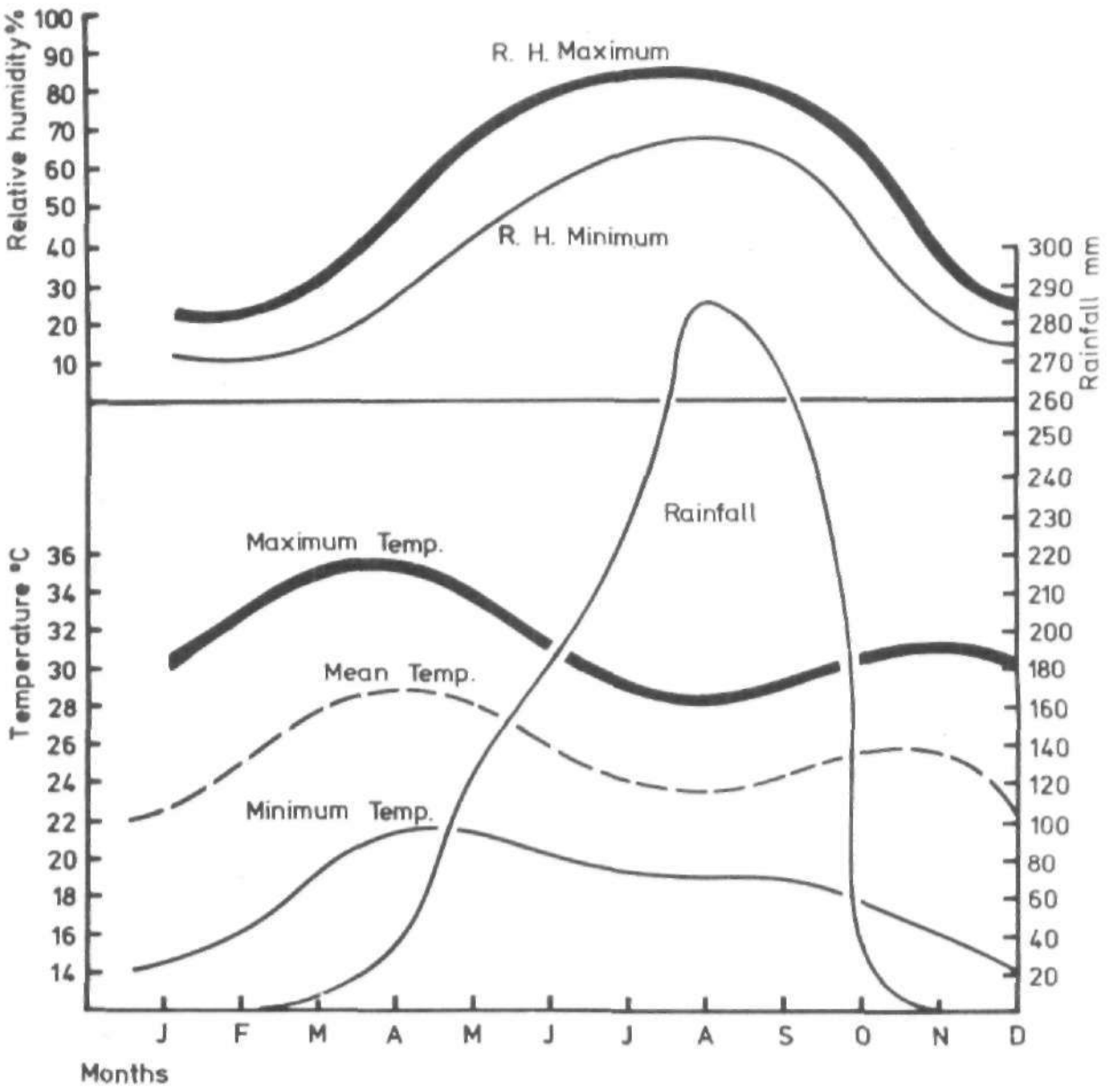


FIG. 2

MEAN MONTHLY DISTRIBUTION OF PERCENT MARKET FOWLS INFECTED WITH BORRELIA ANSERINA IN NORTHERN NIGERIA.

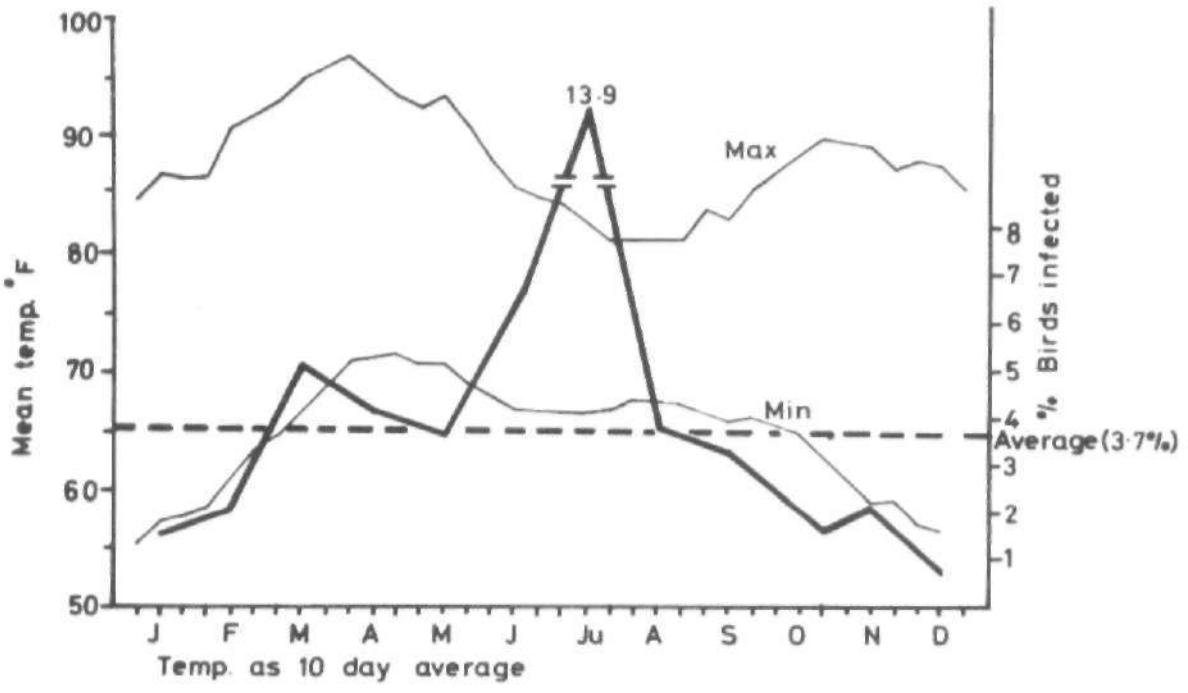


FIG. 3

MEAN MONTHLY DISTRIBUTION OF AVIAN SPIROCHAETOSIS
OUTBREAKS IN NORTHERN NIGERIA.

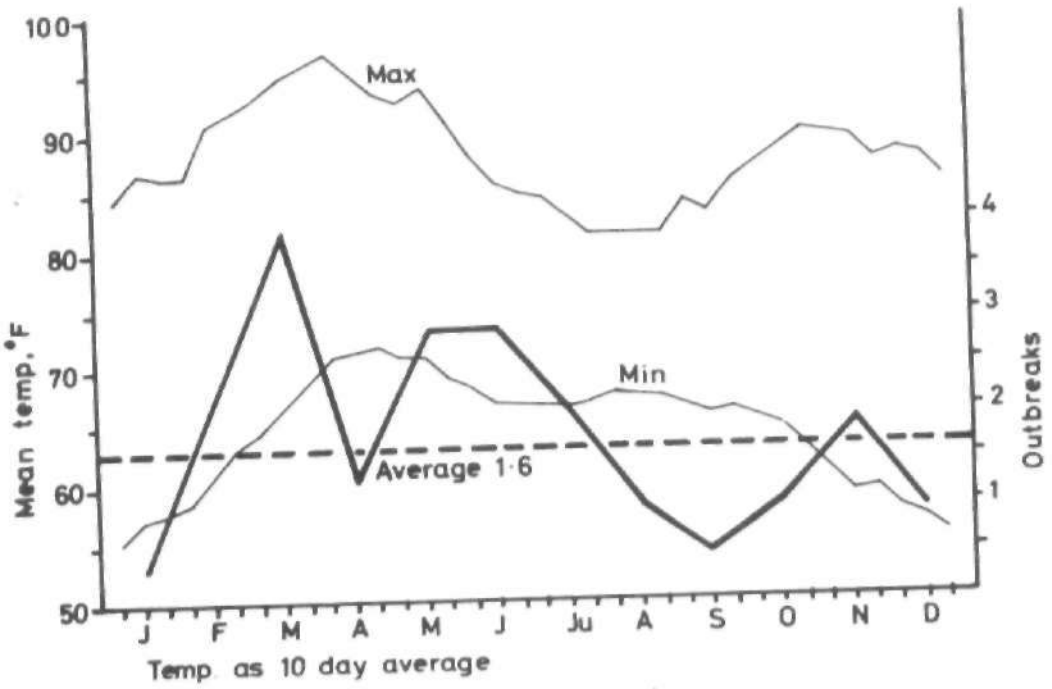


FIG. 4

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: INFLUENCE OF AGE ON EXPERIMENTAL *BORRELIA ANSERINA* INFECTION

SUMMARY

Various age groups of white leghorn (WLH) and Rhode Island red (RIR) fowls from tick and spirochaete free flocks were inoculated with a strain of Borrelia anserina that had been isolated in northern Nigeria. Age did not influence infection but did affect mortality which was higher in younger than older birds of the same breed. Day-old chicks had prolonged spirochaetemias but less pronounced clinical signs as compared to older fowls. There was some indication that RIR may be more resistant to spirochaetosis than WLH. It is concluded that age influences mortality and duration of spirochaetemia but not susceptibility.

INTRODUCTION

The question of an age-related susceptibility of poultry to avian spirochaetosis has not been completely resolved. Cantacuzene (6) reported that chicks (1-4 weeks old) were extremely susceptible to Borrelia anserina infection and invariably died whereas adult fowls were virtually immune. The majority of subsequent studies have supported this view (7,8,11,13,15,20). In India fowls of all ages became infected with B. anserina but mortality was lowest in 6-12 month old birds (17). Mortality was higher in chicks and poults than in older birds when they were exposed to B. anserina of turkey origin (14).

In contrast clinical signs were found to be more severe and mortality greater in adults than in chicks when they were infected with a southwestern United States strain of B. anserina. Experimental inoculation of 2 week old chicks resulted in a mortality of only 10% whereas 5 of 6 adults died from B. anserina infection (16). In a comprehensive review of Borrelia infections, it was suggested mortality may be higher in adult than younger birds (9).

It has been reported that in Nigeria young fowls infected with B. anserina are more severely affected than older ones (19), MacFie and Johnston (12) in their study on spirochaetosis in southern Nigeria did not report the influence of age on B. anserina infection. From the plates and case histories in their paper, however, it would seem they were dealing with young growing and adult birds.

In a clinical study conducted in northern Nigeria (4) spirochaetosis was rarely found in birds under 10 weeks of age and the disease was considered to be a problem primarily of older birds. Several possible explanations were postulated, one of which was the possibility of an age-related resistance in young fowls. This study was undertaken to test this hypothesis and also re-examine the general question of the influence of age on B. anserina infection.

MATERIALS AND METHODS

Method of Exposure. During routine examination of blood samples from indigenous fowls (5) several isolates of B. anserina were obtained. One isolated during November, 1973, was designated strain H. The morphology of strain H and clinical disease it produced were considered typical for B. anserina in northern Nigeria.

The strain was maintained in the laboratory by passage through susceptible fowls. When spirochaetemia was high but intravascular clumping of spirochaetes had not started 4-5 ml of blood was withdrawn from the heart or jugular vein into a sterile, evacuated tube containing sodium citrate. The blood was stored under refrigeration (4-6^{o}C) and the strain passaged every 3-4 weeks by inoculating each of 2 susceptible fowls with 0.5 ml blood intramuscularly (IM). Continued passage did not noticeably alter the isolate.

This procedure was also used to obtain infective blood for each trial. Freshly collected blood was always used except for trial B where the blood had been refrigerated overnight. Strain H was used in all trials except the pilot study in which an earlier field isolate was used. The dosage and route of exposure for each trial are presented in Table 1.

Description of Birds. All experimental fowls were from tick and spirochaete free flocks. Those used in the pilot study were Harco x white leghorn (WLH) crosses produced by a local flock owner. In the remaining trials both WLH and Rhode Island red (RIR) breeds were used (Table 1). Young WLH were hatched from eggs produced by a WLH breeding flock maintained at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, while adult WLH were purchased from a local poultry keeper. The RIR came from the North Central State Poultry Production Unit, Kaduna. Adult RIR were from the same source but had been raised at the Faculty of Veterinary Medicine or were purchased from a local poultry keeper.

Diagnostic Methods. Wet blood smears were periodically examined as previously described (3) throughout each of the trials. Birds in trial E were examined daily for an additional 2 weeks to detect possible relapses. Clinical observations were noted when blood samples were taken.

Statistical Analysis. The Chi-square and t tests were used where appropriate to determine statistical significance.

RESULTS

Pilot Trial (Week Old Chicks). All chicks became infected with B. anserina (100%) and 3 of 4 died (75%) (Table 2). Deaths occurred on post-inoculation days (PI) 4, 9, and 10. Duration of spirochaetemia ranged from 3-9 days (mean 6.5 ± 2.7 days).

Trial A (Day Old RIR Chicks). Although all chicks became infected (100%) with B. anserina and died (100%), the first death did not occur until PI 8. Subsequent deaths occurred sporadically during the following 2 weeks. Duration of spirochaetemia ranged from 5-20 days (mean 12 ± 4.8 days).

Trial B (Day Old WLH Chicks). All chicks became infected (100%) with B. anserina and all died (100%). Deaths began on PI 8 and peaked on the following day. Last deaths occurred on PI 16. Duration of spirochaetemia ranged from 5-13 days (mean 7.4 ± 2.7 days).

Trial C (Day Old WLH Chicks). All chicks became infected (100%) with B. anserina and 40 of 41 died for a mortality rate of 98%. Exact duration of spirochaetemia was not determined. However, from the data a minimum of 1 day and a maximum of 13 days could have occured.

Trial D (17 Day WLH Chicks). Nineteen of 20 chicks became infected (95%) with B. anserina and all infected chicks died for a case fatality rate of 100%. Duration of spirochaetemia ranged from 3-7 days (mean 5.2 ± 1.1 days).

Trial E (5½ Week RIR Fowls). All fowls became infected (100%) with B. anserina but only 5 of 15 died (33%). Duration of spirochaetemia was not determined. Relapses did not occur. B. anserina was not found in any fowl after the initial disappearance of the spirochaetes.

Trial F (9 Week WLH Fowls). All fowls became infected (100%) with B. anserina although one bird did not become spirochaetemic until PI 10. It died 5 days later. Nine of 10 died (90%). Duration of spirochaetemia varied from 4-8 days (mean 5.9 ± 1.1 days).

Trial G-I (Adult Fowls). All fowls became infected (100%) with B. anserina and 11/15 died (73%). Duration of spirochaetemia varied from 3-5 days (mean 4.3 ± 0.9 days).

Clinical Findings. Clinical signs did not differ from those previously described (4) except in groups A-C (day old chicks) and group E. Clinical evidence of illness was less obvious in birds in these groups especially the day old chicks. The only abnormality observed in the chicks was stunted growth. Unless examined closely, the chicks appeared normal even though the blood was teeming with spirochaetes.

Breed. Birds in these trials were uniformly susceptible to B. anserina infection irrespective of breed. However mortality was considerably lower and duration of spirochaetemia longer in RIR than in WLH while the Harco x WLH were intermediate (Table 3).

Route of Inoculation. The method of inoculation did not influence susceptibility. However, mortality was lower and duration of spirochaetemia longer in intraperitoneally (IP) and subcutaneously (SQ) injected birds as compared with IM injections (Table 4).

Statistical Analysis. Statistically significant differences were found when comparing mortality of day old chicks and birds over 1 week of age ($P < .001$), mortality of RIR and WLH ($P < .001$) and mortality of SQ and IM inoculated birds ($P < .01$), duration of spirochaetemia of day old chicks and birds over 1 week of age ($P < .001$) and duration of spirochaetemia of RIR and WLH ($P < .01$). Duration of spirochaetemia in SQ and IM inoculated birds could not be evaluated because of a lack of data for trials C and E.

DISCUSSION

Birds of all ages were found uniformly susceptible to infection with the strain of B. anserina used in this study. There was, however, an age associated difference in mortality, clinical signs and duration of spirochaetemia. In day old chicks 98% of infected birds died, clinical evidence of disease was not obvious and spirochaetemias tended to persist for long periods. In growing fowls and adults, mortality was lower (73-78%), clinical signs were characteristic of spirochaetosis, and spirochaetemia was of shorter duration (Figs. 1, 2, 3). The results of this study further support the finding of Cantacuzene (6) and subsequent workers who found mortality from B. anserina infection to be high in younger birds. They do not support the observations of Rokey and Snell (16) who found mortality to be considerably lower in younger birds than older ones, nor do they support Cantacuzene's further observation that chicks die within 24 hours after onset of spirochaetemia.

Djankov et al. (8) also found a prolonged spirochaetemia to be characteristic of B. anserina infection in day old but not older chicks. They attributed the prolonged spirochaetemia to an inability of day old chicks to produce agglutinins. Factors other than agglutinins, particularly enhanced phagocytosis, were also suspected to be involved in the disappearance of spirochaetes from the circulation because in some birds, spirochaetes disappeared even when agglutinins could not be demonstrated. By comparing cumulative mortalities in this study for day old chicks and older birds infected with B. anserina (Fig. 4), it is evident that the general pattern of mortality is the same regardless of age, except that with day old chicks onset of mortality is delayed by 4-5 days and continues unabated approaching 100%. It would appear then that day old chicks have not yet developed the ability to control and arrest spirochaete infection which is found in older birds. The source of this resistance may well be related to the development of splenic function, particularly antibody

formation and phagocytosis. Considering the prominent role of the spleen in spirochaetosis, it may be of interest to note that antibody producing cells do not develop in the spleen until the 3rd to 7th day after hatching (10, 18).

The high mortality characteristic of B. anserina infection in day old chicks may also stem from the same developmental lag. Without the ability to control and arrest spirochaete infection, spirochaetemias reach high levels and persist until the protective functions develop at which time the spirochaetes are destroyed i.e. crisis occurs. Crisis may be abnormally severe in day old chicks because of the high spirochaetemias that have been allowed to develop. This would result in a high dose rate of physiologically active substances (e.g. endotoxins) if they are released from the destroyed spirochaetes. Experimental studies are needed to examine these possibilities.

From the data it would appear likely that RIR may be more resistant to spirochaetosis than WLH and that breed may be an important factor. It is a common belief in northern Nigeria that of exotic breeds, RIR are more resistant to disease than WLH. Indigenous Nigerian breeds have been reported to be more resistant to spirochaetosis than exotic breeds (19) but this has not been confirmed experimentally. Nigerian cockerels have been shown to be more resistant to Salmonella gallinarum than WLH (2). Differences in the rate and size of bursal development between the two breeds (1) were considered responsible. The same may be true for Nigerian fowls infected with B. anserina. Unfortunately, indigenous birds from tick and spirochaete free sources were not available. Studies on the resistance of these and other breeds to B. anserina are indicated.

Young birds were found to be susceptible to B. anserina in these trials and clinical cases of spirochaetosis should occur in this age group, but they apparently do not. Since younger birds

are not resistant to infection other explanations are needed to account for the apparent age distribution of clinical cases previously reported (4). Possibilities include a) greater owner interest in the more valuable, older bird b) rearing of chicks in separate (e.g. brooding) areas which are likely to be tick free if adults have not been kept there c) preferential feeding of the tick on older birds d) passively acquired maternal antibody. Further studies are needed to determine the factors responsible.

The trials were conducted whenever suitable birds and facilities were available which resulted in some variations in the conditions for each trial. In spite of these differences results of comparable trials were reasonably consistent and the influence age has on B. anserina infections is clearly evident. However, statistical analysis of the data indicated that breed and route of inoculation may also have been important factors. More sensitive techniques for determining spirochaetemia such as the use of buffy coat smears (3) may result in detecting even more profound differences in the duration of spirochaetemia in various age fowls and should be considered in future studies of this nature.

TABLE 1

Protocol for Trials Undertaken to Determine Influence **of Age on Experimental** Borrelia Anserina **Infection.**

Trial	Age	Breed	Number of Birds	Route of Inoculation	Number of Spirochaetes*	Day Blood First Examined	Date
Pilot	1 wk.	Harco x WLH	4	IP	NE	2	Mar. '72
A	1 day	RIR	8	SQ	$9.8x10^5$	3	Jan. '74
B	1 day	WLH	15	SQ	$1.7x10^5$	3	Jan. '74
C	1 day	WLH	41	IM	$3.6x10^5$	2	Aug. '74
D	17 days	WLH	20	IM	$3.6x10^5$	2	Nov. '74
E	5½ wks.	RIR	15	SQ	$9.8x10^5$	5	Dec. '73
F	9 wks.	WLH	10	IM	$3.6x10^5$	1	Aug. '74
G	6-12 mos.	RIR	3	IM	$2x10^4$-10^6**	1	May '74
H	6-12 mos.	RIR	4	IM	$3.9x10^6$	1	Jun. '74
I	6-12 mos.	4 RIR 4 WLH	8	IM	$3.6x10^5$	1	Oct. '74

Abbreviations: WLH = white leghorn, RIR = Rhode Island red, IP = Intraperitoneal, SQ = Subcutaneous, IM = Intramuscular, NE = Not examined.

* Blood containing spirochaetes was evaluated as 1-4+ which has been found to correlate with 10^{5-8} B. anserina/ml by direct microscopic counts.

** One bird each inoculated with 0.1 ml, 1:10 or 1:100 dilution whole blood.

TABLE 2

Influence of age on experimental Borrelia Anserina infection in fowls.

Age Group	Trial	No. Inoc.	Susceptibility	DEATHS First day	DEATHS Last Day	DEATHS Total No.	Days of Spirochaetemia Min.	Days of Spirochaetemia Max.	Days of Spirochaetemia Mean
Day Old	A	8	8(100)	8	22	8(100)	5	20	12 ± 4.8
	B	15	15(100)	8	16	15(100)	5	13	7.4 ± 2.7
	C	41	41(100)	7	16	40(98)	NE	NE	NE
	Total	64	64(100)	7	22	63(98)	5	20	9.0 ± 3.6
1-9 Weeks	Pilot	4	4(100)	4	10	3(75)	3	9	6.5 ± 2.7
	D	20	19(95)	4	8	19(100)	3	7	5.2 ± 1.1
	E	15	15(100)	4	8	5(33)	NE	NE	NE
	F	10	10(100)	6	15	9(90)	4	8	5.9 ± 1.1
	Total	46	45(98)	4	15	36(78)	3	9	5.6 ± 1.3
6-12 mos.	G	3	3(100)	-	-	0	4	5	4.3 ± 0.6
	H	4	4(100)	6	6	3(75)	3	5	4.0 ± 1.2
	I	8	8(100)	5	7	8(100)	3	5	4.5 ± 0.9
	Total	15	15(100)	5	7	11(73)	3	5	4.3 ± 0.9

( ) = %
NE = Not examined

TABLE 3

Comparison between breeds of fowls experimentally infected with Borrelia anserina.

Breed	Number	Infected No. (%)	Deaths No. (%)	Mean Spirochaetemia (Days)
RIR	34	34 (100)	20 (59)	7.5
HARCO X WLH	4	4 (100)	3 (75)	6.5
WLH	90	89 (99)	87 (97)	5.7

Abbreviations: RIR = Rhode Island Red
WLH = white leghorn

TABLE 4

Comparison between routes of inoculation in fowls experimentally infected with Borrelia anserina.

Route	Number	Infected No. (%)	Deaths No. (%)	Mean Spirochaetemia (Days)
SQ	38	38 (100)	28 (74)	7.7
IP	4	4 (100)	3 (75)	6.5
IM	86	85 (99)	79 (93)	5.6

Abbreviations: SQ = subcutaneous
IP = intraperitoneal
IM = intramuscular

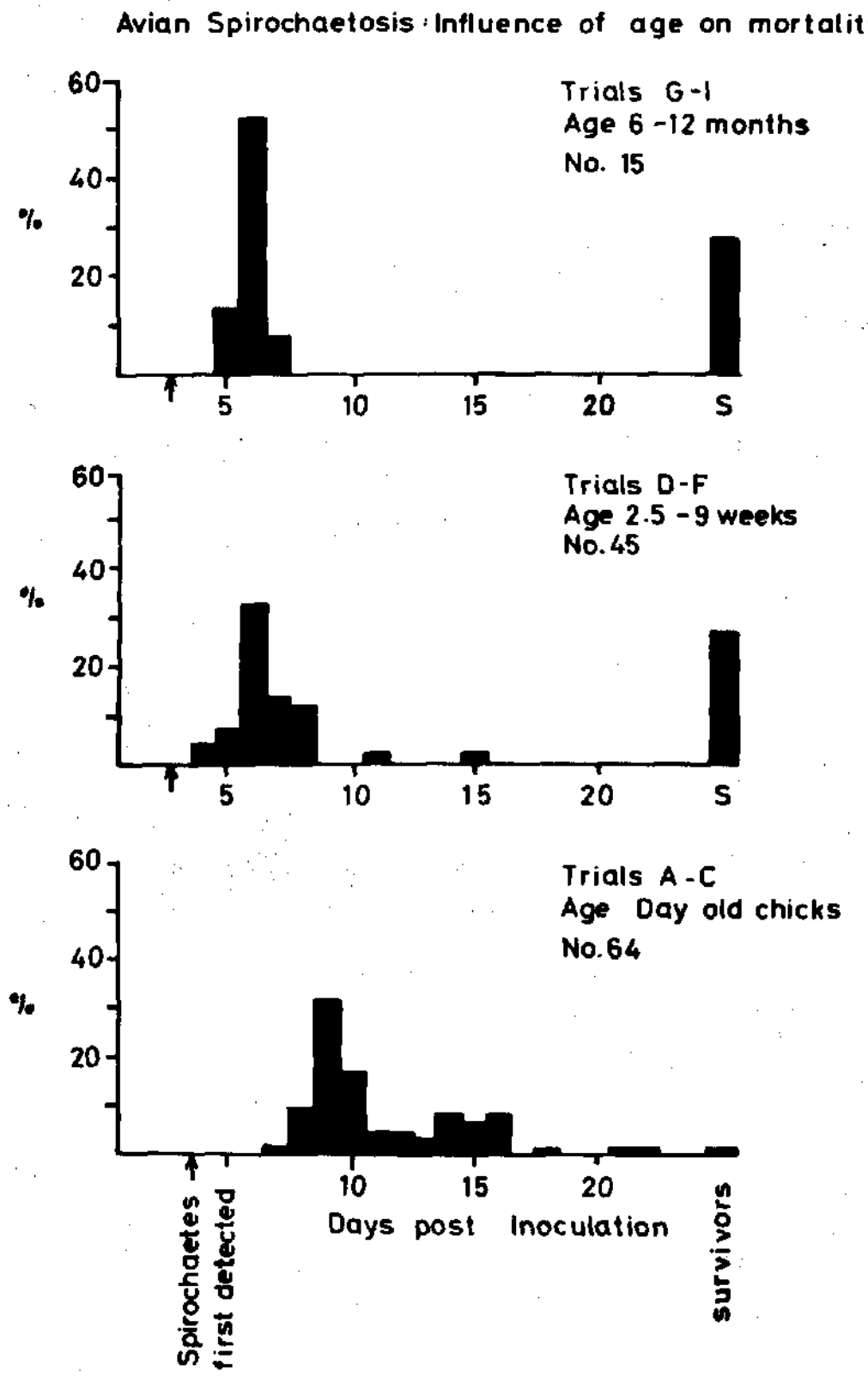
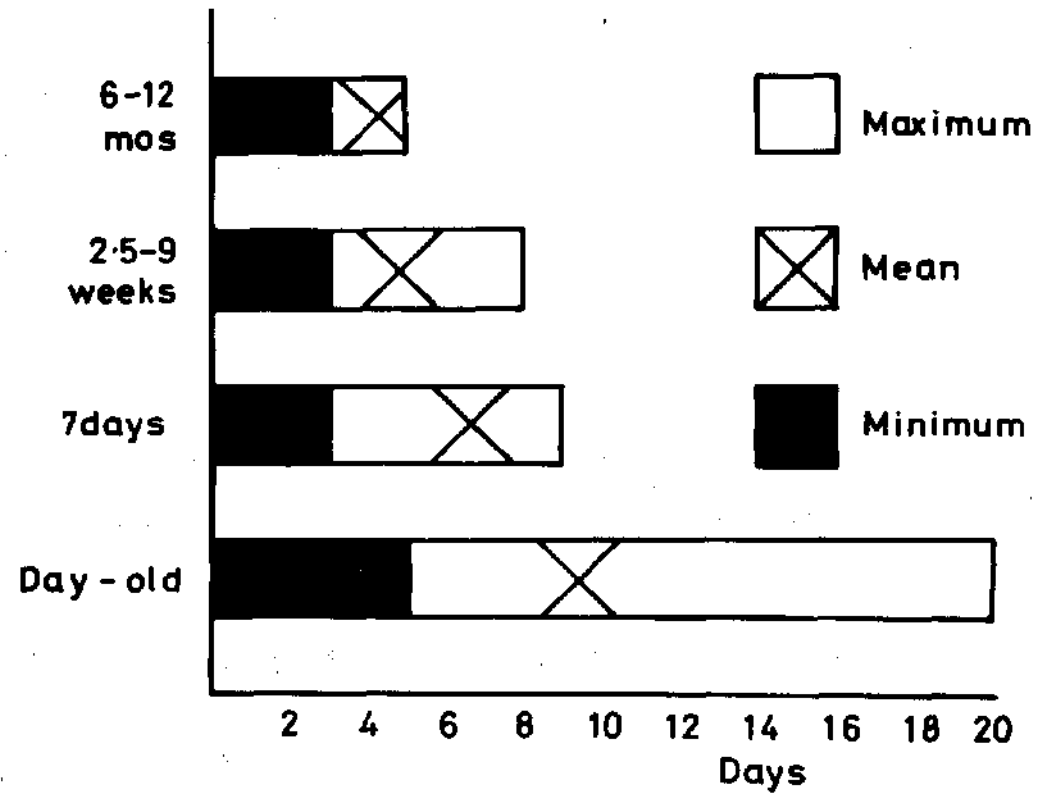

Avian Spirochaetosis · Influence of age on mortality
60
40
20
%
Trials G-I
Age 6 -12 months
No. 15
5
10
15
20
S
60
40
20
%
Trials D-F
Age 2.5 -9 weeks
No. 45
5
10
15
20
S
60
40
20
%
Trials A-C
Age Day old chicks
No. 64
Spirochaetes first detected
10
15
20
Days post Inoculation
survivors

FIG. 1

INFLUENCE OF AGE ON DURATION OF SPIROCHAETEMIA FOLLOWING EXPERIMENTAL BORRELIA ANSERINA INFECTION.



INFLUENCE OF AGE ON SUSCEPTIBILITY, MORTALITY AND DURATION OF SPIROCHAETEMIA FOLLOWING EXPERIMENTAL BORRELIA ANSERINA INFECTION.

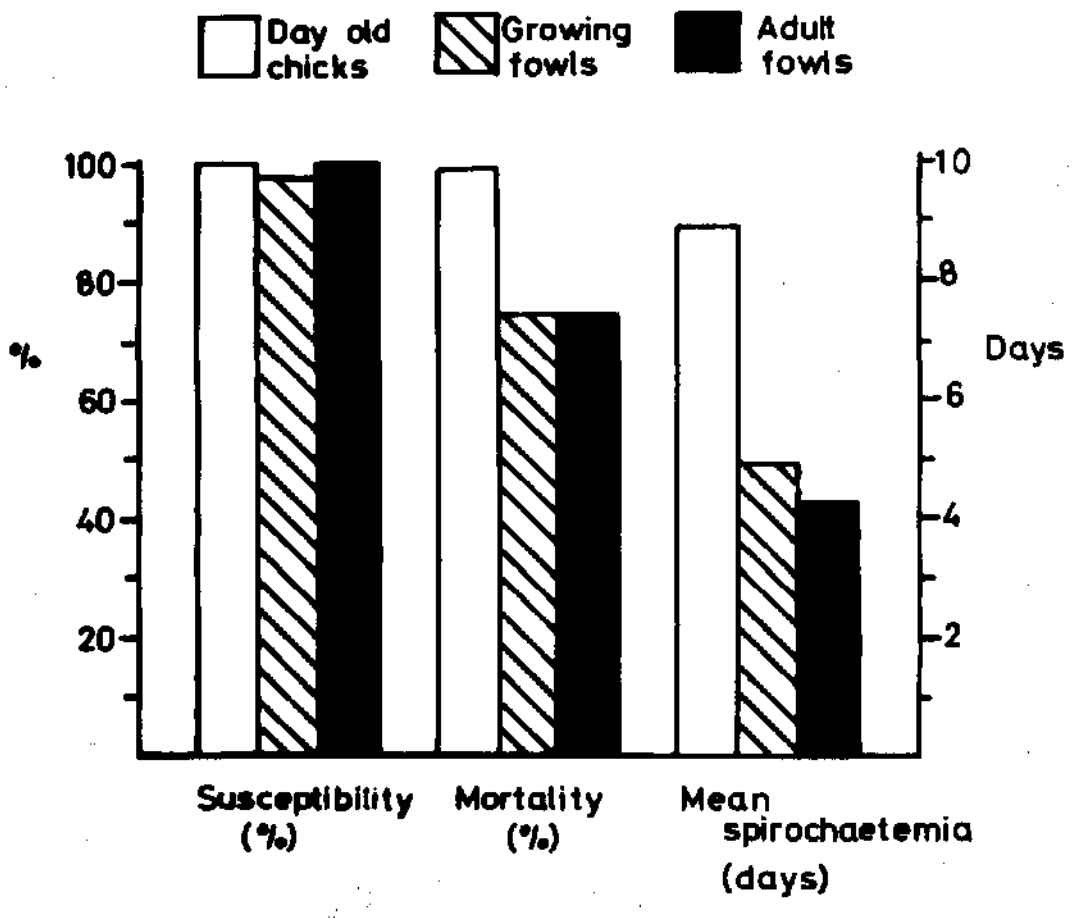


FIG. 3

CUMULATIVE MORTALITY IN VARIOUS AGE GROUPS OF FOWLS FOLLOWING BORRELIA ANSERINA INFECTION.

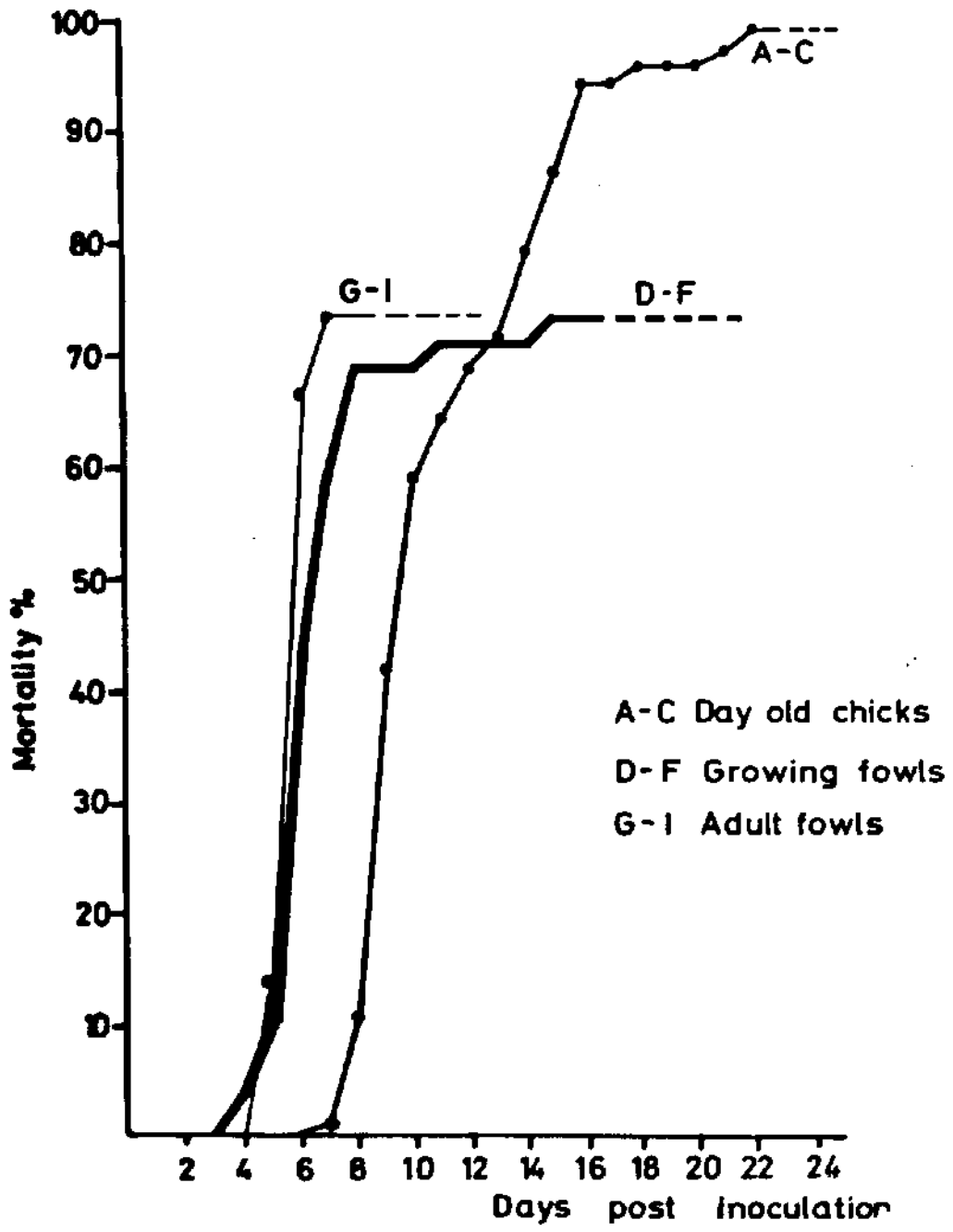


FIG. 4

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: INFECTION OF INTRODUCED AND RESIDENT CHICKS WITH *BORRELIA ANSERINA* AND *AEGYPTIANELLA PULLORUM* IN A TICK INFESTED ENVIRONMENT

SUMMARY

Eight 6-day old Rhode Island red chicks hatched from a tick and spirochaete free flock were placed with a group of 9 crossbred chicks of similar age that had been hatched and were being reared in a tick infested environment. The ticks were known to transmit Borrelia anserina since spirochaetosis was a problem in the flock. Subsequently the ticks were found to also transmit Aegyptianella pullorum. Two distinct patterns of infection with B. anserina and A. pullorum were found. In resident chicks A. pullorum infections occurred first beginning at 3 weeks of age while B. anserina infections did not occur until the birds were 6 weeks old and then continued through 10 weeks of age. In the introduced group B. anserina infections occurred first, within 2 weeks after introduction to the area, and A. pullorum infections first occurred 3 weeks later when the chicks had been exposed to ticks for 5 weeks. Larval ticks were found on all chicks throughout the study. No age preferential feeding habits could be ascertained although it appeared that more larval ticks fed on chicks. Mortality in the resident group as a result of infection with either B. anserina or A. pullorum was nil. In the introduced group, 5 of 8 chicks died during the trial but it was difficult to determine how many of the deaths resulted from infection because of the activity of predatory birds. The resistance of young resident chicks to B. anserina infection could be explained by passively acquired maternal antibodies against the spirochaete.

INTRODUCTION

In a previous report, spirochaetosis was stated to be a problem in fowls over 10 weeks of age in northern Nigeria and rare in birds below this age (2). The possibility of this observation being the result of an innate, age-related resistance in young chickens was studied by using chickens from tick and spirochaete-free sources. It was concluded that age did not influence susceptibility to Borrelia anserina infection but did alter mortality and duration of spirochaetemia (4). Preferential feeding of the tick vector, Argas persicus, on older birds or some other factor in natural infections was considered to be possible explanation. The opportunity presented itself* for introducing some young chicks from a tick and spirochaete free flock into a group of similar aged chicks that had been hatched and were being reared in a tick infested environment where spirochaetosis was known to occur. Observations on these two groups of birds are presented.

MATERIALS AND METHODS

In late November, 1973, eight 6-day old Rhode Island red chicks from a tick and spirochaete free flock were introduced into a group of 9 crossbred chicks of the same age that had been hatched and were being brooded naturally in a tick infested area. All chicks were housed together at night but not confined during the day. Spirochaetosis had caused considerable mortality previously in ducks and fowls in the flock. Some had recovered including the hen that had laid this clutch. During the 10-week period following introduction, both groups of chicks were examined at frequent intervals for larval ticks. Thin blood films were taken and examined as previously described (1).

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*The author wishes to express sincere appreciation to Dr. and Mrs. H. Whittle who made the facilities available for their cooperation with the many requests.

RESULTS

Infections with B. anserina were first found in the introduced group 2 weeks after they came in contact with larval ticks (Fig. 1). New infections continued until the chicks had been in the tick infested area for 6½ weeks. In the resident group, B. anserina infection was not diagnosed until the chicks were 6 weeks of age. Spirochaete infections continued until the end of the trial when chicks were nearly 10 weeks old.

During examination of blood films for Borrelia, concurrent infection with Aegyptianella pullorum was discovered. A. pullorum infection in introduced chicks was not found until 5 weeks after they had been in the tick-infested environment and continued for only an additional 2½ weeks. In the resident group, A. pullorum infections were first observed at 3 weeks of age and continued until the chicks were 8 weeks old. Infections with A. pullorum preceded B. anserina infections in resident chicks whereas the opposite was true for introduced chicks.

Mortality in introduced chicks as a result of infection with either B. anserina or A. pullorum was difficult to evaluate. Four introduced chicks fell prey to predators (black kites) or simply could not be found. Throughout the trial, 5 of the 8 introduced chicks died. No mortality attributable to either B. anserina or A. pullorum infection occurred in the resident group of chicks. The only death in this group happened early in the trial before there was any evidence of infection when one chick was attacked by a black kite.

All chicks in both groups and the hen were examined for larval ticks on the day after the introduced chicks had been mixed with the resident chicks and each time blood samples were taken. Numerous larvae were consistently found on the chicks but only few were found on the hen. None of the birds were ever found completely free of larval ticks.

Virtually no intermingling occurred between the two groups of chicks except when they were together in the poultry house at night. The hen ignored the introduced chicks and they also seemed to have little interest in the hen. While the resident chicks and hen went foraging during the day, the introduced chicks spent the day near the sleeping place. Resident chicks were protected to a large degree by the hen but the introduced chicks were not and easily fell prey to predators. They were particularly susceptible just after introduction or when infected with spirochaetes.

DISCUSSION

The consistent finding of larval ticks on the chicks does not support the suggestion that ticks may preferentially feed on older birds. This had been proposed as a possible explanation for the age distribution of spirochaetosis observed clinically (2). In fact the impression obtained throughout this study was that larval ticks may actually prefer to feed on young birds. This needs to be studied experimentally. It would appear from this study that spirochaetosis should occur as often in young birds as in older fowls. Since this has not been shown to be the case (2) other factors must be responsible for the age distribution of clinical cases.

Two distinct patterns characterized infection with B. anserina and A. pullorum in the two groups. In the introduced group B. anserina infections appeared first and were followed by A. pullorum infection. The opposite was true for resident chicks. These results indicate that resident chicks were resistant to B. anserina infection during the first 6 weeks while introduced chicks were susceptible. Passively acquired maternal antibody against B. anserina has been demonstrated (9). This would appear

to be the most likely explanation for the resistance of resident chicks to B. anserina infection compared with the lack of resistance in introduced chicks. Studies on the ability of passively acquired immunity to provide young birds with resistance against spirochaetosis are indicated. This could explain the reported finding (2) that spirochaetosis occurs infrequently in young fowls (under 10 weeks) in northern Nigeria.

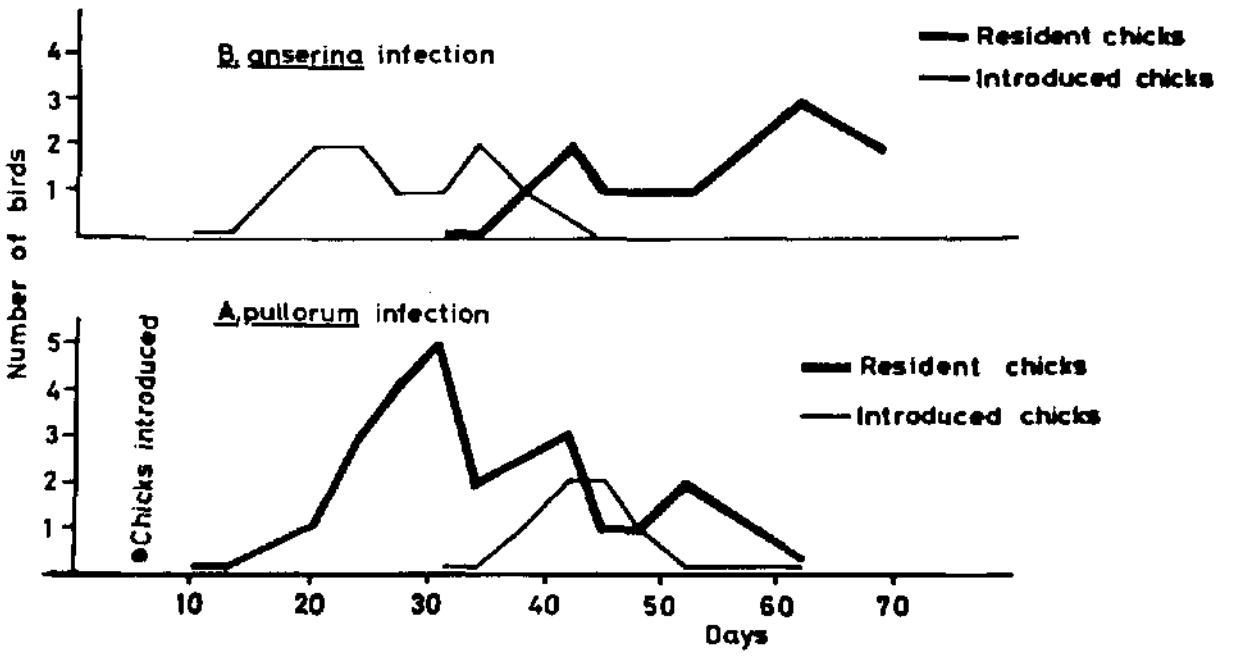
Balfour (5) first reported concurrent infections of B. anserina and A. pullorum in fowls. His work was primarily related to morphology and taxonomic relationship between the two organisms (6). The influence of the two organisms on each other and effect on the host compared to pure infections with each one apparently has not been investigated. A definite interaction between Borrelia and Trypanosoma in mammals has been shown (7, 8) and such an interaction may also occur between Borrelia and Aegyptianella. If such an interaction occurs, its influence in this study was not determined. However, it can be seen that Aegyptianella infections occurred when either passively or actively acquired anti-Borrelia antibodies were probably present.

In this study resident chicks appeared to be more susceptible to A. pullorum infection than introduced chicks. Even when the additional 6 days that resident chicks were exposed to ticks is taken into account, A. pullorum infection in the group of resident chicks still preceded that in the introduced group by at least 2 weeks. No explanation for this increased susceptibility is apparent from the data or literature.

In order to provide introduced chicks with the same mothering afforded to the resident chicks, future studies of this nature would be improved if fertile eggs were added to the clutch and all chicks allowed to hatch together. However, some means of distinguishing between introduced and resident chicks at hatching would have to be devised.

December and January have been found to be the months when B. anserina infections are lowest in northern Nigeria (3). Since this study was conducted during this period it is obvious that natural spirochaete infections do continue to occur during this period although probably less frequently than at other times of the year.

BORRELIA ANSERINA AND AEGYPTIANELLA PULLORUM INFECTIONS IN RESIDENT AND INTRODUCED CHICKS IN A TICK INFESTED ENVIRONMENT.



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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: IMMUNITY IN THE CHICK FROM PASSIVELY ACQUIRED MATERNAL ANTIBODY

SUMMARY

Chicks hatched from a fully susceptible flock and two flocks immune to Borrelia anserina were examined weekly for complement-fixing (CF) antibodies and resistance to challenge with Borrelia anserina. In immune chicks, CF titres progressively decreased and could not be detected past the fourth week. The chicks resisted infection with B. anserina until the 6th week. All control chicks had negligible CF titres and were susceptible to B. anserina infection throughout the experiment. It is concluded that the relatively greater resistance of young fowls to avian spirochaetosis is due to passively acquired maternal antibody and that in the absence of passive immunity they are susceptible to the disease.

INTRODUCTION

Levaditi (12) found embryos and chicks from hens that had recovered from spirochaetosis resistant to Borrelia anserina when exposed to infective blood. He concluded that the immunity was a passive one resulting from antibodies passed from the hen to the egg. Additional studies on resistance of chicks to B. anserina infection as a result of passively acquired maternal antibodies have been reported (8, 11). Perek and Berstein (13) found that chicks hatched from eggs laid by immunized hens had a considerable degree of immunity during the first 9 days of life. Revaccination of these chicks resulted in a 10-fold increase in antibody titre.

In contrast, 6 chicks hatched from eggs laid by hens that had recently been immunized against spirochaetosis did not resist infection when inoculated with virulent blood at 3-5 weeks of age. Four developed spirochaetosis and 2 died (14).

Agglutinius (13) and ability to inactivate spirochaetes (11) have been demonstrated in the blood of chicks hatched from immune hens. Antibodies have been demonstrated in the yolk of eggs from B. anserina immune hens (1, 13). In an earlier study (11), both the white and yolk had been found capable of inactivating B. anserina if the hens were immune to spirochaete infection. Al-Hilly (1) compared antibody levels in the yolk of eggs and serum from convalescent fowls by immobilization and immunodiffusion methods and found lower concentrations of antibody in the yolk.

In northern Nigeria naturally occurring cases of spirochaetosis have been reported to be uncommon in birds less than 10 weeks of age (4). Studies using birds from tick and spirochaete free flocks failed to demonstrate innate resistance of young birds to either natural (6) or experimental (5) infection with B. anserina. In fact, young birds were fully susceptible and suffered a high mortality. However, when chicks were hatched from eggs produced

by hens subjected to constant tick challenge and were reared in the same tick infested environment, they resisted infection with B. anserina until 6 weeks of age and none died from the infection (6). It was suspected that passively acquired maternal antibody may have been responsible. This study was undertaken to determine its role in providing young fowls with resistance to B. anserina infection.

MATERIALS AND METHODS

Source of Chicks. Fertile eggs were collected from 3 separate flocks of white leghorn fowls and hatched at the Faculty of Veterinary Medicine, Ahmadu Bello University. Flocks A and C were maintained at the Veterinary Faculty in separate areas while flock B was kept by a private individual after the primary immunizing series.

Flock A and B fowls were infected with B. anserina strain H (5) at 8 weeks of age. All became infected and were treated by medicating the drinking water with oxytetracycline* at recommended levels for 3 days beginning on the 3rd day of spirochaetemia. Three additional injections of 0.2 ml infective blood were given intramuscularly at weekly intervals following recovery. Spirochaetemia did not result from these subsequent exposures.

Between 16-18 weeks of age, mortality began to occur in flock A. Tick infestation and spirochaetosis were found. The source of the ticks was traced to a fowl being kept at the Veterinary Faculty that had been obtained in Niger. The birds were not treated and the outbreak was allowed to run its course resulting in a 50% mortality. The strain of B. anserina was isolated and designated strain L. The flock remained infested and was constantly exposed to ticks during the period when eggs were collected for hatching.

At 26 weeks of age, each fowl in Flock B was given 1 ml allantoic fluid intramuscularly that contained an estimated 10^9 spirochates/ml. A severe outbreak of spirochaetosis followed resulting in 2 deaths and a cessation of egg production. Sufficient numbers of eggs could not be obtained until 4-6 weeks after inoculation.

*Terramycin, Animal Formula - Pfizer, Ltd.

Flock C was a breeding flock at the Veterinary Faculty that had always been free of ticks and spirochaetes. Chicks from this flock served as controls.

Method of Exposure. Beginning on the day of hatching, 1-4 chicks from each flock were inoculated intramuscularly with 0.2 ml infective blood (strain H) diluted in sterile phosphate-buffered saline with 0.2% glucose to contain 10^3-10^4 motile spirochaetes/ml. Counts of spirochaetes were done in triplicate using darkfield microscopy and a Petroff-Hauser counting chamber.

Serology. Blood was collected from each chick just prior to exposure. After the sera from each chick had been separated, they were pooled to make a composite sample for each flock. Sera were examined for complement-fixing (CF) antibodies as previously described (2).

RESULTS

Chicks from Flocks A and B had high initial CF titres (1:1024) on day 1 that decreased progressively to less than 1:4 at 5 weeks of age remaining negligible ($<$1:4) through the 8th week (Fig. 1). CF titres equal to or in excess of 1:4 were never found in chicks from flock C.

Chicks less than 6 weeks old from flocks A and B resisted spirochaete infection. One of 4 chicks from these flocks exposed at 6 weeks of age became infected and died. All birds from flocks A and B were infected and died during the 7th and 8th week. All chicks from the control flock (C) became infected throughout the trial with a cumulative case fatality rate of 14/18 (78%) during the 8 week period.

The presence of CF antibodies equal to or in excess of 1:4 correlated well with resistance to B. anserina infection except during the 5th week after hatching when CF antibody was not detected but chicks were refractory to infection.

to provide the chick with protection against infection. In their study 4 of 6 survived, 2 without becoming infected which does represent a degree of resistance, particularly if the resistant chicks were the ones exposed at 3 weeks of age. Except for the duration of resistance, their results would not be inconsistent with the results presented here.

Resistance of younger birds to spirochaetosis as a result of passively acquired maternal antibodies provides at least a partial explanation for the paucity of clinical cases in young birds in northern Nigeria (4). In this study, birds were not fully susceptible until 7 weeks of age. The longer incubation period following natural exposure compared with experimental inoculation (10) would further tend to increase the age of birds when infected.

The role of passive maternal antibody in providing natural immunization would also appear to be important. In a previous report (6), chicks hatched from eggs laid by an immune hen and exposed continuously to infected ticks experienced infection but no mortality. In this study all chicks hatched from eggs laid by immune hens but only exposed to B. anserina on a single occasion, died during the

7th and 8th weeks. Although the difference may have been influenced by the degree of exposure i.e. natural vs experimental inoculation, it would appear more likely that if passively immune chicks were exposed frequently to B. anserina there could be a point where the waning level of antibody would allow infection to occur but modify the infection to prevent death. The chick would develop a naturally acquired active immunity.

If natural immunization does occur in this type of situation, then closed flocks where replacement chicks are produced by natural brooding should not have a problem with spirochaetosis after the initial outbreak even if infested with infected ticks since recovered fowls and their offspring should be immune. Only if susceptible birds were introduced into this flock or fowls from this flock were introduced into a susceptible flock would a problem with spirochaetosis result. From clinical observations in northern Nigeria (4) and reports from other parts of the world (7, 9) this would appear to be the case. Spirochaetosis should then be considered a greater problem when the status quo of flocks is constantly being disturbed. This situation is most apparent in market fowls in northern Nigeria which offers yet another reason why spirochaetosis is so common in these birds (3).

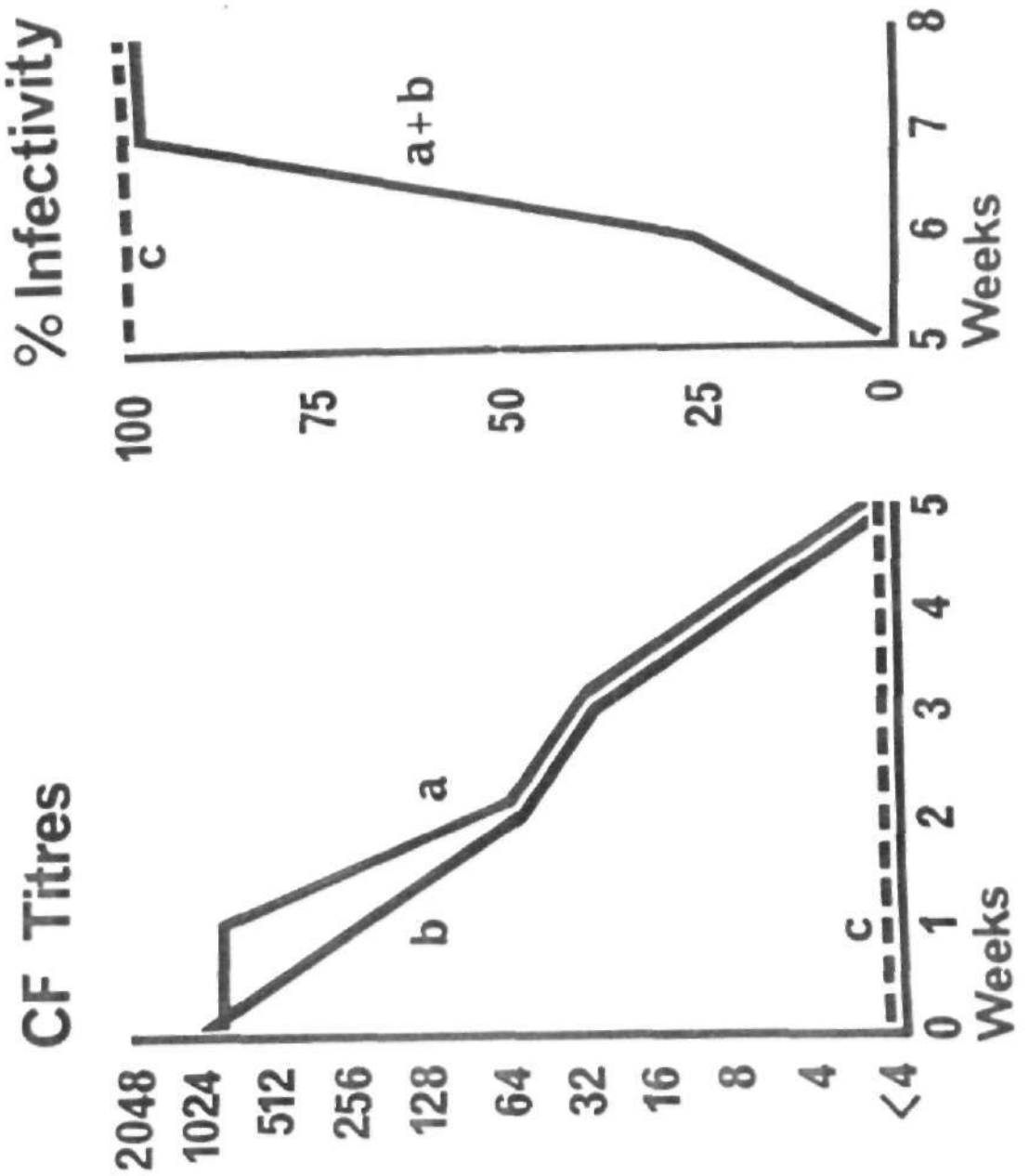
Mortality has been reported previously to be greater in younger fowls infected with a Nigerian isolate of B. anserina (5). Further support for this finding is provided by the control group of chicks used in this study in which 14 of 18 died. Of the 4 survivors, 3 were over 4 weeks of age.

All chicks in which CF titres equalled or exceeded 1:4 were found capable of resisting B. anserina infection. There was a period, however, when chicks from immune flocks resisted infection but CF antibodies were not detected. Complement-fixing antibodies may have been sufficient to provide protection but could not be detected with this test, or more likely, CF antibodies were not the only ones responsible for resistance. Studies detecting

different types of antibodies could show different results.

Because of the common occurrence in northern Nigeria and high mortality characteristic of avian spirochaetosis, a vaccination programme has been suggested (3) as an addition to currently used control methods. It was further suggested that vaccination be done between September and January so that fowls would have developed sufficient immunity to protect them during March through July when the disease is most common (3). From this study, vaccination of hens before the laying season (generally March through September) would provide protection of the hens and could also provide a degree of protection to hatched chicks. If vaccination of adult fowls against spirochaetosis is undertaken in northern Nigeria, the possible protective influence on the chick should be investigated.

COMPLEMENT-FIXING ANTIBODY TITRES AND SUSCEPTIBILITY OF CHICKENS FROM IMMUNE HENS TO BORRELIA ANSERINA INFECTION AT WEEKLY INTERVALS AFTER HATCHING.



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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA:

DURATION OF IMMUNITY

SUMMARY

Eleven fowls which had recovered from avian spirochaetosis caused by a Nigerian isolate of Borrelia anserina and which had been kept in a tick free environment were exposed to the homologous isolate 18 weeks later. One fowl experienced a transitory spirochaetemia of 2 days duration. The spirochaetemia was accompanied by an increase in cloacal temperature to 109°F and an abrupt fall in packed cell volume of the blood. Control birds inoculated simultaneously developed typical avian spirochaetosis except mortality was nil. A flock of 10 fowls that had been immunized with a series of 4 injections of B. anserina and a booster dose 14 weeks later were exposed to the homologous isolate 5 months after the booster dose and they completely resisted infection. The use of buffy coat smears was found to be advantageous for detecting low spirochaetemias. It is concluded that birds which recover from spirochaetosis caused by Nigerian strains of B. anserina probably possess at least some resistance to avian spirochaetosis for at least 18 weeks following recovery.

INTRODUCTION

Duration of immunity to Borrelia anserina following a variety of vaccination procedures has been studied by several investigators (6,7,8,11,14,15). Fowls have been proven immune for periods of 6-18 months after vaccination. When vaccinated birds were constantly exposed to infected ticks, immunity was considered to be lifelong (9). The duration of immunity in fowls that have recovered from natural infections has been less extensively studied. It has been reported that immunity in these birds can last as long as 13 months (5) or may even be lifelong (10). Knowles et al. (12) reported recovered fowls to be solidly immune but did not report the duration of this immunity. McNeil et al. (13) were unable to infect an adult turkey on 2 occasions during an 18 month period after recovery.

In this paper, the immunity of a naturally recovered and an experimentally immunized flock kept in tick and spirochaete free environments for 18 weeks and 5 months respectively after recovery or immunization was tested by exposure to B. anserina.

MATERIALS AND METHODS

Flock A. Fifteen 5½ week old Rhode Island red (RIR) chicks were inoculated subcutaneously with 0.05 ml whole blood containing B. anserina, strain H (3). All became infected and 5 died. On post-inoculation day (PI) 7, two drops of blood from an infected chick with high spirochaetemia were instilled into each eye of 3 additional chicks from the same flock. They all became infected, 1 died and 1 was sacrificed for another study. The remaining bird recovered and was added to the 10 birds that had previously recovered. The flock was maintained free of ticks and spirochaete infection. Eighteen weeks after recovery each fowl was inoculated intramuscularly with 0.1 ml whole or diluted blood containing approximately 2×10^4, 10^5, or 10^6 viable B. anserina as sub-

sequently determined by titration in chick embryos. Three additional RIR from the same original flock that had not had prior exposure to B. anserina were inoculated in the same way and served as controls Pack cell volumes (PCV), cloacal temperatures, clinical signs of disease and spirochaetemia determined by wet smears and buffy coat smears (1) were recorded for each of the 14 birds daily. Serum samples were taken on PI-2, PI 4, and PI 14. Unfortunately these were lost in a subsequent power failure.

Flock B. The flock consisted of 10 adult white leghorn (WLH) fowls which had been previously immunized and used for a source of B. anserina, strain H, antiserum as previously described (2). The flock was free of ticks and natural infections of spirochaetosis had never occurred in the birds. Five months after the 1st exposure of B. anserina each bird was given 3.6×10^5 viable spirochaetes by intramuscular injection of 0.2 ml whole blood diluted 1:100 in sterile phosphate buffered saline (pH 7.2) containing 0.2% glucose. Four susceptible adult WLH and 4 susceptible adult RIR were inoculated at the same time and served as controls. Cloacal temperatures, buffy coat smears, and clinical signs were determined daily.

RESULTS

Flock A. One of the 11 recovered fowls had a transitory spirochaetemia on PI 4 and 5. This fowl had received 10^3 spirochaetes. On PI 4 spirochaetes were only found in buffy coat smears while on PI 5 they were found in both buffy coat and direct wet smears. The fowl did not show clinical signs of infection, although the cloacal temperature was 109°F on both days (Fig. 1). This was approximately one degree above its pre-exposure baseline temperature. The temperature fell to 107°F after infection and gradually returned toward the pre-exposure level. The PCV dropped on the initial day of infection, recovered somewhat the following day

and then fell again after spirochaetes could no longer be found in the blood. The other 10 recovered fowls that did not become infected did not show any marked deviations in PCV or cloacal temperatures.

All 3 of the control fowls became infected. The disease in the control birds was typical of avian spirochaetosis except that none of the fowls died as a result of infection. The fowl that received 10^5 spirochaetes became infected and recovered one day earlier than the 2 that had received 10^3 and 10^4 spirochaetes. The onset of spirochaetemia was first detected in buffy coat smears of 2 of the control fowls. In the third fowl spirochaetes were found in the wet smear and buffy coat on the same day, however, the organisms were much more numerous in the buffy coat which was found to be teeming with organisms. Spirochaetemia lasted for 5 days in each of the birds. Changes in the mean PCV of control group fowls were similar to those of the fowl from the recovered group that became infected. After spirochaetemia began, the PCV fell rapidly, recovered slightly and then continued to fall. The highest cloacal temperature recorded in the control group was 110°F which occurred in one fowl on the second day of spirochaetemia.

Flock B. All the immunized fowls in this group resisted exposure to B. anserina while all the control fowls developed acute spirochaetosis and died.

No marked deviations of the mean cloacal temperatures of the immunized fowls occurred although a slight increase was observed on PI 4 which was the same day that the mean cloacal temperature of the control group was highest (Fig. 2).

Spirochaetemia was first detected in 2 control fowls by buffy coat smears only, while in the remaining 6 fowls both wet and buffy coat smears were found positive simultaneously. The mean cloacal temperature began to rise even when spirochaetes could

only be found in buffy coat smears of one fowl. The mean cloacal temperature continued to increase and reached a peak on the second day, fell on the third day, rose again on the fourth day but not as high as initially, then fell to near normal just before death. The curve produced was biphasic with the first peak exaggerated.

DISCUSSION

In this study it was found that fowls which had recovered naturally from avian spirochaetosis induced by a Nigerian isolate of B. anserina and kept in an environment free of fowl ticks were at least partially resistant when exposed at 18 weeks after recovery. It was further shown that an immunized flock was fully resistant to infection with the homologous strain of B. anserina for at least 5 months.

All fowls in flock A which had recovered naturally from avian spirochaetosis were protected against mortality at 18 weeks. However, one had a transitory spirochaetemia for two days. A marked rise in body temperature and fall in PCV accompanied the spirochaetemia in the infected fowl even though very few spirochaetes appeared in the circulation. Although only one bird, this finding provides additional support for the observation presented in companion studies that spirochaetes have a tremendous capacity to affect physiological functions even when present in small numbers (4).

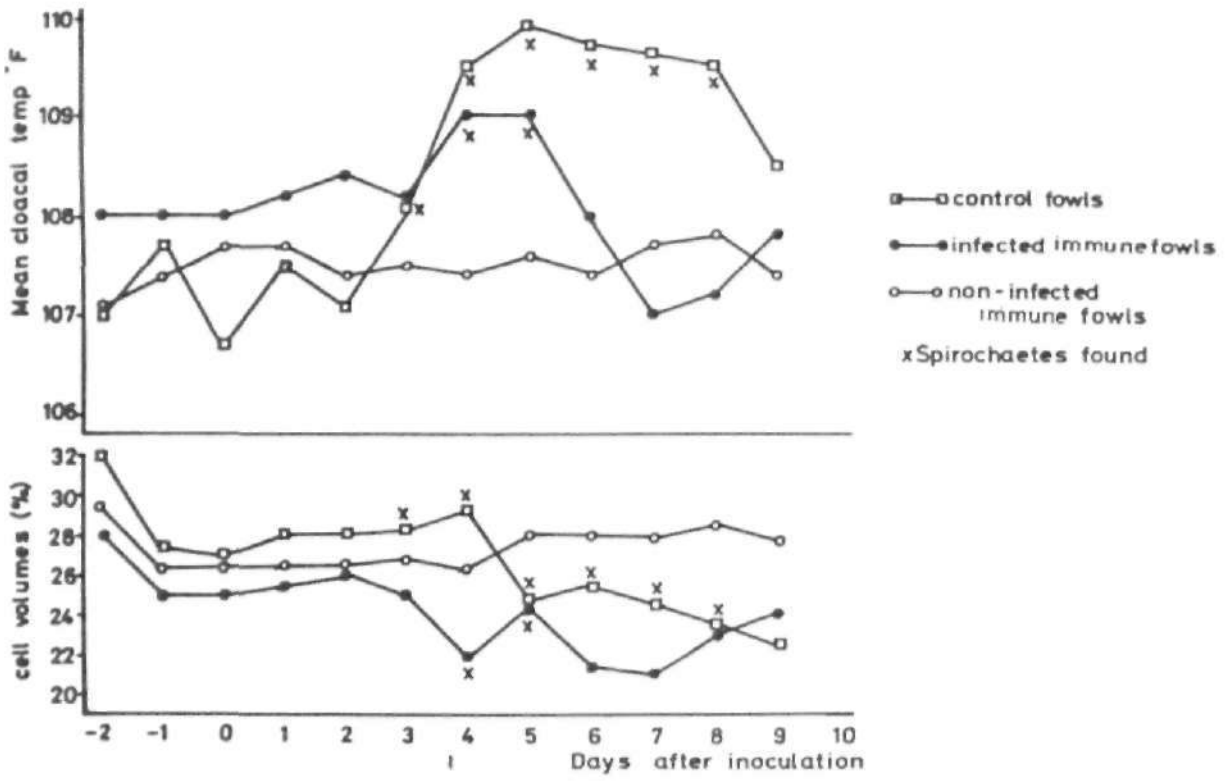
It could not be determined if this particular fowl had initially been infected subcutaneously or intraocularly but there is no evidence from the avian spirochaetosis literature that route of infection influences immunity provided the fowl becomes infected and recovers. Also, the original course of infection and percent mortality were identical for the two groups even though exposed by different routes. Nonetheless, the role route of infection may play in immunity should be examined.

The initial drop in PCV that occurred in all recovered and control fowls in flock A probably resulted from the blood drawn on PI-2 for serum since this change occurred before the birds were exposed to B. anserina.

The immunization procedure used in flock B was extensive and would not be of any practical value for immunizing large numbers of birds in the field. However, the results do show that immunization against spirochaetosis is possible for fowls in northern Nigeria. Developing and testing of various vaccines remains to be accomplished. When this flock was exposed, a slight temperature rise contemporary with a temperature rise in the infected control birds was found. Whether this occurred as a result of exposure or was due to other factors could not be determined. No evidence of spirochaetemia was obtained but the possibility that a few spirochaetes may have been multiplying in tissues remains as a possible explanation.

The advantages of using buffy coat smears (1) to detect spirochaetemia in experimental studies was clearly demonstrated in this study. Onset of spirochaetemia was first detected by this method in the recovered bird that experienced a transitory infection and also in 4 of the 11 fowls in the control groups. This finding supports the suggested applicability of the test for early detection of spirochaetemia.

BORRELIA ANSERINA INOCULATION OF FOWLS THAT HAD RECOVERED FROM AVIAN SPIROCHAETOSIS 12 WEEKS EARLIER.



BORRELIA ANSERINA INOCULATION OF FOWLS THAT HAD BEEN IMMUNIZED AGAINST SPIROCHAETOSIS 5 MONTHS EARLIER.

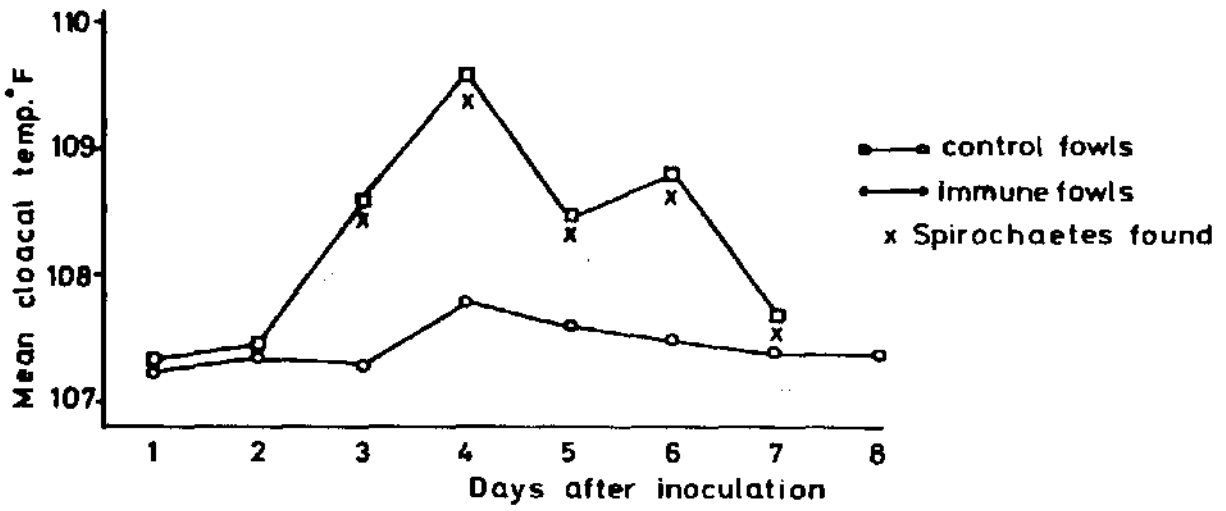


FIG. 2

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA:

BORRELIA ANSERINA INFECTION IN CHICK EMBRYOS

SUMMARY

Embryos between 6-18 days of age could be infected with Borrelia anserina when inoculated via the appropriate route, yolk sac or allantois. Inoculation into the yolk sac of 6-9 day embryos was easier and more likely to result in infection. The ability of spirochaetes to penetrate either the shell or the shell membrane of the air space could not be demonstrated. When 6 and 9 day embryos were infected, deaths began 3-4 days later, reached a peak on the 5th day and continued until the 7th or 8th day. Mortality of infected 6 and 9 day embryos was 100%. Maximum numbers of spirochaetes occurred on the 5th day when mortality was highest. Spirochaetes were associated with the blood and could be demonstrated in blood contaminated allantoic fluid or smears of heart blood. A striking marbled appearance of the liver, splenomegaly and nephritic changes were typical findings in embryos that had died of spirochaete infection. Sodium penicillin in amounts of 100-2000 units/embryo suppressed spirochaete growth but was not effective in eliminating the infection. Inoculation of 15 and 18 day embryos resulted in chicks hatching with spirochaetemia. Approximately half of these chicks recovered which is in sharp contrast to the 100% mortalities occurring in younger embryos and day old chicks. It would appear likely that the study of B. anserina infections in developing fowl embryos could be useful in elucidating basic mechanisms of spirochaete pathogenicity and role of the host immune system in Borrelia infections.

INTRODUCTION

Levaditi, 1906 (13), reported that Borrelia anserina would grow in fertilized fowl eggs that had been incubated for 3-4 days. Growth could not be established in unfertilized eggs, fertile eggs from immune hens or when the spirochaetes were placed in the albumin rather than the yolk. Growth rarely occurred in eggs that had been incubated for only 1 or 2 days. Spirochaetes continued to multiply in the vascular system and tissues of the embryo for 6-8 days at which time the embryo died. In contrast to infection in adult fowls certain events, intravascular aggregation of spirochaetes, crisis, disappearance of spirochaetes and recovery never occurred in embryos. The liver was reported to contain the greatest number of spirochaetes and to be most affected, characterized by intense inflammatory, degenerative and haemorrhagic lesions. Levaditi further compared avian spirochaetosis of embryos with hereditary syphilis of man.

A number of subsequent investigators have reported successful cultivation of B. anserina in developing fowl embryos. Cultivation in turkey embryos has also been reported (16). A spirochaete causing mortality in pheasants was successfully cultivated in fowl embryos (14). Although this organism was found to differ somewhat from a turkey isolate of B. anserina, it was suggested that it was probably just a different serotype. A strain of B. anserina isolated from ducks has been grown in fowl embryos (18). The technique has found wide application in diagnosis (9, 12, 14), maintenance of cultures (15, 16), and vaccine production (4, 5, 7, 8, 11, 17, 19, 20, 21). McKercher (15) presented a detailed description and results of a simplified technique of inoculating the yolk sac of the fowl embryo with infective blood. The growth of B. anserina in embryos would also appear to have potential value for studying the pathogenesis and immunologic aspects of avian spirochaetosis.

Cultivation of mammalian borrelias in developing fowl embryos has been reported (3, 6). This has been found to be particularly

useful in the diagnosis of B. recurrentis infection (louse-borne relapsing fever) since relatively few laboratory animals are susceptible to this organism. Immune tolerance to B. hispanica has been studied using fowl embryos (10).

Although the technique of growing B. anserina in fowl embryos has been extensively used and widely applied, only the original report of Levaditi and observations of a few subsequent investigators (7, 8, 12) deal with the effect of B. anserina on the developing embryo. The purpose of this paper is to report the results of studies on B. anserina infection in fowl embryos.

MATERIALS AND METHODS

Strain of Borrelia anserina. Strain H (2) was used for all experiments.
Source of Eggs. Fertile eggs were collected from a breeding flock of white leghorns that had been maintained at the Faculty of Veterinary Medicine, Ahmadu Bello University. There was no history of tick infestation or avian spirochaetosis in the flock during the previous 3 years.
Incubation of Eggs. Eggs were incubated in a Robbins "Hatchomatic" (R) at the Faculty of Veterinary Medicine, Ahmadu Bello University. During some experiments inoculated eggs were kept in a bacteriology incubator that had been modified for incubating eggs. All experimmental eggs were candled daily. Any dead embryos were examined for presence of B. anserina. Deaths during the first 48 hours were considered to have resulted from inoculation technique.
Inoculation. Incubated eggs were determined to be fertile by candling. The size of the embryo was noted and any under or over sized embryos were not used. The eggs were placed large end up in a fiber egg tray and the upper 1/3 of the egg thoroughly disinfected with either 70% alcohol or tincture of iodine and allowed to dry. In the center of the air space, the shell was pierced with a heat-sterilized egg pick or tip of a 21 gauge needle that had been forced

through a rubber stopper. The inoculum was infected into the yolk sac or allantois through a 1 inch 23 gauge needle fitted to a 1 ml tuberculin syringe. The hole was sealed with either cellophane tape, colloidin or melted wax and the egg returned to the incubator. Routes and dosages used for the studies are presented in Table 1.

Examination. Embryos were examined on various post-inoculation days (PI) by first disinfecting the upper 1/3 of the egg with 70% alcohol and removing the shell over the air sac with sterile forceps. To examine blood for spirochaetes, the shell and chorioallantoic membranes were pinched with a pair of sterile rat tooth forceps until a drop of blood appeared. The membrane was removed and blood from the ruptured allantoic vessels allowed to mix with the allantoic fluid. The bloody allantoic fluid was aspirated and the embryo and yolk placed into a sterile Petri dish. In some experiments samples of yolk were examined in which case they were diluted approximately 1:10 with physiological saline. The embryo was dissected and in some trials wet smears from the heart and liver were prepared and examined. Smears were examined by darkfield microscopy. Chicks that hatched after being inoculated as embryos were examined for spirochaetemia by wet smears as previously described (1). Spirochaetemia was determined at X300 (HPF) and rated as negative (0/10 HPF), + (1-10/10 HPF), ++ (1-5/HPF), +++ (5-50/HPF) or ++++ (over 50/HPF).

Statistical Analysis. The t-test was used where appropriate.

RESULTS

Age, Route of Inoculation and Determination of Spirochaete Infection in the Embryo. (Trials 1-5).

Trial 1. Nineteen of the 21 inoculated embryos became infected with B. anserina. The 2 embryos that failed to become infected had been inoculated by the allantoic route; one on the 9th day and the other on the 12th day of incubation. Spirochaetes were found in the yolk of 9 (47.4%) embryos, allantoic fluid of 17 (89.5%) embryos, liver of 17 (89.5%) embryos and heart blood from all infected embryos

examined (Table 2). The number of spirochaetes in the allantoic fluid varied directly with the degree of haemorrhage that occurred and was lower in embryos that appeared to have been dead for longer periods of time. Spirochaetes were usually most numerous in heart or liver smears. One inoculated embryo was found to have unilateral anophthalmia with a deviation of the maxilla. Three of the 4 control chicks hatched and were all free of spirochaetes.

Trial 2. All 11 embryos became infected and died between PI 4 and PI 7 (Table 2). Spirochaetes were found in the yolk of 5 embryos, allantoic fluid of 8 embryos and liver and heart smears from all embryos. Spirochaetes were most numerous in heart blood.

Trial 3. One embryo died 2 days after inoculation and was found to be contaminated when examined. No spirochaetes were found and it was discarded from the trial. The remaining 27 embryos all became infected and died (Figs. 1,2). Spirochaetes were found in the allantoic fluid of 21 (77.8%) embryos. Generally the fluid was clear and spirochaetes numerous, however, in one sample the allantoic fluid was turbid and only a few spirochaetes were found after the fluid had been diluted. In the remaining embryos in which spirochaetes were not found in the allantoic fluid, the fluid was very turbid. When examined by darkfield microscopy, numerous, small, highly refractile globules were found.

Trial 4. Of the 31 six day embryos, two died on PI 1 from environmental causes. The other 29 became infected with spirochaetes. One egg was accidentally broken on PI 3. Although the embryo was alive and infected with spirochaetes, it was discarded from the trial. Six of the 39 nine day embryos died on PI 1 from environmental causes and one died on PI 3 of unknown cause. It was negative for spirochaetes. The remaining 32 became infected. Spirochaetes were found in the allantoic fluid or heart smears from all infected embryos. The age at death is shown in Figures 1 and 2.

Spirochaete Infection in Older Embryos and Hatched Chicks (Trials 5-7).

Trial 5. Thirteen chicks hatched out of the 36 embryonated eggs. All 5 hatched from the control group, 5 from the eleven 18 day embryos and 3 from the twenty 15 day embryos. Two chicks which were not infected drowned the day after hatching. One chick in each of the inoculated groups was found to be infected with B. anserina when first examined on the second day after hatching. The chick that had been infected as a 15 day embryo (chick A) became negative for B. anserina on the 6th day after hatching while the chick that had been infected as an 18 day embryo (chick B) became negative on the 15th day after hatching. All 11 chicks became infected when inoculated with B. anserina at 4 weeks of age. The nine chicks that had not been infected at hatching all died of spirochaetosis between PI 3 and PI 6. Chick A had a prolonged illness and died 11 days after inoculation. This chick had a massive spirochaetemia during most of the period. Chick B experienced a mild infection and recovered on PI 5. It was the only chick to recover during the trial.

Trial 6. Twenty-four chicks hatched from the thirty-seven 18 day embryos that had been inoculated. Twenty-one of these were spirochaetemic on the day of hatching. An additional chick tried to hatch and was helped from the shell on the 2nd day after the other chicks had hatched. It was also spirochaetemic but died the following day. It, along with the 3 non-infected chicks, was not included in the remainder of the trial. Eleven of the twelve 18 day control embryos hatched. One died on the 3rd day after hatching from a non-specific yolk sac infection. All control chicks were negative for spirochaetes throughout the trial.

Two distinct periods of mortality occurred; one between PI 5-7 and a second between PI 13-15 (Fig. 3) for a case fatality rate of 10/21 (47.6%). Spirochaetes were extremely numerous in the blood.

The microscopic field (X300) would be found teeming with spirochaetes even in diluted blood. All surviving chicks remained spirochaetemic until PI 8. On PI 9 four chicks were negative for spirochaetes, however, spirochaetemia was detected in 2 of these on PI 10. One remained spirochaetemic through PI 12 but was negative on PI 14. After PI 10 there was a progressive decline in the number of chicks with spirochaetemia (Fig. 3). All chicks that died were spirochaetemic before death. Of the 10 chicks that died, 5 were examined for spirochaetes at postmortem and all were found positive. Further observations were not possible because the chicks were stolen from the laboratory on PI 22.

Trial 7. A total of 28 exposed and 7 control chicks hatched. Four of the exposed chicks hatched late and had to be assisted out of the shell, however, they all survived. At hatching 6 chicks were infected. Eight additional chicks became spirochaetemic on the 2nd and 3rd days after hatching but one died on day 3. Of the remaining 13 infected chicks, 10 (76.9%) died (Fig. 3). Spirochaetemia in the recovered chicks ended on days 13, 15 and 16. The duration of spirochaetemia for the 13 infected chicks was 9.8 $\pm$ 2.5 days whereas the duration of spirochaetemia for the 3 infected chicks that recovered was 12.3 $\pm$ 1.5 days. The difference was found to be statistically significant (P .001).

Invasiveness of Borrelia anserina (Trials 8-9).

Trial 8. All embryos died, the control embryos on PI 2 and PI 7 and the exposed embryos on PI 2,3,4,5 and 14 (hatching date). Spirochaetes were not found in any of the dead embryos. Five control and 1 exposed embryos that died on PI 2 were culturally negative for aerobic bacteria.

Trial 9. None of the 12 day or 18 day embryos died. On PI 5, four of the exposed 12 day embryos were examined and found negative for spirochaetes. The remaining exposed embryos and control embryos were used in trial 7. Thirty-six of the forty 18 day embryos exposed to B. anserina and 13 of the control embryos hatched. All were negative for spirochaetes at hatching on days 1 and 2 after hatching.

Effect of Penicillin on B. anserina infection of Embryos (Trials 10-12)
Trial 10. Between 18 and 64 hours after inoculation, 29 living and 2 dead embryos were examined for B. anserina and found negative. At 88 hours 5/6 embryos were found infected with spirochaetes. On PI 5 two dead embryos were found to have died on contamination and 1 living embryo examined was infected with spirochaetes (++). When embryos were given sodium penicillin, none examined on the subsequent day were found free of spirochaetes. On the second day one embryo that had received 100 units penicillin and one which had received 500 units were negative. One that had received 1000 units was positive although the number of spirochaetes was low. On the third day infected embryos were found in the group that had received 100 units but not in the embryos that had been given 500 or 1000 units. All uninoculated embryos were positive for spirochaetes during this same period.
Trial 11. On PI 4 two embryos were examined and found to have +++ and ++++ spirochaetemias. The remaining 37 embryos were given penicillin on the basis of this finding. On PI 5, the day after penicillin had been given, 10 dead embryos and 3 viable embryos were examined. All were positive for B. anserina. On PI 6 four dead and 2 viable embryos were examined. Spirochaetes could not be found in one dead embryo but the remaining 5 were positive. Between PI 7 and hatching, 8 additional dead embryos were examined but no spirochaetes were found. Extensive liver damage was found in most of the dead inoculated embryos. Six inoculated and 2 control embryos that had died were examined bacteriologically but no aerobic bacteria were found. Only 2 control and none of the inoculated embryos hatched at the end of the trial. All remaining inoculated and control embryos were examined but no spirochaetes were found.
Trial 12. On PI 6, the day following treatment with penicillin, 9 infected embryos died. Seven were negative for spirochaetes, 1 had numerous non-motile spirochaetes and the other had (1+) viable spirochaetes. No control embryos died. The following day one inoculated embryo was dead but spirochaetes could not be found.

Embryos were not examined again until PI 12, and 7th day after receiving penicillin, at which time 4 inoculated and 2 control embryos were found dead. Examination could not be done because of extensive autolysis. Two dead embryos were found on PI 13 and both had a few spirochaetes present in the blood. Twelve of the 18 control but only 3 of the 31 inoculated embryos hatched. One of the latter was weak and had to be assisted out of the shell. All 3 inoculated penicillin treated chicks had high spirochaetemias. Of the remaining 12 embryos that died at the time of hatching, spirochaetes were found in 11. Control chicks were not infected and appeared normal.

Development of Spirochaetosis in the Embryo (Trial 13).

The number of spirochaetes increased progressively until they reached maximum number of 3.2×10^8/ml at the 120 hours (Fig. 4). One inoculated embryo died at 60 hours with a massive haemorrhage at the injection site. At 108 hours, 2 dead embryos were both positive for B. anserina. At 120 hours (PI 5), 24 embryos were dead and at 132 hours, 4 embryos were dead. All dead embryos at 120 and 132 hours were positive for B. anserina.

Noticeable tissue changes were not detected in viable embryos until 108 hours. Accumulations of a stringy, opaque substance suspected to be fibrin was present in varying quantities in the extra-embryonic tissues. In some embryos the chorioallantoic fluid was pale gray and jelly-like in consistency. It was frequently turbid because of fine particulate matter from the yolk that had leaked into it. Haemorrhages involving the vascular bed of the extra-embryonic tissues were occasionally present. Infected embryos were of normal size and externally did not differ from control embryos.

In viable infected embryos the liver was swollen and usually had a slight greenish discolouration. In two embryos, small, dark yellow, wedge-shaped areas occurred along the posterior margin of the liver. These were separated from the remainder of the liver by an intense zone of inflammation and appeared to be infarcts. The spleen of infected embryos was 2-3 times larger than the spleen of control

embryos. It was usually between 1/2 and 2/3rds the length of the proventriculus but larger in diameter. Kidneys appeared slightly swollen but otherwise normal.

In dead infected embryos there was often an extensive accumulation of blood between the skull and the brain (Fig. 5). This would appear to have resulted from passive congestion after death. The most obvious change in the liver was numerous foci that occurred throughout the organ as well as marginally. These were similar to those seen in viable embryos. At times as much as 1/5th of the distal part of the liver was sequestered from the remainder. In other embryos the foci were diffusely scattered throughout the liver giving it a marbled appearance (Figs. 6, 7). In the spleen and kidneys numerous minute foci gave the organs a fine granulated appearance (Fig. 7). Macroscopic lesions were not observed in other organs.

DISCUSSION

The results of this study have shown that 6, 9, 12, 15 and 18 day embryos were susceptible to B. anserina infection when inoculated via the appropriate yolk sac or allantoic routes. They did not become infected when the organisms were placed on the shell (7 day embryo) or air space membrane (12 and 18 day embryos). Spirochaete infection could easily be determined by first examining the bloody allantoic fluid and, if this was negative, heart blood from the embryo. Sodium penicillin in amount 100-2000 units/embryo was not toxic to the embryos but also was not effective in eliminating the spirochaete infection. Maximum growth of B. anserina in 9 day embryos occurred on the 5th day following exposure which was also the day of highest mortality.

A marbled appearance of the liver, splenomegaly and nephritic changes were typical findings in embryos that had died of spirochaetosis. Minute foci scattered throughout the spleen and kidneys gave the organs a fine granular appearance. Tissue changes were similar but less pronounced in viable infected embryos.

In trials 1-4 it was determined that infection could be established by either the yolk sac or allantoic routes. The former route was found to be the simplest and most consistent as previously reported (15). Failure of 2 embryos to become infected via the allantoic route could have been the result of faulty technique rather than resistance to the spirochaetes.

Except for some embryos sacrified during these trials, all embryos known to be infected died. There was little difference in the duration of spirochaete infection in 6 and 9 day embryos. Of 42 six day embryos that died, the duration of spirochaete infection was 5.4 $\pm$ 1 day whereas of 66 nine day embryos that died the duration of spirochaetemia was 5.1 $\pm$ 1.1 day. The distribution of deaths in the two groups did show some difference although the greatest number of deaths occurred on PI 5 in both groups. Deaths of 6 day embryos tended to occur over a shorter period resulting in a sharp peak on PI 5 while deaths in 9 day embryos occurred over a longer period (Fig. 1).

The number of spirochaetes in the allantoic fluid was related to the amount of bleeding that occurred when the chorioallantoic membrane was removed. In viable embryos, considerable haemorrhage resulted when this was done and spirochaetes were numerous. In dead embryos, particularly those that had died several hours earlier, there was little or no bleeding and few if any spirochaetes would be found. Organisms could usually be demonstrated in liver smears and were always found in heart smears. They were uncommonly found in the yolk or in allantoic fluid when it was contaminated with yolk. It would appear that the organism is definitely associated with the blood and is probably rare or absent in the yolk and extra-embryonic fluids. A simple method of determining spirochaete infection was devised by first examining the blood contaminated allantoic fluid and if this was negative, then the embryo was dissected and heart blood examined.

One inoculated embryo in these experiments was found to have unilateral anophthalmia and a marked deviation of the maxilla. This was subsequently found to be a common malformation in the flock and unrelated to spirochaete infection.

The high non-specific mortality that occurred in trial 4 was caused by a malfunction of the heating unit in the incubator. Similar problems arose in trials 8 and 11.

In trials 5-7 older embryos were inoculated with B. anserina, allowed to hatch, and the infection studied in the hatched chicks. Hatchability was low but this may also have been related to mechanical problems with the incubator. Infection was more difficult to establish in older (15-18 day) embryos. Probably the embryos that failed to become infected were not properly inoculated. The allantoic route was used but it is doubtful if the inoculum was actually deposited in the allantois of many embryos because of the stage of development. If the inoculum did not penetrate the shell membrane of the air space, infection would not have resulted. This is discussed in more detail later.

One of 3 chicks exposed as 15 day embryos was infected at hatching. The chick remained spirochaetemic until 6 days of age. When re-exposed to B. anserina at 4 weeks of age it developed a prolonged spirochaetemia (11 days) and eventually died. The course of illness was similar to that seen when day old chicks are infected (2). This chick may have been at least partially tolerant of B. anserina. A companion infected chick that hatched at the same time but had been exposed as an 18 day embryo remained infected for 15 days after hatching. When exposed at 4 weeks of age it had a mild infection of 5 days duration and recovered. It would seem unlikely that it possessed any degree of tolerance. It had probably developed a low level of active immunity during the 15 day period of spirochaetemia after hatching. The nine control chicks exposed at 4 weeks of age became infected, had a typical course of spirochaetosis and died.

It was not clear why most of the chicks in trial 7 did not become spirochaetemic until after hatching whereas the chicks in trial 6 were spirochaetemic when they hatched. Both were inoculated as 18 day embryos and the trial 7 embryos received a larger inoculum, 0.1 ml compared to 0.05 ml for trial 6 embryos, which contained more spirochaetes (++++ compared to ++ for trial 6 embryos). Although it is doubtful that the infective allantoic fluid that had been placed on the shell membrane of the air space when these were 12 day embryos conferred any resistance to trial 7 embryos, it does remain as a possibility. A more likely explanation is that because of incubator conditions, chicks in trial 7 hatched one day earlier than expected and spirochaetemia had not had time to develop to detectable levels by the day of hatching. Also, it could have been possible that some unrecognized fault in technique could have reduced the viability of spirochaetes in the inoculum of trail 7 embryos.

The overall case fatality rate for infected chicks exposed as 18 day embryos in trials 6 and 7 was 20/34 (58.8%). This is considerably below the 100% case fatality rate that has been found when day old chicks have been infected with B. anserina (2). It can also be seen that the case fatality rate was higher in trial 7 (76.9%) where several of the chicks did not become spirochaetemic until after hatching than in trial 6 (47.6%) where all chicks were spirochaetemic at hatching. This would indicate that during this particular period of 18 days before hatching, or perhaps slightly earlier, the embryo acquires considerable resistance against mortality resulting from B. anserina infection. The reason for this ability of the embryo to recover from infection was not determined. It may be related to immune tolerance. Whatever the source of the resistance, its elucidation may well provide important information on the pathogenesis and cause of mortality of avian spirochaetosis and probably other Borrelia infections.

In trial 6, spirochaetemia was not detected in 4 chicks on PI 9 whereas on PI 10, two of these 4 chicks were found spirochaetemic.

Although these could represent relapses, it is more likely that the use of direct wet smears was not sensitive enough to detect spirochaetemia on PI 9 in these 2 chicks.

In trials 8 and 9 it was surprising that the Borrelia were not found capable of infecting embryos when placed on the shell of 7 day embryos or shell membrane of 12 or 18 day embryos in view of the well known capacity of B. anserina to penetrate host and vector tissues. In trial 8, considerable difficulty was experienced with the incubator which resulted in the death of all exposed and control embryos before hatching. It is possible that since incubator conditions were not adequate to support the embryo, they may not have been adequate to support the spirochaete either. In trial 9 only 10% of exposed 12 day embryos were examined on PI 5 which, may not have allowed sufficient time for the spirochaete to penetrate the membrane and infect the embryo. The same might have applied to the chicks hatched after exposure as 18 day embryos since they were only examined for 3 days after hatching. This seems unlikely for PI 5 was the day when mortality was highest when B. anserina had been inoculated by other routes. Also, it might have been possible to infect the embryo via the shell or shell membrane routes if a larger inoculum had been used. Although it would appear doubtful that B. anserina can penetrate the shell or shell membrane and infect the embryo a definite statement to this effect can not be made on the results of these trials alone and additional studies are needed.

If, as was indicated, B. anserina can not penetrate the shell membrane, this was probably the reason many exposed 18 day embryos failed to become infected. In these embryos it is highly likely that the inoculum was deposited on the shell membrane and not within the embryo tissues since the air space is quite large at this stage of development. It would appear necessary to introduce the organism through the shell membrane before infection of the embryo could be expected.

It has been suggested that trials testing the susceptibility of B. anserina to various antibiotics might be done more economically and conveniently by using infected embryos (15). Also because of the high mortality in embryos following infection with viable B. anserina, a way was sought to allow the infection to develop but then abort it before mortality occurred in the hope of producing chicks tolerant to B. anserina at hatching. Trials 10-12 were conducted to see if recovery of the embryo from B. anserina infection could be effected by using penicillin. The results of the trials were somewhat varied but each was generally unsuccessful. Penicillin levels of 100 units/embryo were considered ineffective. At concentrations of 500-2000 units/embryo a high number of embryos died on the day following treatment. When examined, these embryos were found to have extensive liver damage. Unfortunately, none of these trials had a full complement of controls i.e. uninoculated, untreated; inoculated, untreated; and uninoculated, treated embryos. In trial 10 lever damage was observed in the dead inoculated, untreated controls but was not considered to be as extensive as that found in dead treated embryos. In trial 11 which had uninoculated, untreated controls only non-specific mortality related to incubation occurred. No liver damage was noted in these while extensive liver damage was found in the dead inoculated, treated principals. In trial 12 which had uninoculated, treated controls no mortality occurred until just prior to and at hatching. Liver damage was not observed in these embryos whereas the inoculated, treated embryos that died on the day following penicillin treatement had the same extensive liver damage found in the embryos of previous trials. It would appear then that liver damage occurred in all embryos infected with B. anserina, which is consistent with the findings in the subsequent trial, but that it was more extensive in B. anserina infected embryos that died following treatment with penicillin. It seems likely that penicillin treatment may have precipitated and increased the severity of liver damage in B. anserina

infected and treated embryos. When uninoculated control embryos were given doses of 2000 units mortality did not result which suggests that penicillin alone in that concentration was not sufficiently toxic to have killed the infected embryos.

It was further found that although spirochaetes could not be demonstrated in the embryos a few days after treatment they often reappeared later causing mortality. It would appear that the concentrations of penicillin used were sufficient to suppress but not eliminate spirochaete infection and the penicillin was acting bacteriostatically rather than bactericidally. There must have been some reason why the organism reappeared but whether it was because the penicillin naturally broke down, was metabolized by the embryo, the Borrelia acquired resistance or some other factor could not be determined. It would appear feasible to use infected embryos for antibiotic testing but much work is needed to establish suitable techniques and it is doubtful if this procedure would be of any great advantage over using day old chicks.

Spirochaetosis is just as severe a disease in embryos as it is in chicks. If we accept that 10-12 day embryos are immunologically incompetent, then the organism would appear to have an inherent ability to cause mortality which does not require an active immune response by the host. The same organs, liver, spleen, and kidney, that are primarily affected in older birds were also affected in the embryo, however, the liver damage was considerably greater in embryos than in fowls. The liver changes resembled recent infarcts while the minute foci observed in the spleen and kidneys appeared to be either colonies of spirochaetes or areas of cell proliferation. Microscopic studies of tissues are indicated and may help to resolve whether this inherent ability to kill stems directly from action of the spirochaete or indirectly through a toxin released at death. The former would seem to be most likely since deaths corresponded with peak numbers of viable spirochaetes in the embryo and not at some

TABLE 1 (continued)

9	12 18	41 40	0.1 ml AF ++++	Air space membran	30 15	As above, chicks hatched from 18 day embryos, 1-3 days after hatching	Same AF as trial 12. Uninfected 12 day embryos used in trial 7
Effect of Penicillin on B. anserina Infection of Embryos (10-12)							
10	6	68	0.05 ml AF ++	y/s	0	AF and heart blood from dead and viable embryos, days 1-3 post pen	On PI 5, 28 embryos given 100,500 or 1000 units pen.* 10 embryos left untreated
11	9	39	0.05 ml AF +++	y/s	10	Dead embryos and selected viable embryos, daily until hatching	On PI 4, given 1000 units pen., 8 dead embryos cultured for bacteria
12	6	53	0.1 ml AF ++++	y/s	20	All dead embryos, blood of survivors after hatching	On PI 5, 31 test and 20 controls given 2000 units pen
Development of Spirochaetosis in the Embryo (13)							
13	9	62	0.1 ml AF ++++	y/s	12	Beginning at 24 hours, 3 viable embryos every 12 hours (except 4 at 132 hours), 2 controls at 24 hour intervals, counts by Petroff-Hauser chamber and darkfield microscopy, all examined for gross pathology	

Age = days of incubation y/s = yolk sac inoculation +++ = 5-50/HPF
IM = intramuscular a/c = allantoic inoculation ++++ = over 50/HPF
AF = allantoic fluid ++ = 1-5 spirochaetes/HPF
*Sodium penicillin (Mycofarm, Delft, Lot 818 reconsistuted and diluted with water for injection, Dumex) administered via a/c.

TABLE 2

Borrelia anserina in fowl embryos inoculated at different ages and by different routes.

Embryo Age	Route	Embryo No.	Day Examined*	R E S U L T S Allantoic Fluid	Yolk	Liver	Heart
6 day (Trial 1)	y/s	1	4**	+++	+	+++	NE
		2	5**	++	+	+++	NE
		3	5	+	Neg	++	NE
		4	5	++	Neg	++	NE
		5	6**	++++	Neg	+++	NE
		6	6	+	Neg	+++	NE
6 Day (Trial 2)	y/s	7	4	+	Neg	++	+++
		8	4	+	Neg	++	+++
		9	5	++	Neg	+++	++++
		10	5	+++	+	++	++++
		11	5	Neg	+	+	++
		12	5	Neg	Neg	+	++
		13	5	+	Neg	+	++
		14	5	+	Neg	+	++
		15	6	++++	++	++	++++
		16	6	Neg	+	++	+++
		17	7	++	+	+++	++++
9 Day	y/s	18	5	++	+	++	++
		19	6**	++	+	+	+++
		20	6**	++	+	++	+++
		21	6**	++	+++	+++	++++
		22	6	++	+	+++	+++
9 Day	a/c	23	4	Neg	Neg	Neg	NE
		24	5	Neg	Neg	++	++++
		25	5	+++	Neg	++	++
		26	6	+	+	++	+++
		27	6	+++	+	++	++
12 Day	a/c	28	6	Neg	Neg	+	++

TABLE 2 (continued)

29	6	+	Neg	+	+++
30	7	Neg	Neg	Neg	Neg
31	8	++	Neg	Neg	+
32	8	+++	Neg	Neg	+++

* No. days after inoculation
**Embryo alive when examined
NE Not examined
y/s = Yolk sac inoculation
a/c = Allantoic inoculation

Neg = 0 spirochaetes/10HPF (X300)
\+ = 1-10/10HPF
++ = 1-5/HPF
+++ = 5-50/HPF
++++ = over 50/HPF

DISTRIBUTION OF DEATHS IN FORTY-TWO 6 AND SIXTY-SIX 9 DAY EMBRYOS INFECTED WITH BORRELIA ANSERINA.

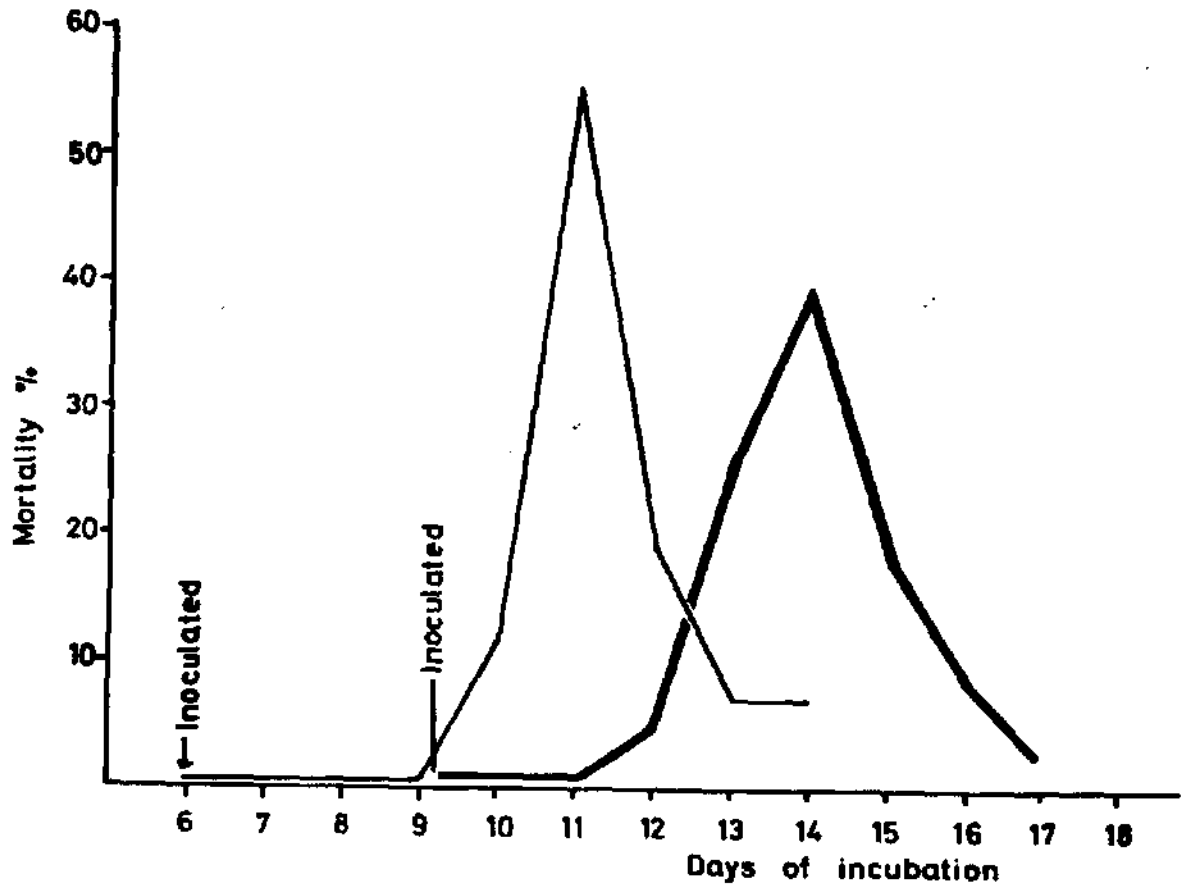


FIG. 1

CUMULATIVE MORTALITY IN 6 AND 9 DAY CHICK EMBRYOS INOCULATED VIA THE YOLK SAC WITH BORRELIA ANSERINA.

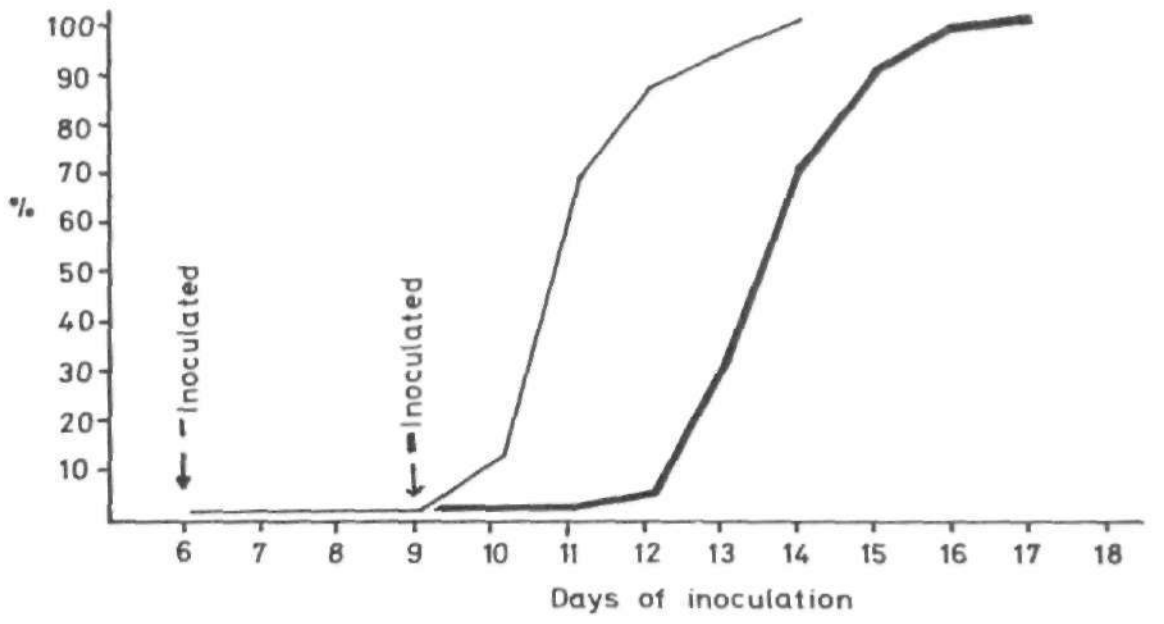


FIG. 2

MORTALITY AND INFECTION RATES IN CHICKS THAT HAD BEEN INOCULATED WITH BORRELIA ANSERINA AS 19 DAY EMBRYOS.

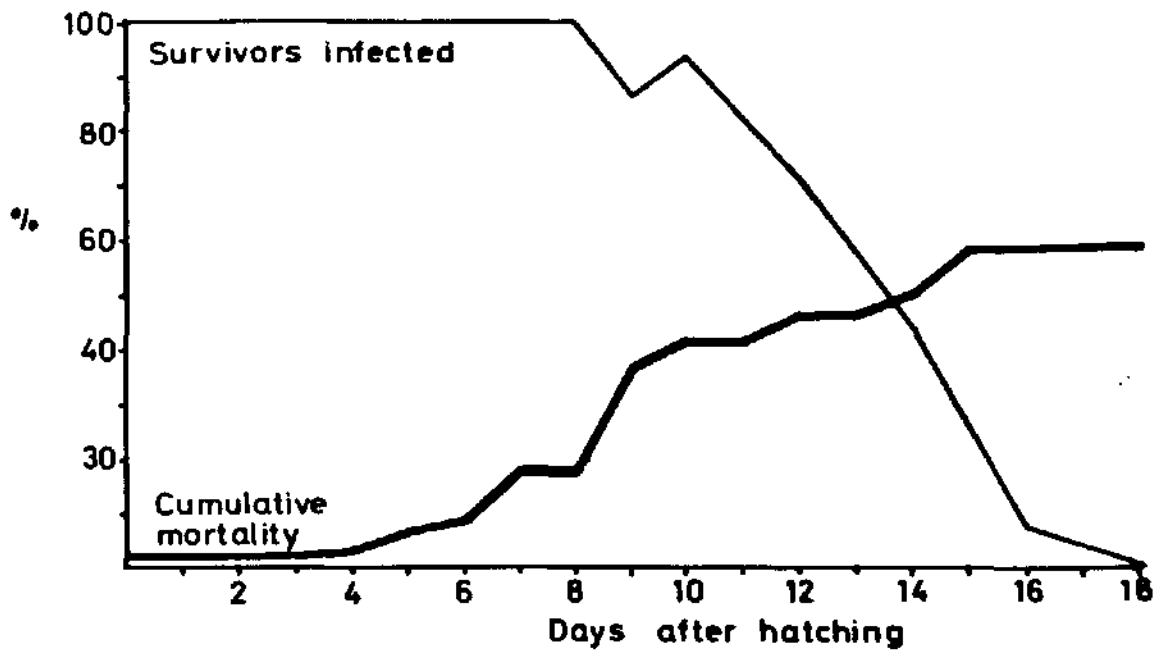


FIG. 3

GROWTH OF BORRELIA ANSERINA IN 9 DAY EMBRYOS.

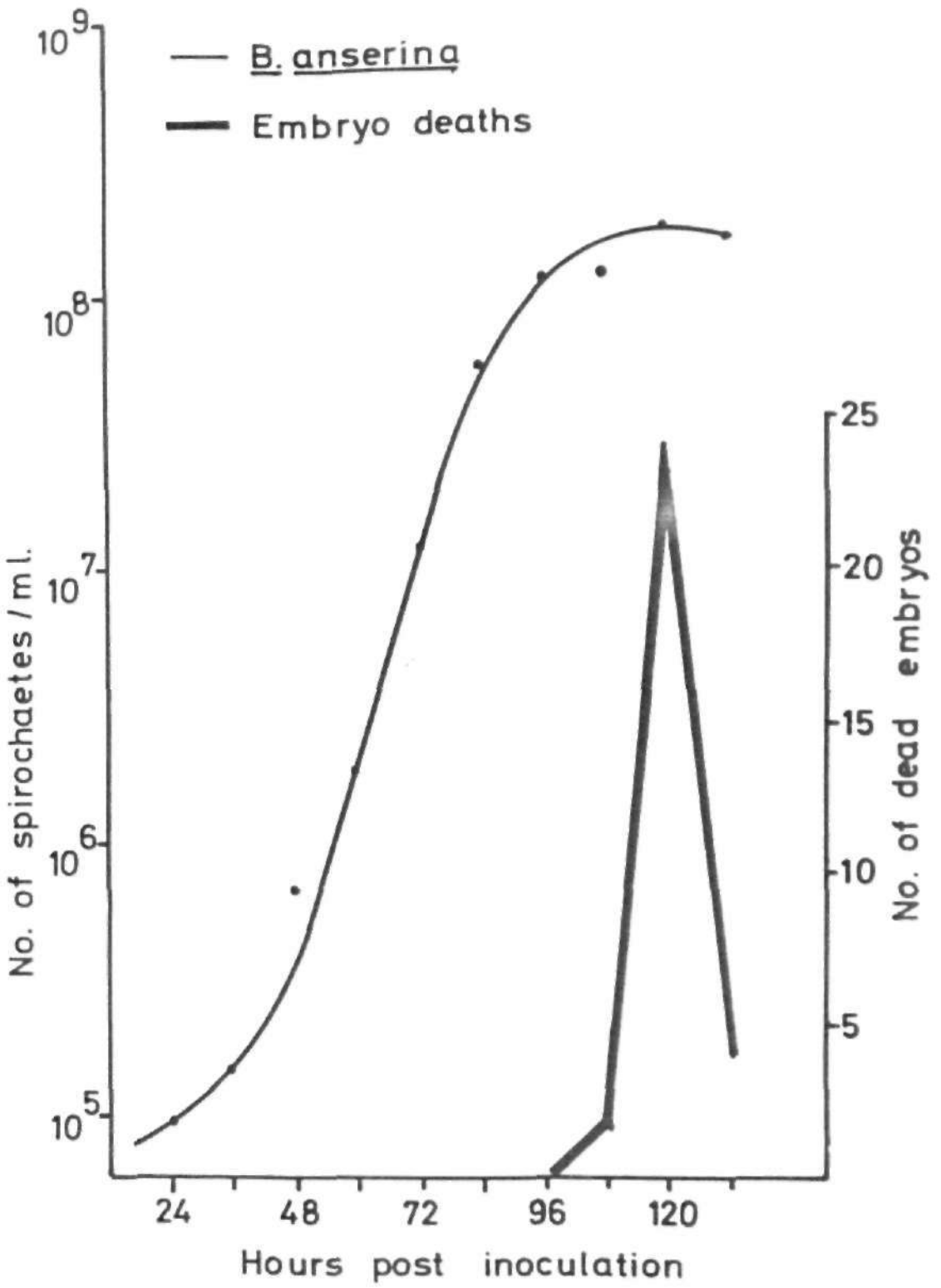


FIG. 4



Figure 5. Three embryos that died 120 hours after inoculated with Borrelia anserina on the 9th day of incubation. A large blood clot is present over the brain beneath the skull. This was found in dead embryos but not living ones.

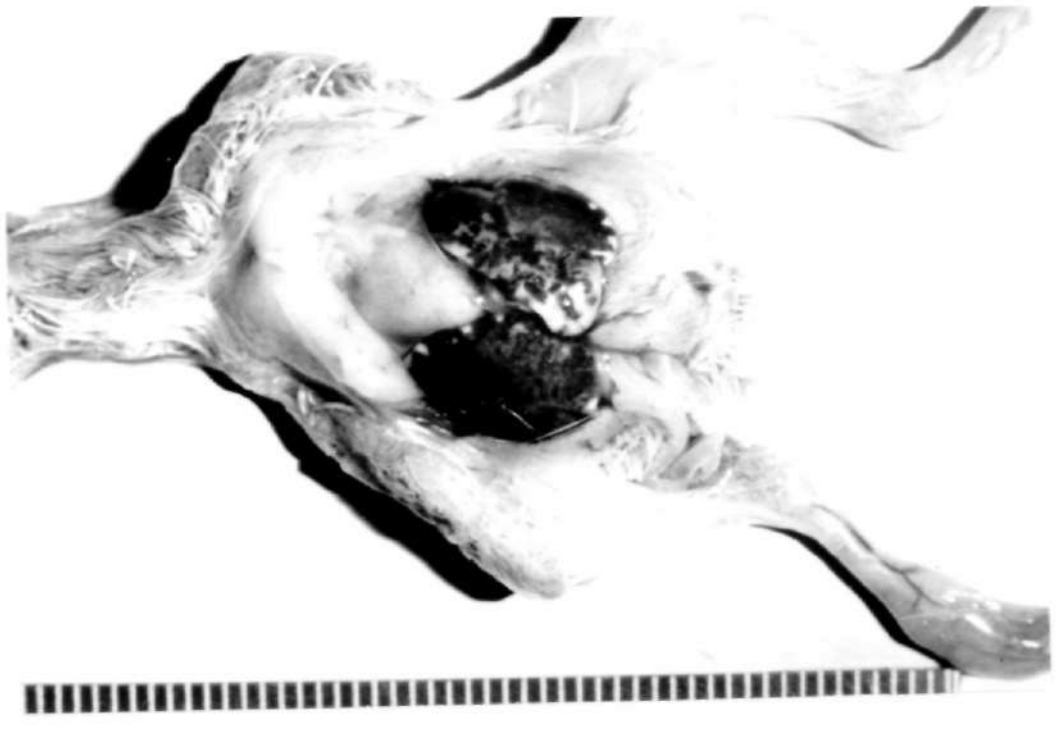


Figure 6. An embryo that died 120 hours after inoculated with Borrelia anserina on the 9th day of incubation. A number of marginal, wedge-shaped lesions are apparent in the liver of this embryo. Note the extensive damage involving nearly ½ of the left lobe of the liver. (Scale in mm)

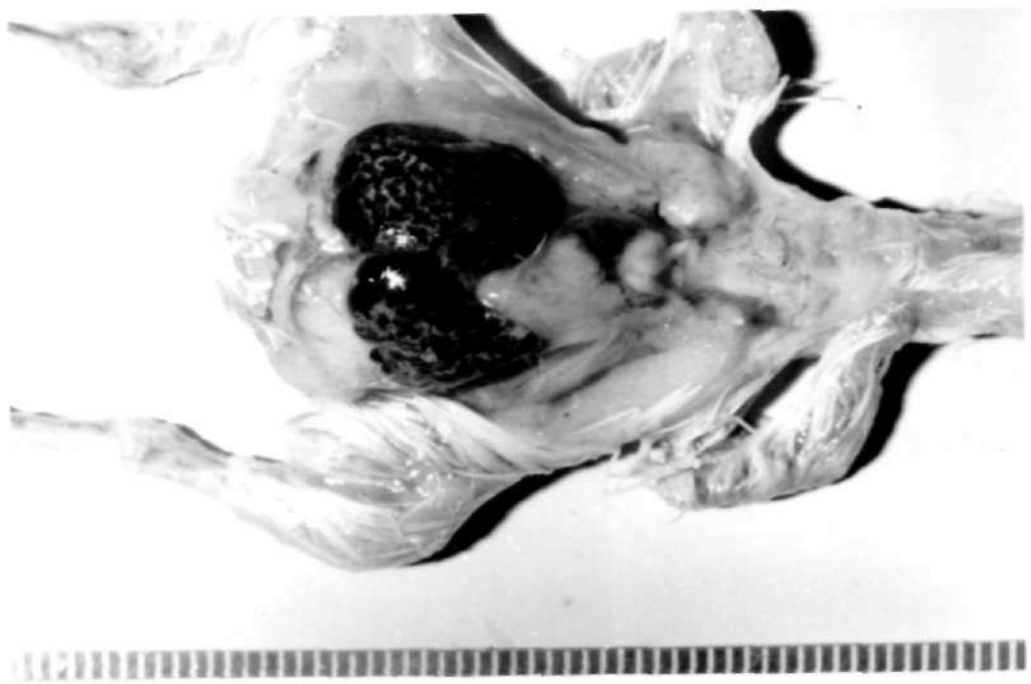


Figure 7. An embryo that died 120 hours after inoculated with Borrelia anserina on the 9th day of incubation. Extensive liver damage has given the organ a marbled appearance. Lesions of this type and marginal lesions along the borders of the liver were characteristic of B. anserina infection in the embryo. (Scale in mm)

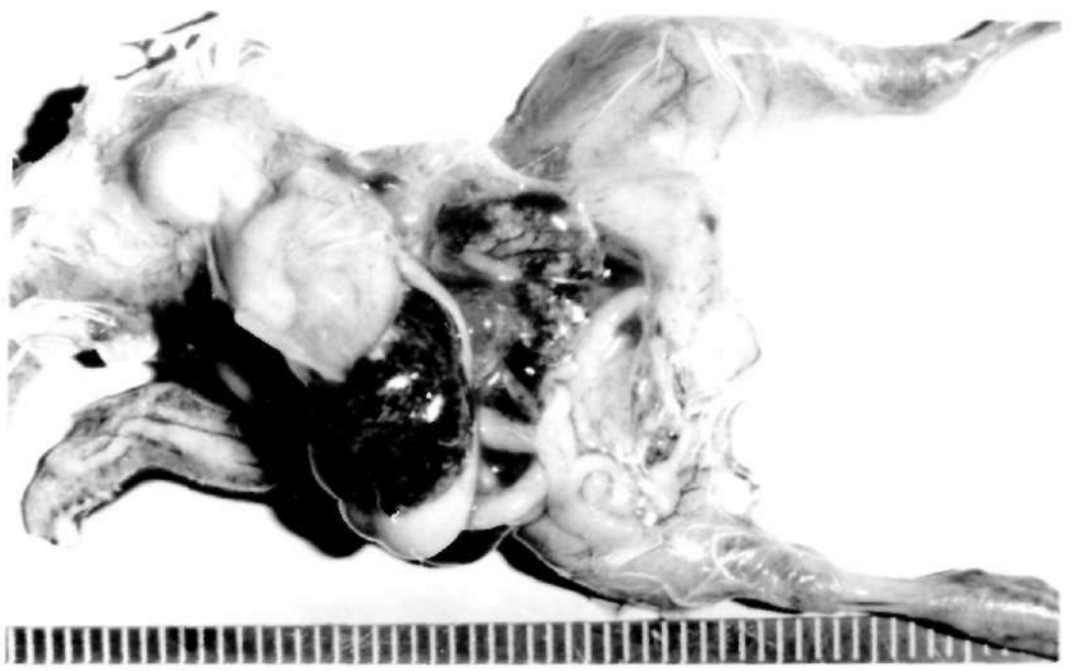


Figure 8. An embryo that died 120 hours after inoculated with Borrelia anserina on the 9th day of incubation. An enlarged spleen, swollen kidneys and marbled liver can be seen. Note the fine granular appearance of the spleen and kidneys and the small marginal lesions on the border of the liver. (Scale in mm)

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: EXPERIMENTAL *BORRELIA ANSERINA* INFECTION IN ADULT FOWLS

SUMMARY

Thirty-one adult fowls experimentally infected with Borrelia anserina were used in a series of studies to examine the development of avian spirochaetosis. Changes occurring initially included decreased appetite, excess bile in the faeces, inactivity, and an abrupt, marked increase in body temperature. These generally coincided with the demonstration of B. anserina in blood but sometimes preceded it. Diarrhoea containing excess bile and urates followed within 12-24 hours and was probably the result of anorexia since it could also be produced by starving non-infected fowls. Clinical signs became more severe until the fowls were somnolent or comatose prior to death. The body temperature curve was usually biphasic in fowls that died whereas body temperatures tended to remain high throughout spirochaetemia in fowls that recovered. The loss of body weight was rapid, progressive and more severe than in starved fowls. The maximum weight loss was 28.8% with the mean of 20.7% for 14 fowls that died. Dehydration may have been responsible for at least part of the weight loss and probably was the main stimulus for increased thirst. Haematological changes supported clinical evidence of anaemia which developed during the later stages of the infection. A few spirochaetes were found in the aqueous humour when spirochaetemia was high but they did not appear to persist in the eye. However, spirochaetes were found to persist in the brain of one fowl that had recovered 6-7 days earlier. Complement-fixing antibodies were found in low titre in some fowls that were still spirochaetemic and in higher titres in recovered fowls. The size of the spleen increased until spirochaetes began to clump and disappear from the circulation whereas the liver did not increase in size until terminally. It was suggested that the changes in the spleen may have been the result of cellular proliferation followed by necrosis while the liver changes may

have occurred in response to an endotoxin released from lysed spirochaetes. Renal changes also suggested the presence of a toxin terminally. Other frequent tissue changes included catarrhal enteritis and bile staining of the gastric and intestinal mucosa, small hyperaemic foci in intestinal mucosa, petechial haemorrhages of the caecal tonsils, and inflammation of the proventriculus/ventriculus junction.

INTRODUCTION

Avian spirochaetosis, a septicaemia caused by Borrelia anserina, has been reported to be a common disease of poultry in northern Nigeria (5). Clinical and field observations on the disease in northern Nigeria have been reported (4). In the latter study it was noted that clinical and postmortem findings were often variable depending upon the stage of the disease. Since these aspects are of considerable importance in diagnosis and understanding of the disease process, investigations were conducted on experimentally infected adult fowls. The results of these investigations are presented.

MATERIALS AND METHODS

Study 1. Twenty adult (8-9 months) Rhode Island red (RIR) cockerels from a tick and spirochaete free flock were used. The fowls were identified and serum samples collected and tested for immobilizing antibodies before inoculation as follows: 0.1 ml serum from each experimental fowl was mixed with 0.9 ml serum/saline mixture. This mixture was prepared by diluting 0.1 ml serum that contained 8.65×10^7 motile spirochaetes/ml from a donor fowl infected 3 days earlier with B. anserina, strain H (6) in 0.8 ml phosphate buffered saline (pH 7.2) containing 0.2% glucose (PBS/G). Known positive and negative B. anserina sera as well as a saline control were prepared in the same way. The resulting 1:10 dilutions of experimental and control sera were incubated along with the saline control for 30 minutes in a 37°C waterbath. Aliquots from each sample were placed on slides and examined for spirochaete motility using darkfield microscopy. The immobilizing capacity of the diluted serum was judged as ++++ if only 0-10% of the spirochaetes remained motile, +++ if 20-40% remained motile, ++ if 40-60% remained motile, + if 60-80% remained motile and negative if over 80% were still motile.

Eighteen fowls were inoculated intramuscularly with 1.7×10^7 B. anserina. Fowls were selected by random process--all numbers were placed in a container and picked blindly by an attendant who had no knowledge of the condition of the fowls--and were examined and exsanguinated according to the following schedule: 1 at post-inoculation hour (PIH) 24, 1 at PIH 48, 1 at PIH 60, 2 at PIH 72, 2 at PIH 84, 2 at PIH 96, 2 at PIH 108, 2 at PIH 120, 2 at PIH 132, 1 at PIH 144, 1 at PIH 288 (day 12) and 1 at PIH 504 (day 21). A new consecutive accession number was assigned after the fowls had been selected. Two fowls were left uninoculated and exsanguinated on PIH 0 and 96 respectively. Experimental fowls were always examined between 8-10 a.m. and 8-10 p.m. At each interval selected fowls were examined for body temperature, carcass, spleen and liver (without gallbladder) weights and gross tissue changes. All fowls were observed for clinical signs at each period. A blood sample was collected in EDTA and the presence of spirochaetes was determined by examining direct wet and buffy coat smears (1). The packed cell volume was determined by standard technique. Haemoglobin was determined by a slightly modified cyanmethaemoglobin method where a minimum interval of 2 hours elapsed before samples were examined spectrophotometrically. It had previously been determined that this minimum interval was necessary to obtain reproducible results but longer intervals up to 12 hours did not appreciably differ from 2 hour readings. The blood was kept at 4^oC for 2-12 hours until the cells had settled. Plasma was aspirated, appropriately diluted in PBS/G and spirochaetes were counted using a Petroff-Hauser counting chamber and darkfield microscopy. Counts were done in triplicate and averaged if they were within 10% of the mean. Counts outside this range were discarded and samples recounted.

Samples of cerebrospinal fluid (CSF) collected via the cisterna magnum and aqueous humour aspirated from the anterior chamber of the eye were examined for spirochaetes by darkfield microscopy. Serum

samples were collected and examined for complement-fixing (CF) antibodies by a modified direct test (3). Portions of the cerebrum, cerebellum and medulla of the brain from fowl 18 killed at 12 days, approximately 6-7 days after recovery, were ground in PBS/G as 20% suspensions and 2 and 5 ml amounts respectively inoculated subcutaneously into 2 susceptible 6 week white leghorn (WLH) chickens. The chicken receiving 2 ml brain suspension was subsequently exposed to a field isolate of B. anserina 18 days later.

Study 2. Four susceptible (Nos. 21-24) and 1 previously recovered (No. 20) adult (18 mos.) RIR cockerels were inoculated intramuscularly with 4×10^6 B. anserina, strain H. Fowls were given feed and water ad lib. throughout the study. They were examined twice daily between 8-10 a.m. and 8-10 p.m. Body temperature, body weight and clinical signs were determined. A blood sample (2 ml) was collected each morning from a jugular or wing vein and added to 0.1 ml 10% EDTA. Haemoglobin, packed cell volume, spirochaetemia and number of spirochaetes were determined by the same methods used in study 1. In fowls that died a postmortem was conducted and the gross pathology, carcass, spleen and liver weights determined.

Study 3. Five susceptible adult WLH cockerels (10 mos.) were used. Three were inoculated intramuscularly with 2×10^5 B. anserina while the remaining 2 were left uninoculated. At PIH 36 the exposed fowls were observed to be anorexic. At this time feed was removed from the 2 uninoculated fowls and witheld for 4½ days. Birds were examined and samples taken as in study 2.

Study 4. Additional observations were made on 2 groups of experimentally infected adult fowls that had served as non-immune controls for a previous experiment (7). Group A consisted of 3 adult RIR (6 mos.), 2 hens and 1 cockerel while group B consisted of 4 adult WLH (7 mos.) cockerels and 4 adult RIR hens (11 mos.). In group A, body temperature, packed cell volume and spirochaetemia were determined daily. In group B, body temperature, body weight and

spirochaetemia were determined daily. Determination of spirochaetemia in both groups was done by both direct wet and buffy coat smears. At postmortem, carcass, spleen and liver weights were determined.

RESULTS

Study 1. A serum sample from one experimental fowl immobilized about 20% of the spirochaetes and it was rated + while all others were negative.

Clinical evidence of infection was first observed at PIH 48. Food consumption was decreased. Formed, bile stained, stools were being passed. Body temperature was slightly increased and spirochaetes were found in both direct wet and buffy coat smears (Fig. 1). In general, clinical signs increased in severity until PIH 120-132. No fowls died of spirochaetosis during the study. At PIH 132, one fowl had a peculiar hopping gait. It would take 2 or 3 steps and then hop on the left leg. It had drooped wings but otherwise appeared reasonably well and was observed eating. It was exsanguinated and found to be still spirochaetemic. The onset, duration and severity of clinical signs have been summarized in Table 1.

Body temperatures increased sharply and peaked at 111°F on PIH 60. They moderated but were still elevated between PIH 72-96, dropped to 107.8°F at PIH 120, increased sharply at PIH 132 and returned to normal at PIH 144. Body temperature was below normal in the fowl examined at PIH 288 returning to normal on PIH 504 (Fig. 1).

Spleen weights compared with body weights generally increased during the earlier stages of the infection, reached maximum size at PIH 96 and then progressively declined until PIH 288. Liver weights were fairly constant until the later stages of the infection increasing sharply at PIH 96 and remaining elevated until PIH 144. After PIH 144 liver weights decreased progressively through PIH 504. The liver:spleen ratio was relatively constant until PIH 96 when it increased sharply and remained elevated until PIH 504, peaking

on PIH 288 (Fig. 2).

Tissue changes were first observed at PIH 48 when splenomegaly and catarrhal enteritis were observed. At PIH 60 catarrhal enteritis was found along with a few focal pinpoint areas of hyperaemia in the intestinal mucosa and a few haemorrhages in the caecal tonsils. The latter changes were consistent findings even in control birds. Post-mortem findings are summarized in Table 2.

Spirochaetes were found in both the buffy coat and wet smears of all spirochaetemic fowls. The maximum number of spirochaetes found was 2.9 X 10^7/ml at PIH 96. Clumping of the organisms in fowls after PIH 96 made counting difficult (Fig. 1). Spirochaetes were found in the aqueous humour of 5 fowls between PIH 96-132 that also had high spirochaetemias. They were never numerous and never found in birds without spirochaetemia (Fig. 1).

All attempts to collect CSF samples resulted in considerable contamination of the samples with blood and it was not possible to evaluate them.

A CF titre was detected in two fowls that were still spirochaetemic--1:16 in fowl 9 at PIH 96 and 1:64 in fowl 11 at PIH 108. Titres equal to or over 1:256 were found in recovered fowls at PIH 144, 288 and 504 (Fig. 1). The results of packed cell volume and haemoglobin determinations are presented in Figure 3.

One of the 2 WLH fowls that had been inoculated with brain suspension from the fowl examined at PIH 288 became spirochaetemic 6 days after receiving 5 ml subcutaneously and died 3 days later. The other chicken that had received 2 ml brain suspension did not become spirochaetemic. Eighteen days after exposure it was inoculated with blood from a field case, became infected and died 6 days later.

Study 2. Fowl 20 which had recovered from avian spirochaetosis previously did not become spirochaetemic during the study. However, a moderate body temperature rise occurred on day 3 which correlated

with maximum body temperatures in infected fowls (Fig. 4).

A sharp rise in body temperatures occurred early in the infection and coincided with the onset of spirochaetemia. In fowl 21, body temperature decreased gradually until recovery and then fell to below normal levels. In fowls that died, the temperature curve was strongly biphasic (Fig. 4). Maximum temperature recorded was 111.4°F from fowl 22 at PIH 84. Fowl 24 had a temperature range of 106-109.2°F which was generally lower than the other fowls.

In general the onset of clinical signs correlated with the onset of spirochaetemia. Clinical signs became more severe as the disease progressed. This data is presented in Table 3.

Haematological values declined progressively in spirochaetemic fowls. In fowl 21 they increased slowly and had reached near normal values by 4 weeks after recovery. An abrupt decrease in haematological values was found for fowl 21 on day 19 (Fig. 5).

Body weights decreased progressively in infected fowls and continued to decrease in fowl 21 for several days following disappearance of B. anserina from the circulation. A maximum loss of 28.6% of the body weight was found in fowl 21 on day 13. Mean weight loss of the 3 fowls that died was 18.1% (Fig. 4, Table 4).

Spirochaetes reached maximum numbers of 7.7×10^7/ml on day 5. Clumping of the organisms made reproducible direct counts impossible after day 6 (Fig. 5).

At postmortem an enlarged, mottled spleen; swollen pale, friable liver; catarrhal enteritis and swollen, pale kidneys were consistent findings. In fowl 22 a large area of old haemorrhage in the left pectoral muscle, marked dehydration, crop filled with green coloured fluid, small erosions at the proventriculus/ventriculus junction, minute foci of hyperaemia in the jejunal mucosa and a few petechial haemorrhages in the cecal tonsils were also found. A distinct zone of inflammation between the proventriculus and ventriculus was observed in fowl 23 and the ventriculus mucosa was found to be roughened and irregular in fowl 24. Spleen and liver weights were

considerably above normal values (Table 4).

Study 3. A progressive weight loss occurred in both starved and infected fowls but the loss was significantly greater ($p < .05$) in infected as compared with starved fowls on day 5 (Fig. 6). Little change occurred in haematological values of starved fowls compared with pre-exposure levels (Fig. 7). Body temperatures were not elevated and showed a diurnal pattern in starved fowls as compared with infected fowls (Fig. 6). Clinical observations on the two groups are presented in Table 5.

Study 4. In group A fowls the mean body temperature increased when the fowls became spirochaetemic and remained high until spirochaetes had disappeared from the circulation. Mean packed cell volume increased slightly until day 4, the second day of spirochaetemia, then fell abruptly, and continued to decrease throughout the remainder of the study period (Fig. 8). All group A fowls survived. In group B fowls a biphasic temperature curve occurred. Mean body temperature was elevated when spirochaetes were present in the circulation. Mean body weight decreased progressively. Weight loss from day of exposure ranged from 17.4-28.8% with a mean of 22.7% (Fig. 9). Spleen and liver weights are presented in Table 4.

DISCUSSION

A total of 36 adult fowls were used during the studies of which 5 were uninfected controls. In the first study a number of fowls were infected and then selected at random to be sacrificed at given intervals between 1-21 days after inoculation with *B. anserina*, strain H. In the remaining studies, the fowls were inoculated and samples collected from each fowl at given intervals after exposure. Both types of studies are subject to error. In the former, it has to be assumed that all fowls respond to the spirochaete uniformly and the infection progresses in the same way in all fowls. This, of course, does not occur because of individual

host variation so by the random selection process, it may have been possible to select fowls that were in a more or less advanced stage of the infection. Large numbers of fowls examined at each time period would increase the likelihood of having a more consistent study. In the second type of study, the collection of samples can interfere with the normal host reponse. This is particularly true when blood samples are taken at frequent intervals. In this type of study, suitable control fowls may help to interpret changes in infected fowls. It would have been advantageous to have had more birds in each experiment and to have been able to conduct a wider battery to tests, particularly changes in plasma enzymes and leucocytes. Histologic changes are under investigation and will be presented later.

The earliest clinical evidence of B. anserina infection was a decrease in appetite and the passing of formed stools that were bright green in colour from excessive bile pigments. These changes occurred just prior to or coincided with demonstration of spirochaetes in the circulation. The early signs were subtle and probably would not be detected even by the most observant flock owner. Within 12-24 hours, the birds became inactive and anorexic, body temperatures rose sharply, and greenish-white or greenish-yellow diarrhoea occurred. The latter contained little or no ingesta but only bile and excess urates. It tended to be mucoid early in the infection but profuse and fluid terminally. A similar type of diarrhoea was found in starved non-infected fowls. Other than weight loss, no other clinical signs comparable to those in infected fowls occurred in starved fowls. It seems likely that the greenish-white or greenish-yellow diarrhoea that is typical, not only of avian spirochaetosis, but of most acute fowl diseases is probably the result of anorexia.

As the number of spirochaetes increased in the circulation, clinical evidence of infection became more obvious. Affected fowls

became increasingly inactive, dull and unresponsive, reluctant to move, somnolent and comatose just prior to recovery or death. The comb and wattle became pale in most but not all birds during the later stages of the infection. Cyanosis either involving the tips of the comb or as in one fowl large blotches on the comb was found occasionally. Jaundice was observed in a few fowls during the last 24-36 hours before recovery or death. Anorexia persisted until recovery or death in all infected fowls and excessive consumption of water was a prominent clinical finding in about half the birds infected. The latter tended to be more characteristic of birds in studies 1 and 3 than in the other studies. It did not appear to be related to body temperature because thirst was more obvious in the latter stages of the infection when body temperatures had usually moderated to some degree. It may have been related to the marked loss of body weight which could have been due, at least in part, to dehydration. General paresis was common to all fowls during the latter stages of the infection but a definite disturbance of the locomotor system was only observed in one fowl. In the single recovered fowl that was studied, clinical signs slowly disappeared. The diarrhoea persisted for at least 4 days after spirochaetes had disappeared from the circulation. Pallor of the comb and wattles was still noticeable on day 11. The bird continued to lose weight for at least a week after spirochaetes had disappeared from the blood until it had lost 28.6% of its original body weight.

A rapid and progressive loss of body weight was a characteristic finding in B. anserina infected adult fowls. When body weights were examined during the morning and evening, losses were generally greater during the night than during the day (Figs. 4,6). This further suggests that at least part of the loss of body weight may have been the result of dehydration from elevated body temperature and diarrhoea. During the day, the fowls would

consume water to partially compensate for the loss and therefore not lose as much weight as during the night when they would not be drinking. Dehydration could also account for the significant difference in the loss of body weight between starved and anorexic infected fowls. Presumably the starved fowls would remain properly hydrated even in the absence of feed. The overall mean loss of body weight in 14 fowls that died following experimental B. anserina infection was 20.7% (Fig. 10).

Two patterns of body temperature changes were apparent and were usually associated with survival or death. In birds that survived B. anserina infection, the body temperature remained elevated for the duration of spirochaetemia. In birds that died body temperature dropped after the primary peak, rose again later in the course of infection and fell terminally producing a distinct biphasic pattern (Fig. 11). This suggests that a continuous elevation of body temperature may be important as a survival mechanism in B. anserina infected fowls. It has been suggested (9) that the inability to mount a febrile response may be one reason for the high mortality in experimentally infected day old chicks (6).

The reason for the high initial temperature rise that occurs prior to or at the onset of spirochaetemia is unknown but probably would not be due to an endotoxin in view of the scant number of organisms in the circulation. If endotoxin is elaborated, it should be in highest concentration terminally when lysis of the spirochaetes is occurring, but body temperatures are usually much lower at this point than initially. It would appear that even small numbers of B. anserina can release or stimulate endogenous release of a potent pyrogen but its release is not continuous nor proportionate to the number of spirochaetes in the circulation.

An alternate possibility is that the initial multiplication of B. anserina in the circulation may be controlled by available host defense mechanisms, e.g. phagocytosis, organisms are lysed

and an endotoxin elaborated which is potent and causes an abrupt febrile response. In birds that subsequently recover, host defenses are adequate, lysis occurs more or less continuously, the number of organisms is controlled and a continuous elevated temperature curve results. In birds that fail to recover, host defenses may be initially adequate but overwhelmed and the organisms proliferate while the host defense mechanisms are strengthened and probably augmented by antibody formation to a point where large numbers of spirochaetes are destroyed over a limited period of time resulting in the release of a considerable quantity of endotoxin. This theory would account for the biphasic temperature curve. The decreased body temperature that occurs terminally may be just a manifestation of endotoxic shock. A similar theory has been postulated for the high mortality that occurs in day old chicks (6). Obviously a great deal needs to be learned about the febrile response and its relationship to mortality in B. anserina infected fowls.

From the limited haematological data, anaemia was found to be a characteristic finding during the later stages of avian spirochaetosis. This is consistent with earlier reports (13,14,16) and provides an explanation for the pallor noted clinically in most infected fowls. When blood samples were taken from the same birds at frequent intervals there was some evidence that slight decreases in PCV and haemoglobin resulted from sampling alone but the decreases in infected fowls were considerably greater. The abrupt fall in PCV and haemoglobin reported for fowl 21 (study 2) on day 19 is probably an artifact created by a technical error. Unfortunately the sample had been discarded before it could be re-examined. The cause of the anaemia is unknown but the brown discolouration of the spleen in recovered fowls probably resulted from phagocytized haemosiderin suggesting intravascular haemolysis. Starvation did not appreciably affect haematological values when

compared to infected fowls. Detailed haematologic studies on avian spirochaetosis in Nigeria are indicated and could provide useful information regarding pathogenesis of the disease.

The number of spirochaetes in the circulation of infected fowls reached maximum levels between the 4th and 5th day after inoculation and then decreased. This decrease may have been more apparent than real and may have been a reflection of the counting technique used or the inability to accurately determine the number of spirochaetes after clumping had begun. The maximum numbers found in adult fowls is lower than those reported from B. anserina infected embryos (8) but compares favourably with numbers of B. recurrentis found in the blood of humans with louse-borne relapsing fever (12). If we can assume that the fowl embryo has no or a very limited ability to defend itself against introduced B. anserina and growth of the organism is not impeded, then it would appear that the adult fowl has innate defense mechanisms that can prevent the spirochaetes from proliferating to maximum potential numbers. Certainly antibody formation is important during the later period but cellular functions, particularly phagocytosis, may be an important early function.

A few spirochaetes were found in the aqueous humour of fowls with high spirochaetemias. Although this finding demonstrated that spirochaetes could successfully penetrate the eye during infection, there was no evidence that they persisted in the eye. However, there was some evidence to suggest that at least a few spirochaetes might persist in the brain. When approximately 1 gm of brain tissue from a fowl that had recovered 6-7 days earlier was suspended in PBS/G and inoculated into a susceptible chicken, the chicken became infected 6 days later and died on the 3rd day of infection. Another chicken inoculated with 0.4 gm of the same material failed to become infected and was susceptible to challenge 18 days later with a field isolate. This finding is at variance with findings reported for 2 fowls infected with the same strain of B. anserina which had died

after spirochaetes had disappeared from the circulation and tissues were subinoculated into susceptible fowls (2). This discrepancy suggests that neurotropism and persistence of B. anserina in the brain may occur in certain individuals. Neurotropism as occurs with certain mammalian Borrelia is thought to be an important component of the relapse phenomenon that characterizes these borrelioses.

The finding of low CF titres in spirochaetemic fowls indicates that an antibody response does occur prior to disappearance of the organisms. It seems most likely that antibodies are responsible at least in part for the agglutination, lysis, and disappearance of the spirochaetes. It would appear doubtful if CF antibodies are primarily responsible, however. The inability to find fowls with CF titres at two later periods is probably an artifact created by the type of study as pointed out previously. High CF titres were found in recovered fowls even though exact end points were not determined.

Although splenomegaly has been regarded as a characteristic finding in avian spirochaetosis, these studies indicate that splenomegaly occurs during the earlier stages of the infection while during the later stages of the disease, the spleen decreases in size. The spleen of adult fowls is considered to be approximately 0.2% of body weight (15). This figure agrees with those found prior to the onset of spirochaetemia and in control fowls. In study 1 spleen weights were highest at PIH 96 which was also the period when the highest number of spirochaetes were found in the blood (Figs. 1,2). In 10 fowls that died during studies 2 and 4 spleen weights generally decreases with increasing times of survival (Table 4). It would appear that splenic enlargement, which has been reported to be the result of a proliferation of reticuloendothelial (RE) cells (4), is the result of a stimulation of these cells by increasing numbers of spirochaetes. But, as the organisms reach maximum numbers and begin to agglutinate and undergo lysis, the size of the spleen rapidly diminishes. This may be the result of phagocytosis of the

Borrelia and subsequent necrosis of the RE cells. Vacuolation and necrosis of the splenic RE cells has been observed previously (4) but additional studies are necessary to determine if necrosis occurs at the time when spirochaetes are disappearing from the circulation and if the decrease in spleen size is the result of RE necrosis or some other factor. The size of the spleen continued to decrease after spirochaetemia being less than ½ normal size at PIH 288 in study 1 (Fig. 2).

The size of the liver has been reported to be approximately 2.0% of body weight in healthy adult fowls (11). Liver weights tended to remain fairly constant during the initial stages of the illness until spirochaetes reached maximum numbers and began to agglutinate and undergo lysis at which time the liver weight increased considerably. The reaction could be the result of an endotoxin released from the spirochaetes during lysis. The presence of an endotoxin following lysis of B. recurrentis in cases of human relapsing fever has been suggested (10) but B. anserina endotoxin has apparently not been demonstrated. Following disappearance of B. anserina from the blood, the size of the liver returned to normal and then continued to decrease for at least 2 weeks afterwards. In normal fowls a liver: spleen ratio of approximately 10:1 would be expected. In infected fowls lower values tended to occur early and higher values late which reflects the splenic enlargement found initially and hepatic enlargement found terminally.

In addition to the changes in the spleen and liver, the kidneys were frequently swollen and pale in the later stages of the infection which also indicates the possible action of a toxin.

One of the earliest changes observed was a catarrhal enteritis. The duodenum and upper jejunum were dilated with thick, creamy mucus. The excess mucus may have accumulated because of anorexia or resulted from some action of the spirochaetes. Later, this was

replaced by a "pebbled" appearance to the intestinal mucosa and intense bile staining. Small red foci in the intestinal mucosa were considered to be areas of hyperaemia because free blood was not found in the gut. Petechial haemorrhages of the caecal tonsils were found in several fowls. Both of the latter changes were thought to be agonal but since they were also found in 2 of the 3 fowls that died naturally in study 2 this interpretation may not be correct. Whatever their aetiology, they were subtle changes that could easily be missed without close examination and appear to be of little diagnostic significance. Roughened, irregular erosions of the ventriculus mucosa and the intense bile staining were probably the result of anorexia while the fluid filled crops probably resulted from polydipsia and dehydration. A prominent zone of inflammation between the proventriculus and ventriculus has been observed from fowls in northern Nigeria that had died of a variety of acute viral and bacterial diseases. This area would appear to be particularly sensitive to injurious agents including B. anserina and although the cause of the inflammation is unknown it seems likely that some basic mechanism common to many acute diseases of poultry is probably responsible. Other gross tissue changes included fibrinous pericarditis and large pale areas in the skeletal muscles but these were not common and appear to be infrequent manifestations of avian spirochaetosis in Nigerian fowls.

The results of these studies on experimentally infected adult fowls are essentially consistent with the previously reported observations on naturally infected fowls. They provide a better definition for the stage of the disease at which different clinical and postmortem findings occur and may be useful in explaining some of the variable aspects reported from natural infections.

TABLE 1

Clinical findings in adult fowls experimentally infected with Borrelia anserina (Study 1).

Findings	Hours post innoculation											
	24	48	60	72	84	96	108	120	132	144	288	504
Anorexia		1	3	3	3	3	3	2	1			
Abnormal stool		1	1	2	3	3	3	2	2	1		
Excess urates in stool		1	1	2	2	3	3	3	2			
Elevated body temperature		1	3	3	3	3	3	2	3			
Polydipsia			1	1	2	3	3	2	2	2		
Lethargy, dullness, somnolence			1	2	2	3	3	2	1			
Pale or cyanotic combs				1	1	3	3	3	1	1		
Huddled-up attitude with ruffled feathers				1	2	3	3	1	1			
Regurgitation of fluid crop contents					2	3	3	2				
Jaundiced comb							1	1				
Locomotor disturbances										2		
Spirochaetemia		1	1	2	3	3	3	3	3			

1 = slight 2 = moderate 3 = marked

TABLE 2

Postmortem findings in adult fowls experimentally infected with *Borrelia anserina* (Study 1).

Postmortem Finding	Fowl No./Hours after inoculation																		
	1	2	3	4	5	6	7	C*	9	10	11	12	13	14	15	16	17	18	19
	24	48	60	72	72	84	84	96	96	96	108	108	120	120	132	132	144	288	504
Splenomegaly		x		x	x	x	x		x	x								**	
Mottled spleen					x	x			x	x				x	xB	x	xB		
Catarrhal enteritis	x	x	x	x							x								
Bile stained GI mucosa						x	x		x	x	x	x	x	x	x	x			
"pebbled" intestinal mucosa						x	x		x	x	x	x	x	x					x
Foci of int-mucosal hyperaemia			x	x	x	x		x	x	x	x								
Caecal tonsils-haemorrhages			x	x	x	x	x	x	x	x	x		x	x	x			x	
Proven./ven. inflammation			x		x	x			x			x	x	x	x				
Erosion of ventriculus mucosa											x		x		x			x	x
Swollen liver				x					x	x	x	x	x	x	x	x			
Pale foci-liver									x										
Green ("Bronze") liver												x	x		x				
Jaundice									x	x	x	x	x	x	x				
Fibrinous pericarditis						x													
Kidneys swollen, pale brown									x			x	x		x	x			
Pale areas in muscles													x						
Spirochaetemia	x	x	x	x	x	x			x	x	x			x	x	x			

* uninoculated control ** spleen much smaller than normal B brown discolouration

TABLE 3

Onset of clinical signs in 4 adult fowls infected with Borrelia anserina (Study 2).

Fowl No.	Period (Hr.)	Clinical Finding	Body Temp. (°F)	Spirochaetemia
21	24	Formed stool-excess bile	107.8	Neg
	48	Anorexia	109.4	+*
	72	Diarrhoea-excess bile, urates	110.6	+
	96	Icterus, dullness	109.6	+
	156	Intense icterus, pallor	108.4	+
	168	Somnolence	108.6	+
	192	More alert, marked pallor	106.8	Neg
	264	Diarrhoea still present	105.4	Neg
	312	Formed stool-excess bile	106.2	Neg
	360	Improving, gaining weight	106.4	Neg
22	72	Dullness	111.0	+
	84	Formed stool-excess bile, anorexia	111.4	+
	96	Profuse diarrhoea-excess bile/urates, marked lethargy	110.2	+
	144	Slight icterus, somnolence	108.4	+
	156	Died		
23	72	Cyanosis of comb, anorexia formed stool-excess bile	109.6	+*
	84	Profuse diarrhoea-excess bile/urates, dullness	108.4	+
	96	Marked lethargy	109.2	+
	132	Comatose	108.4	+
	144	Dead in a.m.		

TABLE 3 (continued)

Fowl No.	Period (Hr.)	Clinical Finding	Body Temp. (°F)	Spirochaetemia
24	72	Dullness	108.2	+*
	96	Pallor, formed stool-excess bile, decreased feeding	108.0	+
	108	Anorexia, diarrhoea-excess bile/urates	106.0	+
	144	Dead in a.m.		

*Spirochaetes found only in buffy coat smears

TABLE 4

Spleen and liver weights compared to carcas weights, liver:spleen ratio and percent of body weight loss in 10 adult fowls that died following experimental infection with Borrelia anserina.

Bird No.	Time Post Inoculation (Hrs.)	Spleen/ Body (%)	Liver/ Body (%)	Liver/ Spleen	Weight loss (%)
Study 2					
24	140	.71	3.88	5.46	19.8
23	140	.34	2.99	8.79	16.4
22	164	.39	3.58	9.19	18.0
Study 4					
33	122	.73	5.05	6.92	24.7
30	132	.49	3.65	7.45	21.0
32	140	.58	4.72	8.14	28.8
31	140	.48	4.66	9.71	25.5
35	146	.44	3.52	8.00	17.4
37	158	.28	3.08	11.00	23.1
34	158	.27	3.95	14.63	26.4
Mean	144±12.9	.47±.2	3.91±.7	8.93±2.5	22.1±4.2

TABLE 5

Onset of clinical signs in starved and Borrelia anserina infected adult fowls (Study 3).

Period (Hr.)	Group	Clinical Finding	Mean Body Temp.	Spirochaetemia
36	I	Anorexia	109.3	0/3*
	S	Normal, (feed withheld)	104.7	
48	I	Dullness, formed stools-excess bile	109.1	3/3**
	S	Normal	106.6	
60	I	Diarrhoea-excess bile/urates in 2 fowls, excessive thirst	109.3	NE
	S	Normal	104.5	
72	I	Stretched out flat on ground	109.6	3/3
	S	Diarrhoea-excess bile/urates	105.0	
84	I	Moderate lethargy, pallor in 1 fowl	108.9	3/3
	S	As above	102.7	
108	I	Intense thirst, wet neck feathers, huddled together, pallor, profuse watery yellow-green stool, cyanosis of comb in 2 fowls	106.5	3/3
	S	As above	102.1	
120	I	Same, somnolent or comatose	105.1	3/3
	S	As above	105.4	
132-144	I	All 3 died	-	
	S	As above, (feed replaced)	102.8	

I = Infected group
S = Starved group
NE = Not examined

*numerator = No. spirochaetemic
denominator = No. infected

**Spirochaetes found only in buffy coat smears in all 3 fowls.

EXPERIMENTAL BORRELIA ANSERINA INFECTION
IN ADULT FOWLS. RESULTS OF STUDY 1.

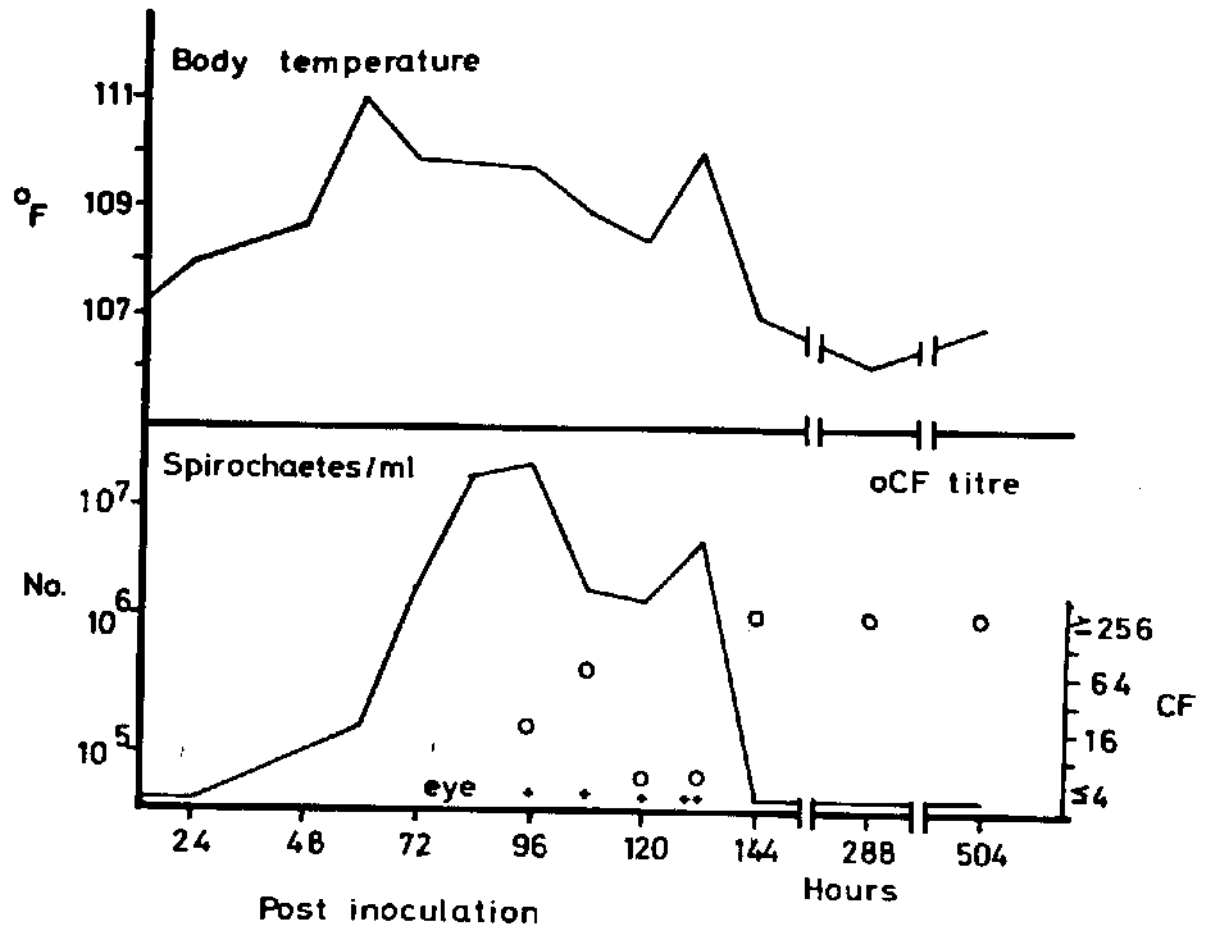


FIG. 1

EXPERIMENTAL BORRELIA ANSERINA INFECTION
IN ADULT FOWLS. RESULTS OF STUDY 1.

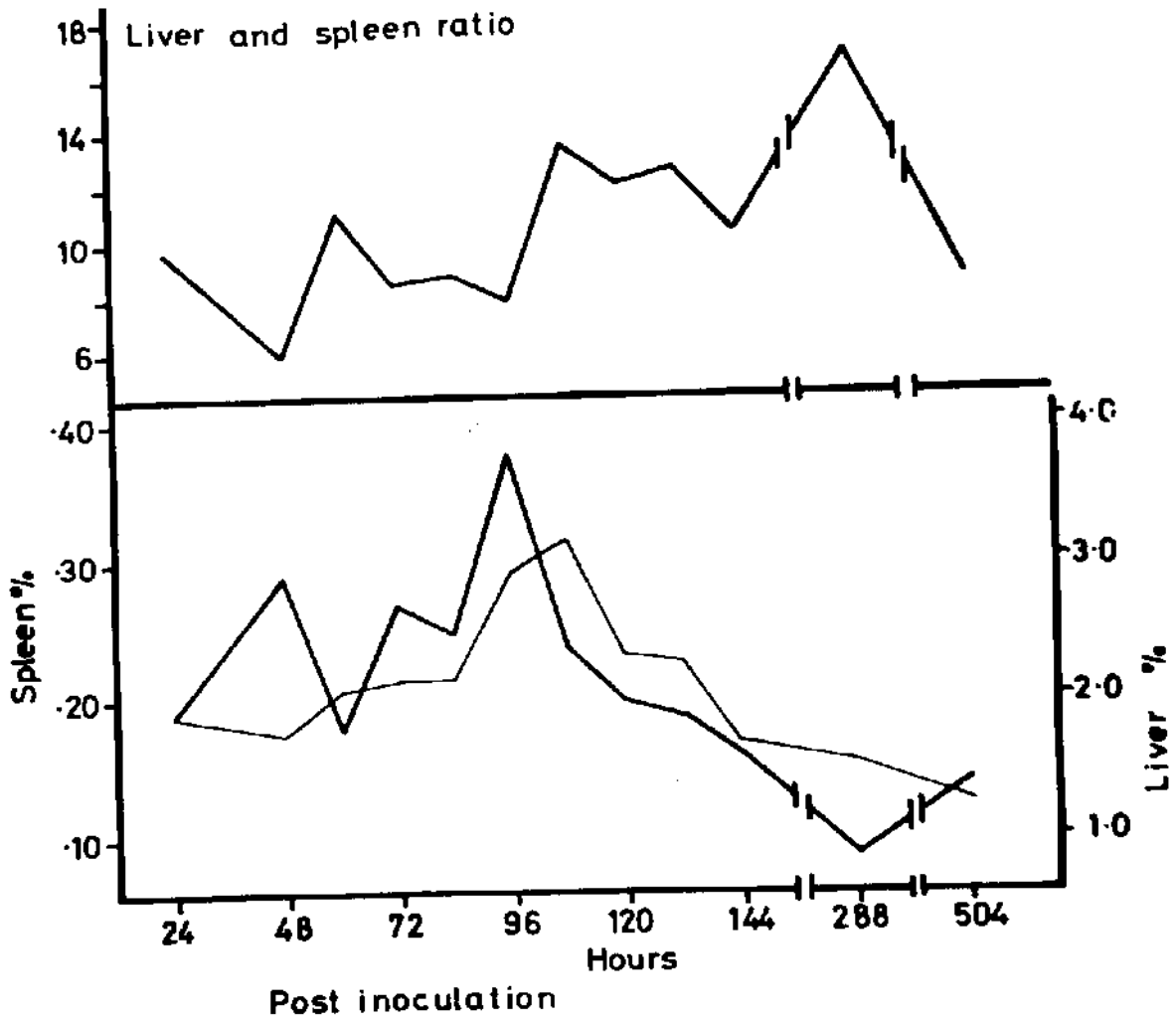
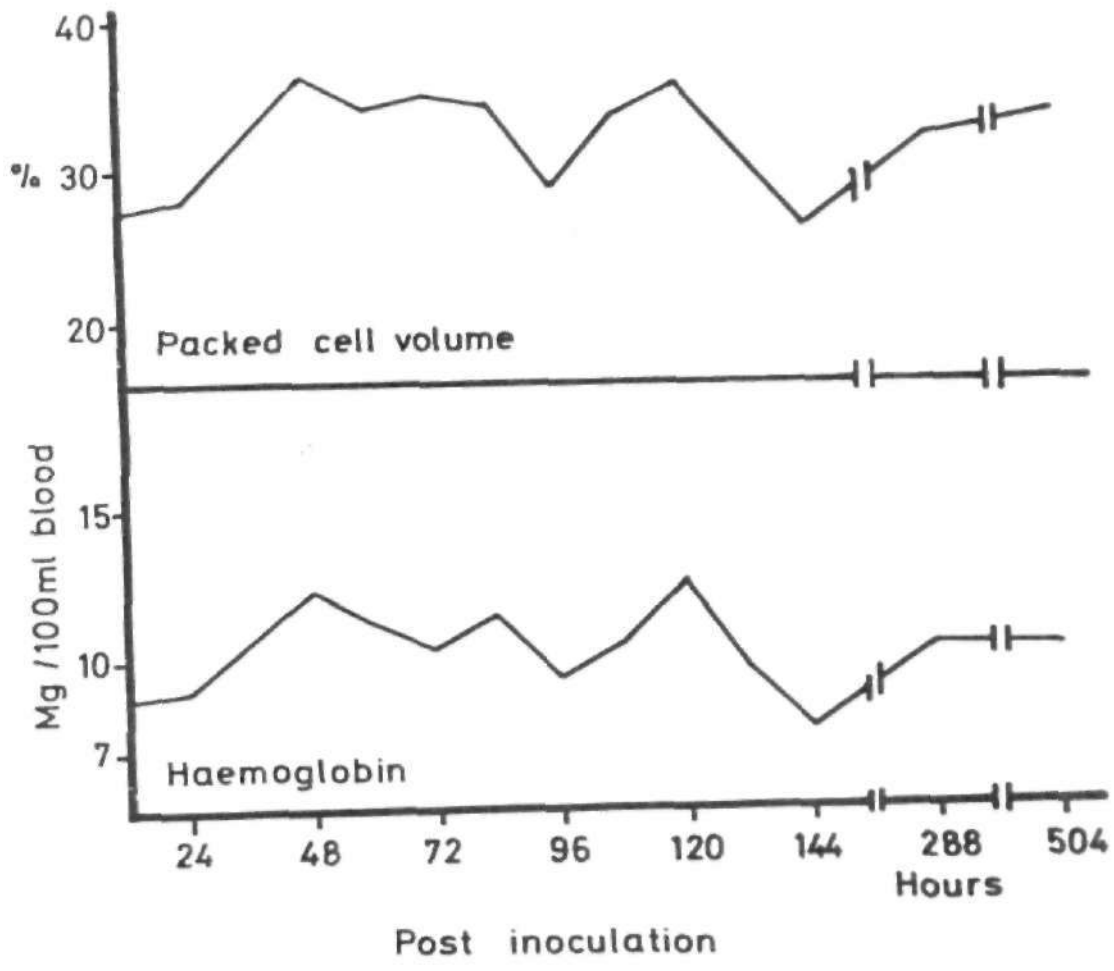


FIG. 2

EXPERIMENTAL BORRELIA ANSERINA INFECTION
IN ADULT FOWLS. RESULTS OF STUDY 1.



BODY TEMPERATURE AND WEIGHTS OF ADULT FOWLS
EXPERIMENTALLY INFECTED WITH BORRELIA ANSERINA.



FIG. 4

HAEMATOLOGICAL VALUES AND GROWTH OF BORRELIA ANSERINA
IN EXPERIMENTALLY INFECTED ADULT FOWLS.

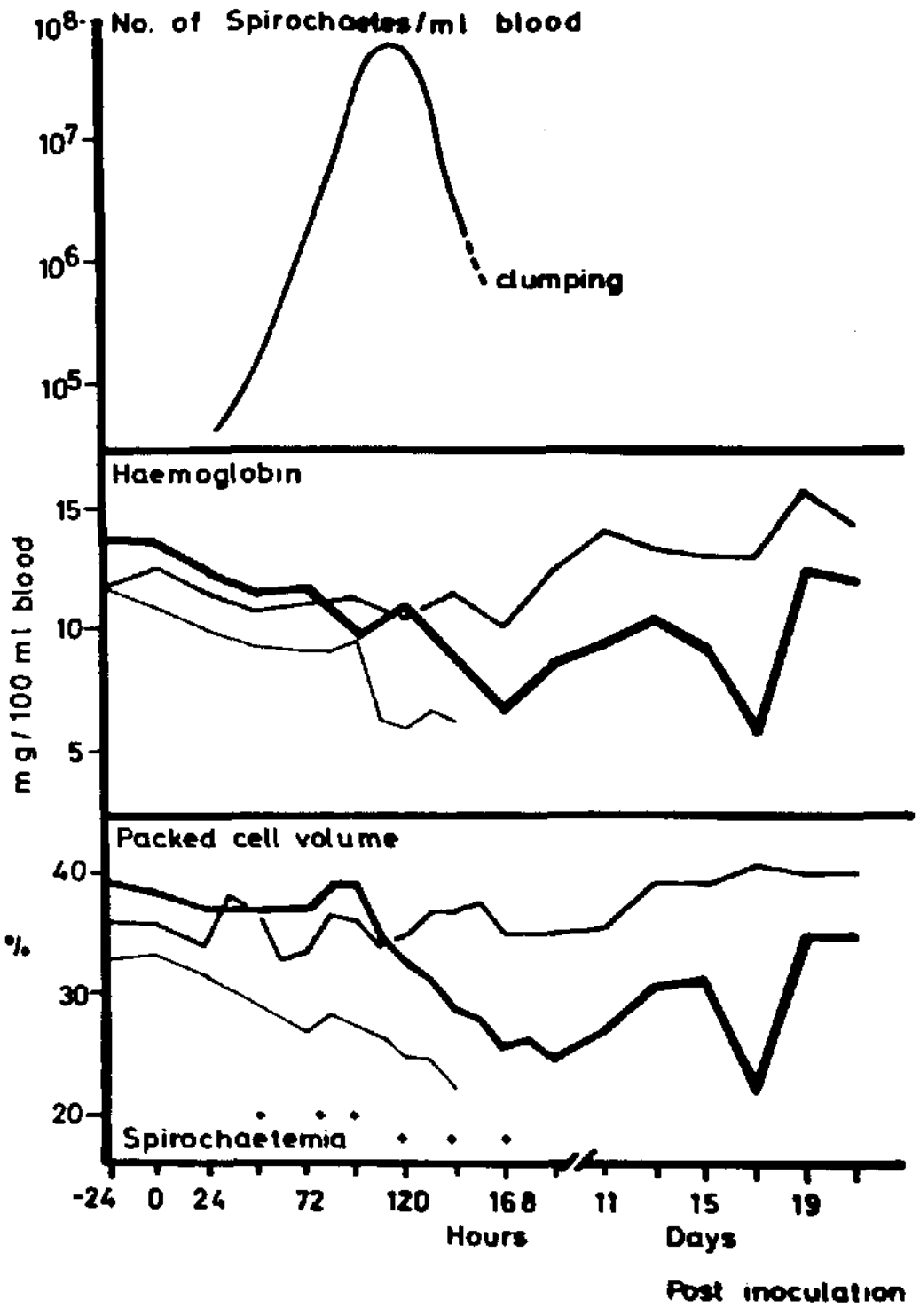


FIG. 5

WEIGHT LOSS AND BODY TEMPERATURE CHANGES IN STARVED BORRELIA ANSERINA INFECTED FOWLS.

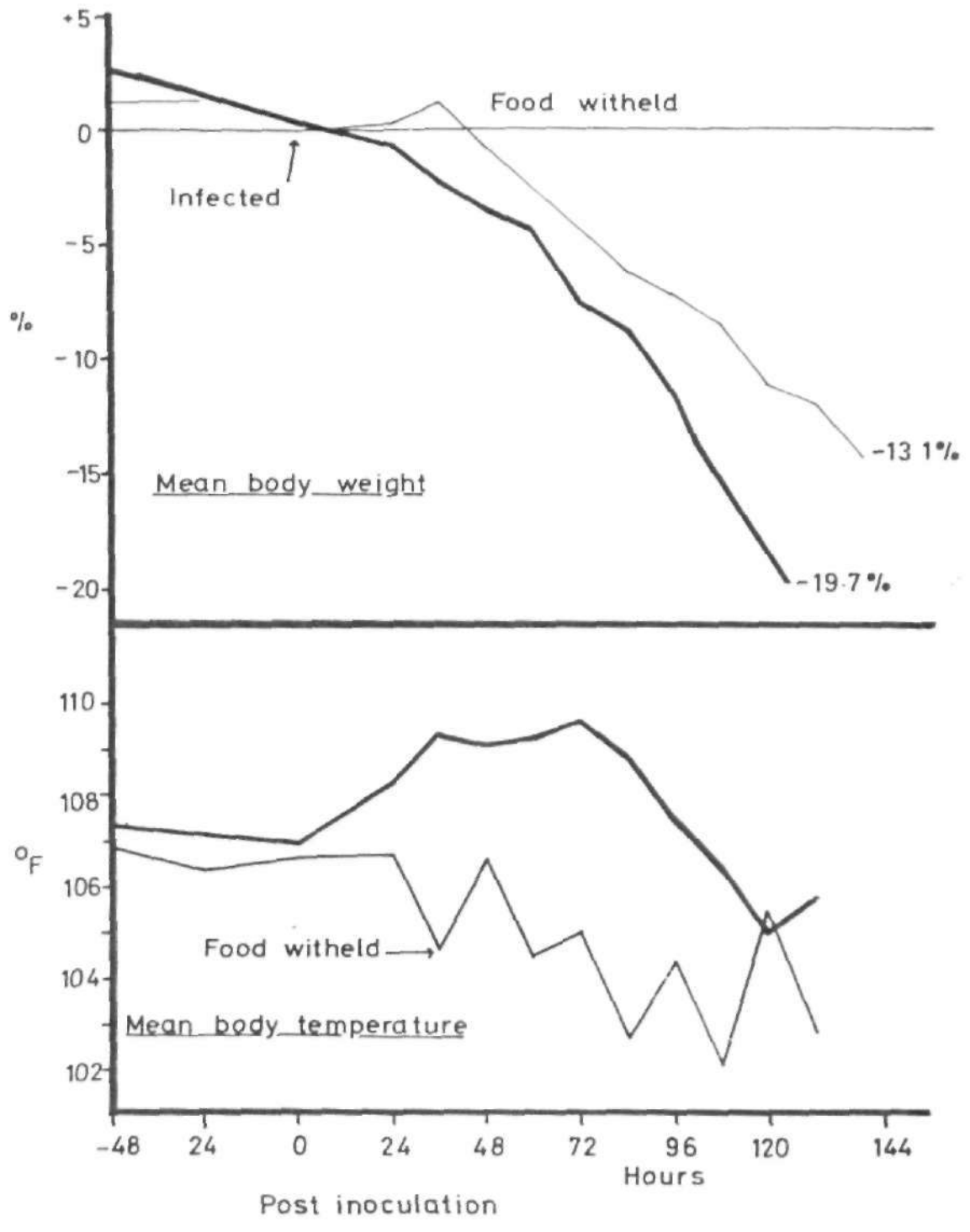
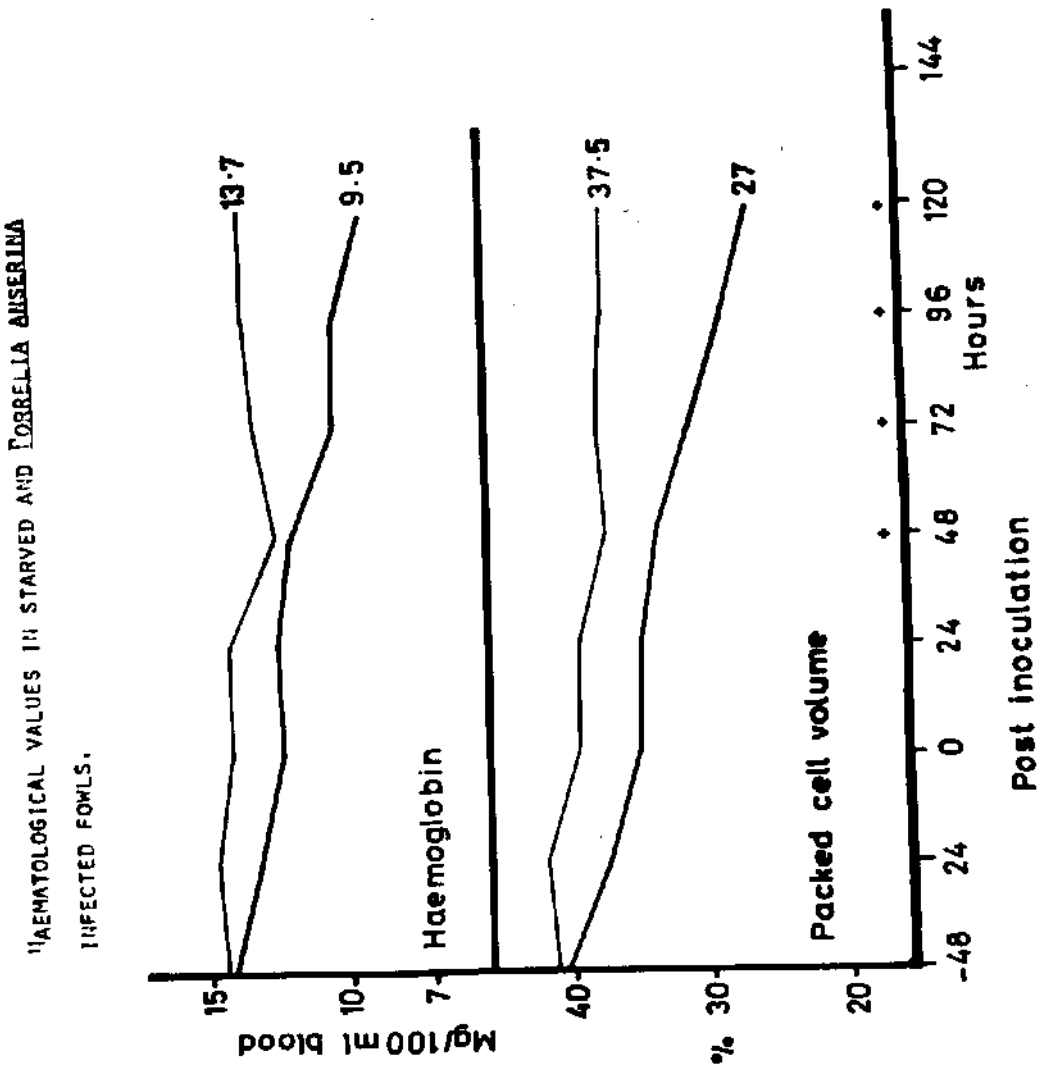


FIG. 6



HAEMATOLOGICAL VALUES IN STARVED AND TOBRELLIA ANSERINA INFECTED FOWLS.

FIG. 7

EXPERIMENTAL BORRELIA ANSERINA INFECTION IN ADULT FOWLS. RESULTS OF STUDY 4, GROUP A.

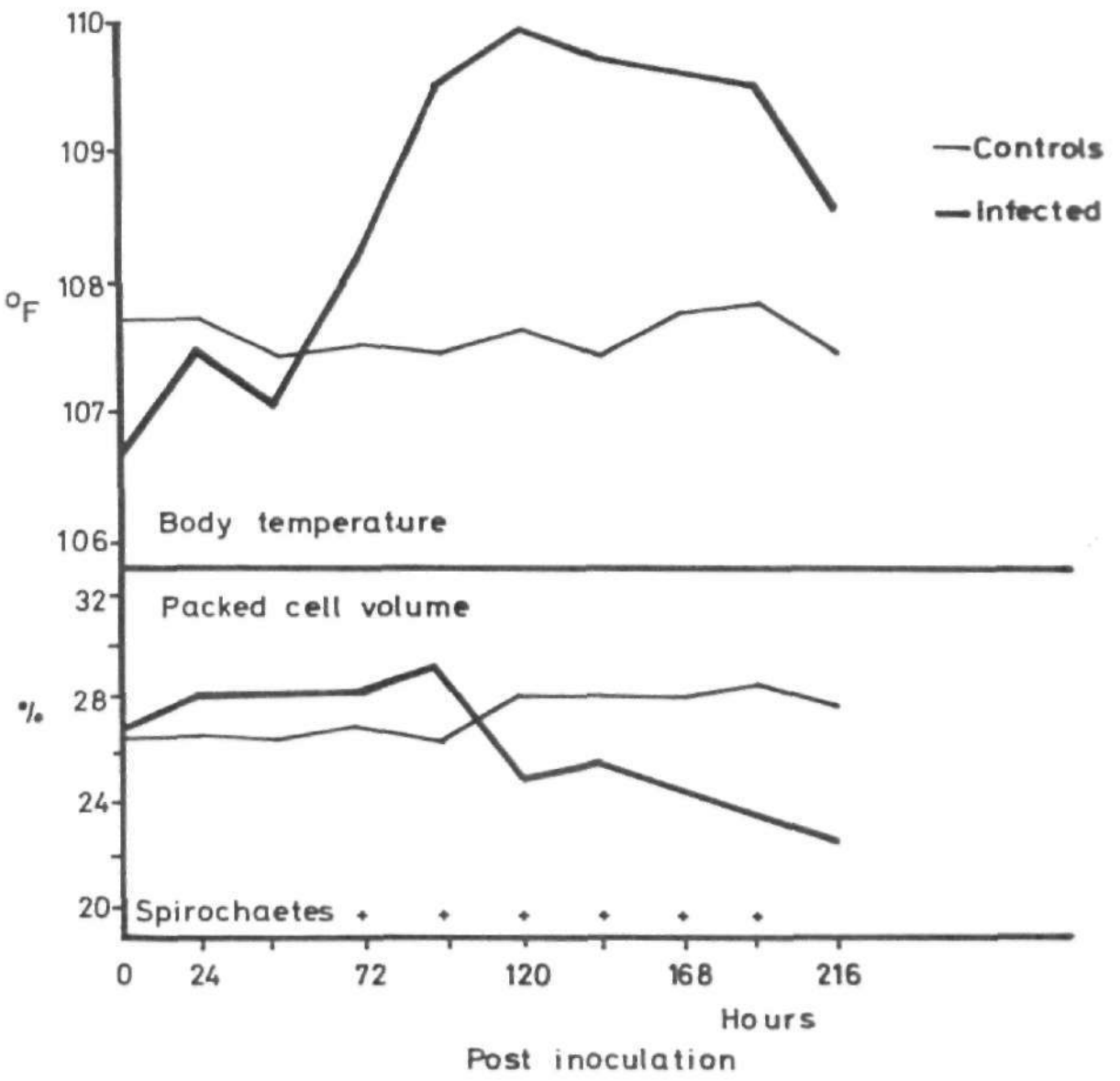


FIG. 8

EXPERIMENTAL BORRELIA ANSERINA INFECTION IN ADULT FOWLS. RESULTS OF STUDY A, GROUP B.

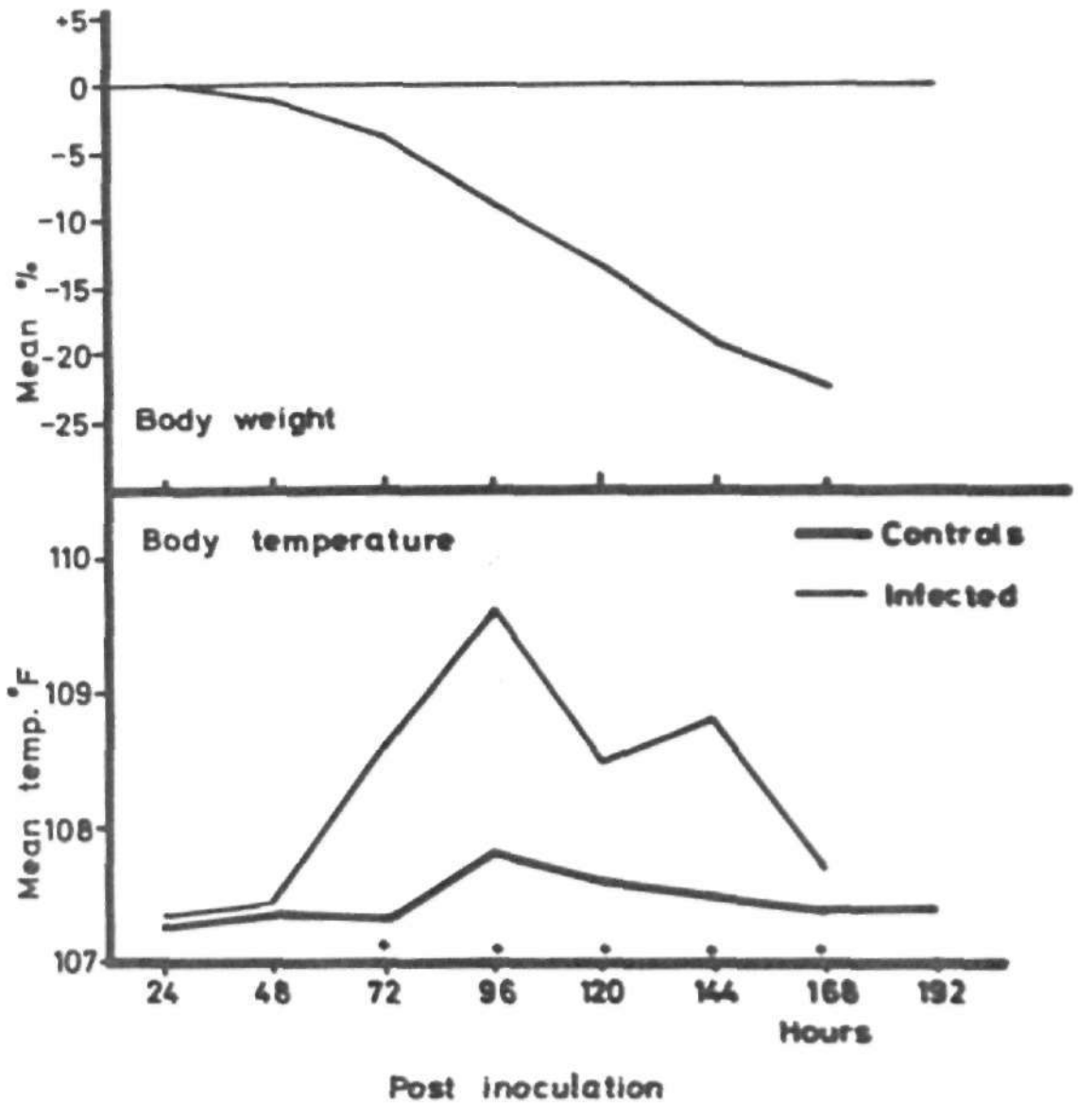


FIG. 9

MEAN CHANGES IN BODY WEIGHT OF 1⁄ ADULT FOWLS THAT DIED FOLLOWING EXPERIMENTAL BORRELIA ANSERINA INFECTION.

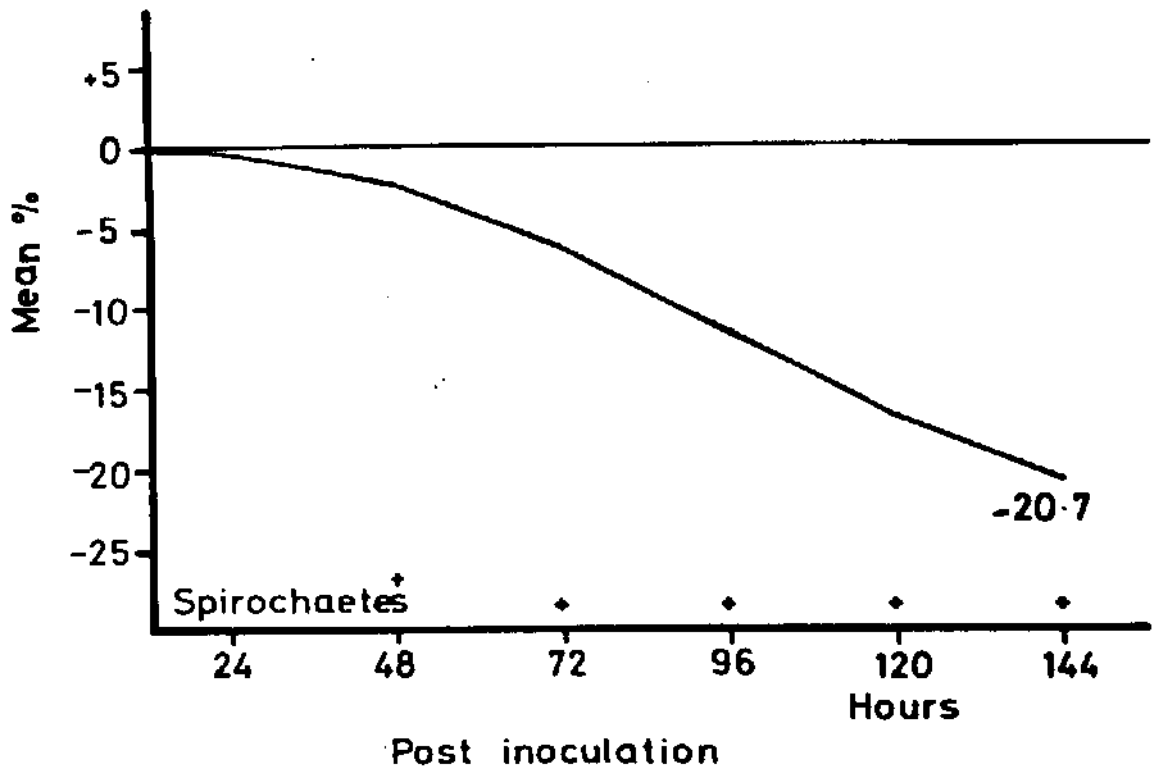


FIG. 10

MEAN BODY TEMPERATURES OF 4 FOWLS THAT SURVIVED EXPERIMENTAL BORRELIA ANSERINA INFECTION AND 11 FOWLS THAT DIED.

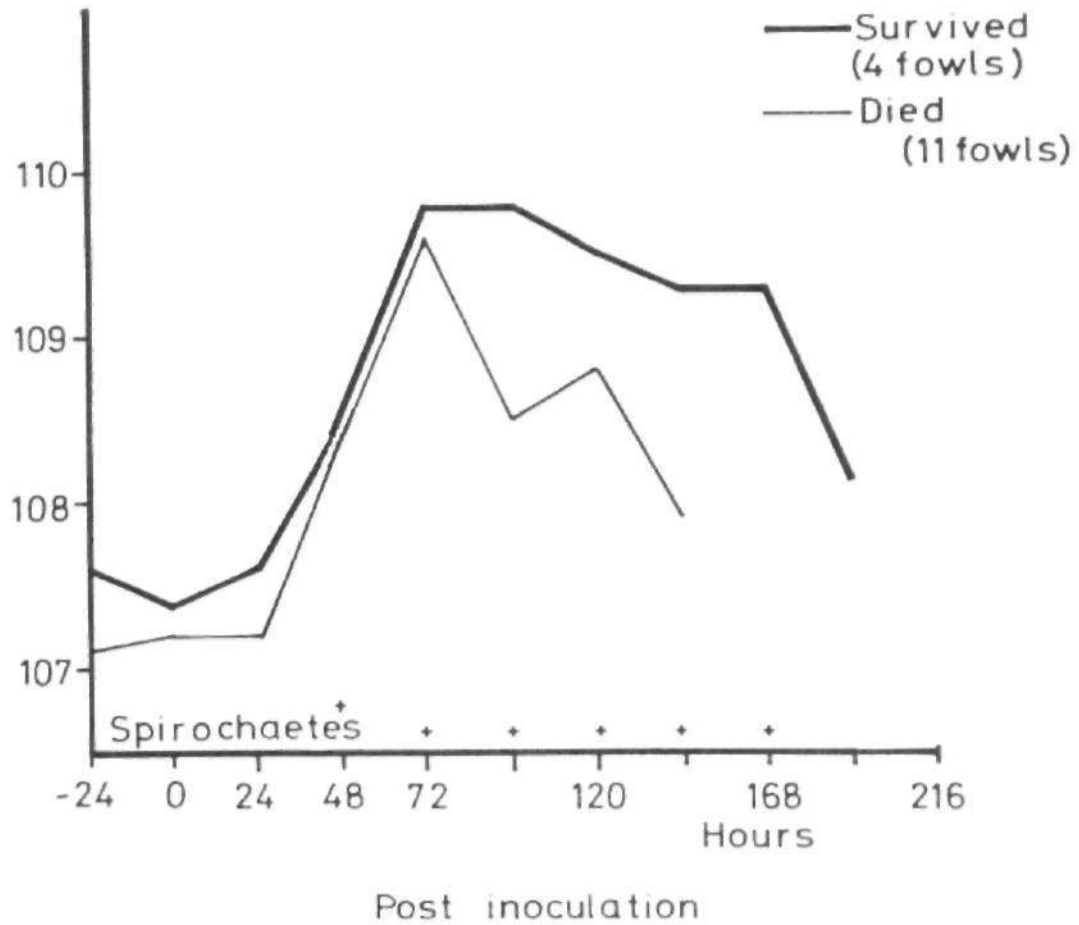


FIG. 11

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AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA: EXPERIMENTAL *BORRELIA ANSERINA* INFECTION IN DAY OLD CHICKS

SUMMARY

Forty-eight day old white leghorn chicks were inoculated with Borrelia anserina and examined daily along with uninoculated control chicks from the same hatching. Exposed chicks had lower mean body temperatures, body weights, packed cell volumes, haemoglobin concentrations and numbers of erythrocytes than control chicks. Failure to grow, slightly depressed appetite and inactivity were the only clinical evidence of infection. Postmortem findings included splenomegaly, hepatomegaly, atrophy of the bursa of Fabricius, muscles and body fat deposits, and in one chick, fibrinous pericarditis. Immune serum (0.5 ml) given subcutaneously to each of 6 infected chicks 12 days after exposure failed to effect cure or precipitate acute clinical signs of spirochaetosis. However, fibrinous pericarditis was found in 4 of 5 serum treated chicks at postmortem. When compared with similar studies in adult fowls, anaemia and splenomegaly were common to both age groups. Clinical signs were less severe, spirochaetemia longer, body weight increased moderately, and involvement of the gastrointestinal tract did not occur in B. anserina infected day old chicks compared with infected adults. From this and other studies it is hypothesized that lysis of the organisms results in the release of potent physiologically active endotoxins. Early host defense mechanisms, including phagocytosis by reticuloendothelial cells and the febrile response, act to reduce the number of spirochaetes early in the infection. Later, antibodies are responsible for the final disappearance of the spirochaetes. In infected birds where the host defense mechanisms are inadequate or lacking e.g. day old chicks, spirochaetes reach higher numbers, remain longer in the circulation and clinical signs are less pronounced because little if any toxin is being released. The birds may die after a prolonged infection because of the intrinsic virulent nature of the spirochaete or the host may develop the ability to attack and destroy the spirochaete in which case the host succumbs as a result of the overwhelming toxaemia.

INTRODUCTION

Considerable differences between Borrelia anserina infection in young chicks and adult fowls have been reported. Blaizot (6) concluded that following infection with the same isolate of B. anserina, young and old birds behaved as if they were different species. Kroo (8) reported that day old chicks experienced a milder disease and prolonged spirochaetemia. He further showed that day old chicks could not be successfully treated using an arsenical compound even though the same treatment was successful in adult birds. Although the spirochaetes disappeared temporarily, they reappeared after a few days in the blood of day old chicks. Chicks that recovered were also found to be susceptible to re-infection whereas recovered adults were immune.

In a previous study on the influence of age on experimental B. anserina infection using a Nigerian isolate, it was found that birds of all ages were susceptible to infection but that day old chicks had a higher mortality and prolonged spirochaetemia compared with birds over 1 week of age (3). This study was undertaken to examine B. anserina infection in day old chicks in greater detail and to compare the results with a similar study conducted on adult fowls.

MATERIALS AND METHODS

Ninety-six day old white leghorn chicks were hatched from eggs collected from a tick and spirochaete free breeding flock maintained at the Faculty of Veterinary Medicine, Ahmadu Bello University. They were arbitrarily divided into two equal groups and maintained separately but under similar conditions in cage brooders with a commercial chick mash and water ad lib. One group was inoculated intramuscularly on the day of hatching with 0.1 ml blood containing 2×10^6 B. anserina, strain H (3). The other group was kept as uninoculated controls.

All infected chicks were examined twice weekly for spirochaetemia. At daily intervals 6 chicks from each group were weighed and the mean body weight calculated except on post-inoculation days (PI) 14 and 16 when insufficient numbers of infected chicks remained. Body temperatures were taken daily via the cloaca from at least 2 birds in each group. All birds in the control and exposed groups were examined for presence of clinical signs. Blood samples were collected in 10% EDTA from two exposed and 1 control chick and the packed cell volume, haemoglobin concentration, number of erythrocytes and spirochaetemia determined. Packed cell volume and number of erythrocytes were determined by standard methods. The method for determining haemoglobin concentrations has been described elsewhere (5). Spirochaetemia was determined by direct wet and buffy coat smears (1). These 3 chicks were then exsanguinated and a postmortem was conducted.

On PI 12 six infected chicks were each given subcutaneously 0.5 ml immune serum prepared against the homologous strain (2). Two control chicks were given 1.0 ml by the same route. They were compared with non-treated infected and control chicks and examined daily for presence of spirochaetes, changes in clinical signs and, in birds that died, gross pathology.

RESULTS

Spirochaetemia was first observed on PI 2 from both exposed chicks that were examined in wet and buffy coat smears. Spirochaetemia was last demonstrated in chicks examined on PI 16. One exposed chick recovered from B. anserina infection between PI 10-13 and was excluded from the remainder of the study.

The body weight of control chicks increased progressively while the body weight of infected chicks increased very little. The initial mean body weights were identical for both groups 26.6 gm. At PI 15 when the maximum mean body weight for infected chicks was found, the mean body weight of control chicks had increased 140.6% to 64 gm while that of infected chicks had only increased 39.8% to

37.2 gm (Fig. 1). By PI 9 the size difference between the two groups was marked and the infected chicks gave the appearance of being emaciated.

There was some fluctuation in body temperature of both groups during PI 1-5. Marked drops in body temperature occurred on PI 5 and 14 in the infected chicks. Body temperatures of infected chicks were consistently lower than those of control chicks after PI 5. The mean difference was, with a single exception (PI 13), always greater than 0.5°F (Fig. 1).

Haematological values were similar in both groups between PI 1-3 after which they tended to increase slightly in the control chicks and decrease slightly in the infected chicks. After PI 7, the values for infected chicks were considerably lower than those of the control chicks (Fig. 2).

The only clinical evidence of infection was the failure to grow as noted above, slightly depressed appetite and inactivity compared with control chicks. Infected chicks had no evidence of diarrhoea. Terminally the chicks became moribund.

Tissue changes included an enlarged, pale liver without mottling or necrotic foci, an enlarged pale brown, mottled spleen, atrophy of the bursa of Fabricius, muscle atrophy and serous atrophy of the body fat deposits. Bursal atrophy became more pronounced and spleens tended to become larger and more brown as the infection progressed. There was no evidence of enteritis or changes in the gastrointestinal tract tissues. Kidneys appeared grossly normal in all chicks except for one infected chick killed on PI 11 in which the kidneys were pale and tubules prominent, containing large amounts of urates. Fibrinous pericarditis was observed in one chick killed on PI 16.

Of the 6 infected chicks treated with immune serum all died and had high spirochaetemias until they died. Serum treated chicks died on the 1st (1), 2nd (2), 3rd (1) and 4th (2) days after treatment. During this same period, 3 of 8 untreated, infected chicks died naturally and the remainder were sacrificed. Five of the 6 infected,

treated chicks were examined for spirochaetes at postmortem and all were found positive. The 2 uninfected control chicks that had been given twice the amount of serum remained healthy. Five of the 6 infected, treated chicks were examined at postmortem. Livers were enlarged and mottled, spleens smaller than non-treated, infected chicks and the bursa of Fabricius was atrophic in all 5 chicks. In 4 chicks there were soft fibrinous masses free in the pericardial sac or adhered to the epicardium.

DISCUSSION

The results of this study agree with earlier studies (3,6,8) and support the premise that considerable differences exist between B. anserina infection in day old chicks and older fowls. By utilizing data obtained in companion studies (3,5) it is possible to arrive at some definite conclusions regarding these differences (Table 1).

It is apparent that day old chicks lack the ability to control and eliminate B. anserina initially but this capability is probably acquired after 1-3 weeks. It has been shown that B. anserina can kill developing embryos (4) which suggests an intrinsic ability to be pathogenic even in the absence of a host response. If this is true then the mortality in day old chicks could in fact not be related to acquired host resistance. With the ability to control and eliminate the spirochaete, there would appear to be an attendant danger of severe illness or mortality such as seen in infected adults. It would seem likely that the destruction of B. anserina by host defence mechanisms--probably phagocytosis initially and antibody formation later--results in the release of potent physiologically active substances (endotoxins?) which are responsible for the severe clinical signs, high febrile response and mortality. Whenever the host defence mechanisms are inadequate, spirochaetes can proliferate and reach a considerable number. If the host then develops or acquires the ability to

destroy the spirochaetes, their destruction could precipitate severe illness and mortality from the large amount of toxin released. This phenomenon is well recognized in human relapsing fever following crisis or treatment. Evidence for a Borrelia endotoxin in relapsing fever at this stage has been presented (7). If the host does not develop or acquire the ability to destroy the spirochaetes, the course of the disease would be, in comparison, mild but the sheer presence of numbers coupled with the intrinsic pathogenicity of the organism and potent toxin that may be released following natural death of the spirochaetes could be sufficient to debilitate or even kill the host. It has been reported (9) that splenectomy, which should remove a good deal of the host's phagocytic abilities, results in a longer, more intense spirochaetemia, but less pronounced clinical signs. This further supports the theory that phagocytosis is initially responsible for controlling the numbers of spirochaetes and their destruction is responsible for the severity of clinical signs.

Certain features appear to be common to both day old and adult fowls; splenomegaly and anaemia. Splenomegaly was also found in infected embryos (4). Stimulation of splenic cells to proliferate would appear to be a common feature of B. anserina infections unrelated to other host responses. The anaemia would also appear to be the result of spirochaete activity and not related to other host activities.

Immune serum was given to 6 chicks in the later stages of B. anserina infection for 2 reasons. To see if antiserum could be useful clinically in curing avian spirochaetosis and to see if acute disease and crisis developed following its use. Neither cure nor crisis ensued. The reason may have been technical. Perhaps different antiserum, dosages, routes, timing, or other variable may have resulted in different findings. The number of treated chicks that had fibrinous masses in the pericardial sac would seem to be higher than would ordinarily be expected. This finding suggests

mild vascular damage. Without larger numbers of experimental chicks it is difficult to assign specific meaning to this observation.

TABLE 1

Comparison between Borrelia anserina infection in day old chicks and adult fowls.

Finding	Day old chick	Adult fowl
Clinical		
Body temperature	Normal to subnormal (102.5-105.5°F)	Elevated (109-111°F)
Body weight	Failure to grow, slight increase (30-40%)	Marked loss (20-25%)
Diarrhoea	Absent	Present
Food consumption	Normal to slight decrease	Nil
Water consumption	Normal	Increased to markedly increased
Activity	Normal to slight decrease Moribund terminally	Marked decrease Somnolent or comatose terminally
Anaemia	Present, later stages	Present, later stages
Spirochaetemia, numbers	Probably high*	Moderate
Spirochaetemia, duration	Prolonged (5-20 days)	Short (3-6 days)
Mortality	High (near 100%)	Moderate (approx. 75%)
Severity	Mild to moderate	Marked
Postmortem		
Spleen	Splenomegaly throughout Mottled, pale brown	Splenogegaly early, normal to subnormal size later. Often mottled
Liver	Hepatomegaly, pale, absence of pattern or foci	Hepatomegaly later Reticulated appearance typical Foci occasionally Size reduced in chronic stage

TABLE 1 (continued)

Finding	Day old chick	Adult fowl
Kidney	Usually normal, rarely pale	Pale, swollen, considerable urates seen late in spirochaetemia, normal otherwise
Intestine	Normal	Catarrhal enteritis, excess bile, hyperaemia in mucosa, petechial haemorrhages in caecal tonsils
Stomach	Normal	Eroded ventriculus mucosa. Inflammation of proventriculus/ventriculus junction.
Heart	Fibrinous pericarditis uncommon	Fibrinous pericarditis uncommon
Muscle	Wasting, emaciation	Large pale areas uncommon
Crop	Normal	Often fluid filled
Other	Bursal atrophy Serous atrophy of fat	

* Empirical observation only, actual counts not done.

INFLUENCE OF BORRELIA ANSERINA INFECTION ON BODY WEIGHT AND TEMPERATURE OF CHICKS INFECTED AT ONE DAY OF AGE.

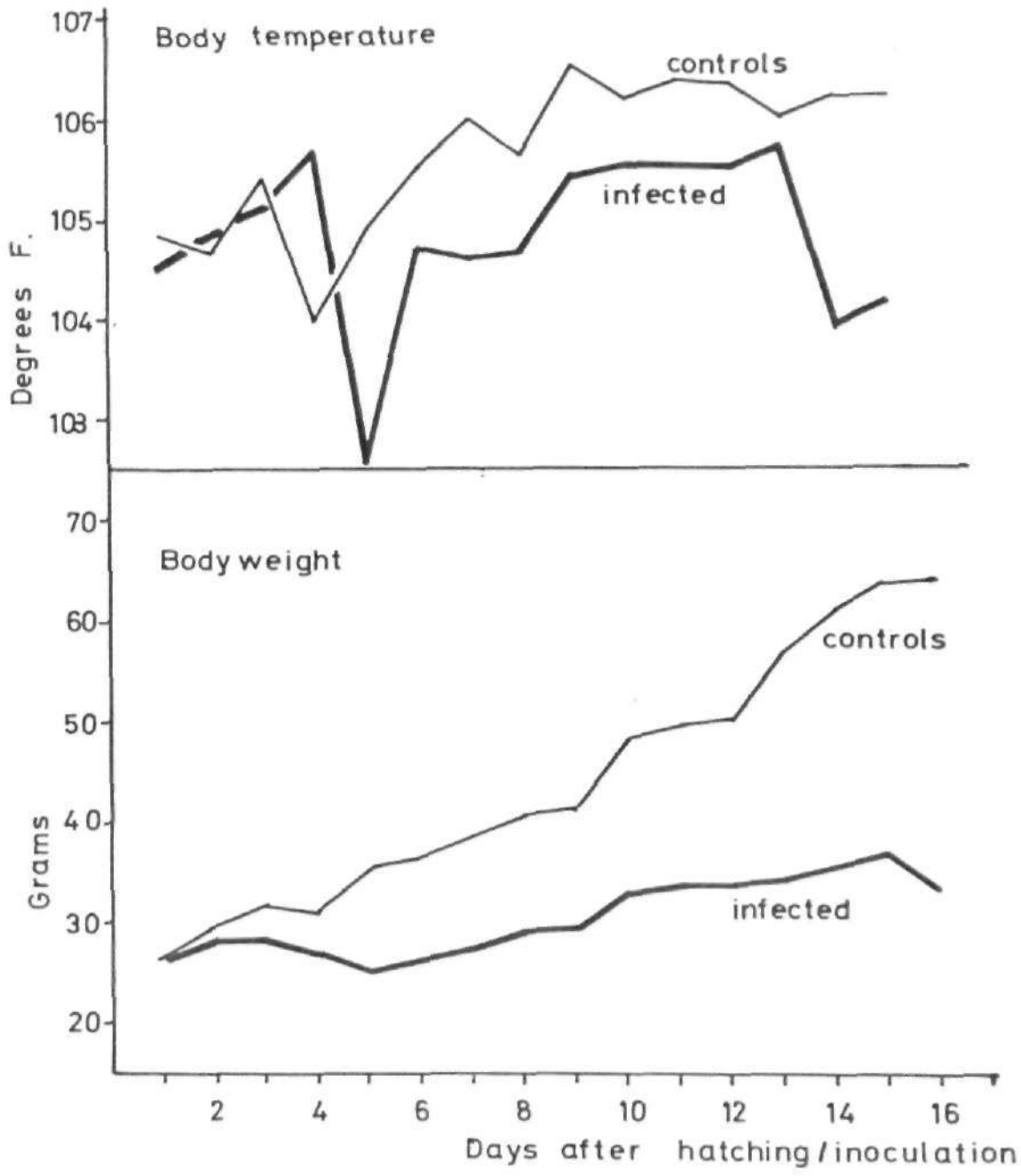
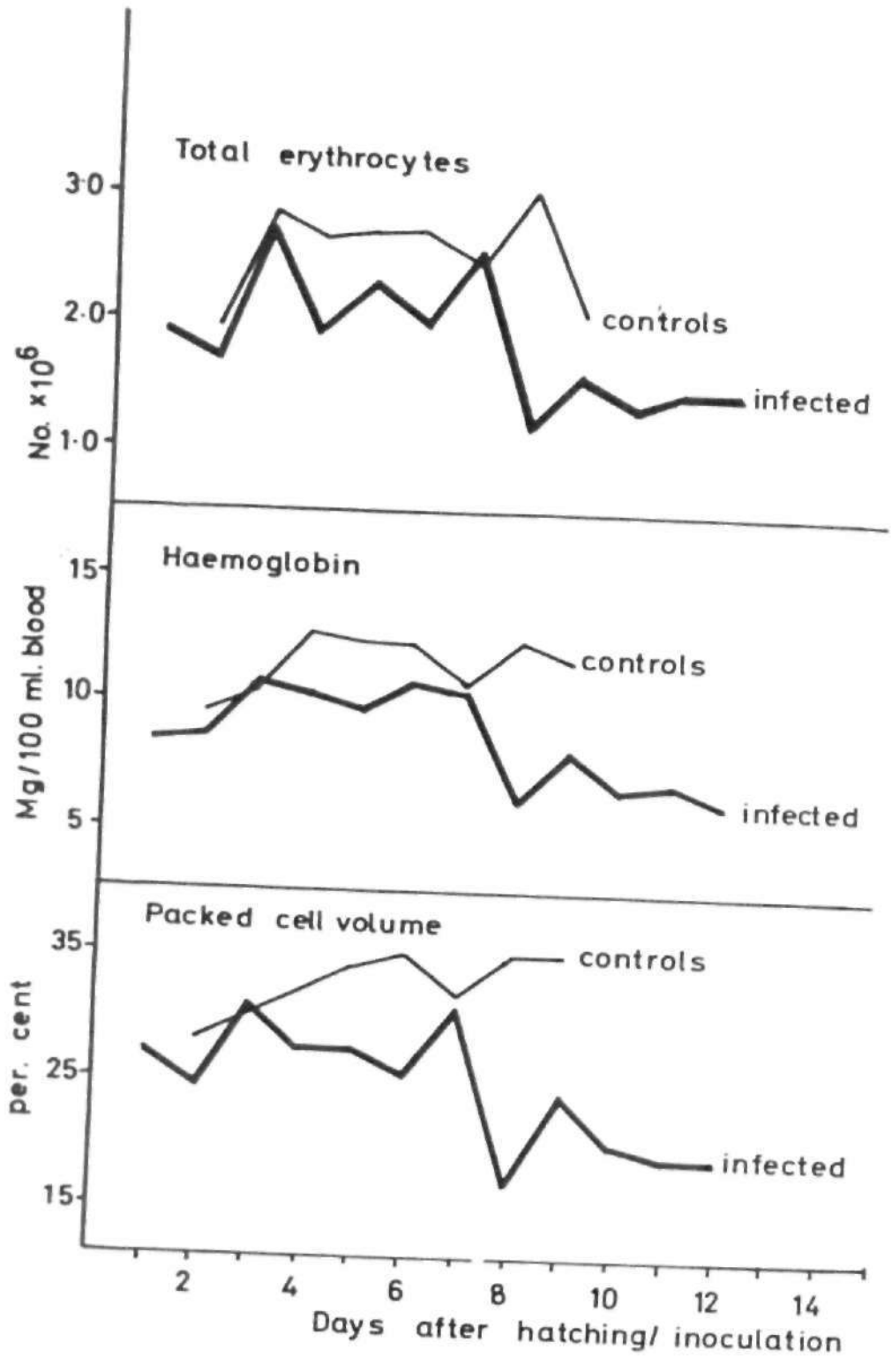


FIG. 1

INFLUENCE OF BORRELIA ANSERINA INFECTION ON HAEMATOLOGICAL VALUES OF CHICKS WHEN INFECTED AT ONE DAY OF AGE.



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APPENDIX

Infectivity of Borrelia anserina from heart clot and brain of fowl carcasses kept refrigerated (3-6°C) or frozen (-10°C) for chicks.

Storage	Time	Tissue	Smears	Inoculated Chick No.	Days Post-Inoculation 1	2	3	4	5	6	7	8	9	10	11	12
REFRIGERATED	5 Days	Brain	Numerous spirochaetes motility 85%	1	Neg	+	++	++++	++++	D						
				2	Neg	Neg	+	++++	D							
		Heart	Numerous spirochaetes motility 30%	3	Neg	Neg	++	++++	++++	++++	++++	++++	++++	++++	D	
				4	Neg	Neg	++	+++	++++	D						
	6 Days	Brain	Numerous spirochaetes non-motile	5	Neg	Neg	++	++	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
				6	Neg	Neg	N	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	15 Days	Brain	Numerous spirochaetes 50% motile	7	Neg	Neg	+	++++	D							
				8	Neg	Neg	++	++++	++++	D						
		Heart	Numerous spirochaetes, 1% motile	9	Neg	Neg	N	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
				10	Neg	Neg	N	+	+++	++++	++++	D				
	14 Hours	Heart	Numerous spirochaetes, non-motile	11	Neg	Neg	NE	+++	++++	D						
				12	Neg	Neg	NE	++++	++++	+++	D					
	32 Hours	Heart	Clumps, difficult to recognize non-motile	13	Neg (D)											
				14	Neg	Neg (D)										
	7 Days	Heart	Very few recognizable spirochaetes, "ghost-like" or beaded filaments	15	Neg	Neg (D)										
				16	Neg (D)											

D = Died of spirochaetosis (D) = Non-specific death Neg = Negative N = Negative
NE = Not examined + = 1-10 spirochaetes/10 high power field (HPF, X300)
++ = 1-5 spirochaetes/HPF ++++ = 5-50 spirochaetes/HPF ++++ = over 50 spirochaetes/HPF

Meteorological Data for Samaru, Zaria, 1971-1974*

Month	Rainfall (MM) 1971	1972	1973	1974	Average **
January	0.00	0.00	0.0	0.0	0.2
February	0.00	0.00	0.0	0.0	1.1
March	3.08	0.25	0.5	11.2	7.7
April	0.00	51.31	21.6	30.0	35.1
May	101.20	152.91	47.0	33.8	120.1
June	27.06	95.76	191.7	144.4	162.0
July	199.98	172.47	191.8	251.9	225.3
August	258.72	325.12	227.5	328.9	285.1
September	168.96	85.34	292.6	247.0	227.3
October	6.60	24.13	1.5	67.6	36.4
November	0.00	0.00	0.0	0.0	1.2
December	0.00	0.00	0.0	0.0	0.1
Total/Means	765.60	907.29	974.2	1114.8	1101.6

* Kindly supplied by the meteorological office, Agronomy Division, Institute for Agricultural Research, Zaria, Nigeria

** Data from 1924-1974

Meteorological Data for Samaru, Zaria, 1971-1974* (continued)

Month	Relative Humidity (%)									
	1971		1972		1973		1974		Average	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
January	16.0	13.0	13.7	9.7	15.3	10.8	20.5	16.1	23.7	13.4
February	15.5	8.9	14.1	9.4	15.5	9.7	17.3	7.1	21.0	11.2
March	33.7	15.0	26.9	14.9	22.0	8.7	16.9	11.0	30.9	14.5
April	34.8	20.3	49.6	30.0	28.8	19.1	52.8	25.3	52.1	27.4
May	59.0	39.7	68.8	45.8	58.2	31.4	59.2	36.8	68.6	44.0
June	67.0	46.5	75.0	59.1	66.4	48.3	64.8	52.5	77.4	57.8
July	77.1	59.6	76.2	60.8	75.2	61.9	82.3	67.4	83.3	67.0
August	80.9	67.6	79.4	66.3	81.9	65.8	79.8	67.9	85.4	71.1
September	77.7	59.7	75.8	58.5	76.2	63.9	78.0	65.2	81.3	63.7
October	48.5	31.8	60.1	45.9	54.7	36.1	65.7	44.1	68.3	43.1
November	21.4	16.3	19.7	16.6	19.8	18.6	24.5	19.1	36.2	21.1
December	19.6	17.2	20.8	15.3	19.7	14.2	21.4	16.4	25.6	15.9
Total/Means	45.9	33.0	48.3	36.0	44.5	32.4	47.8	35.7	54.5	37.5

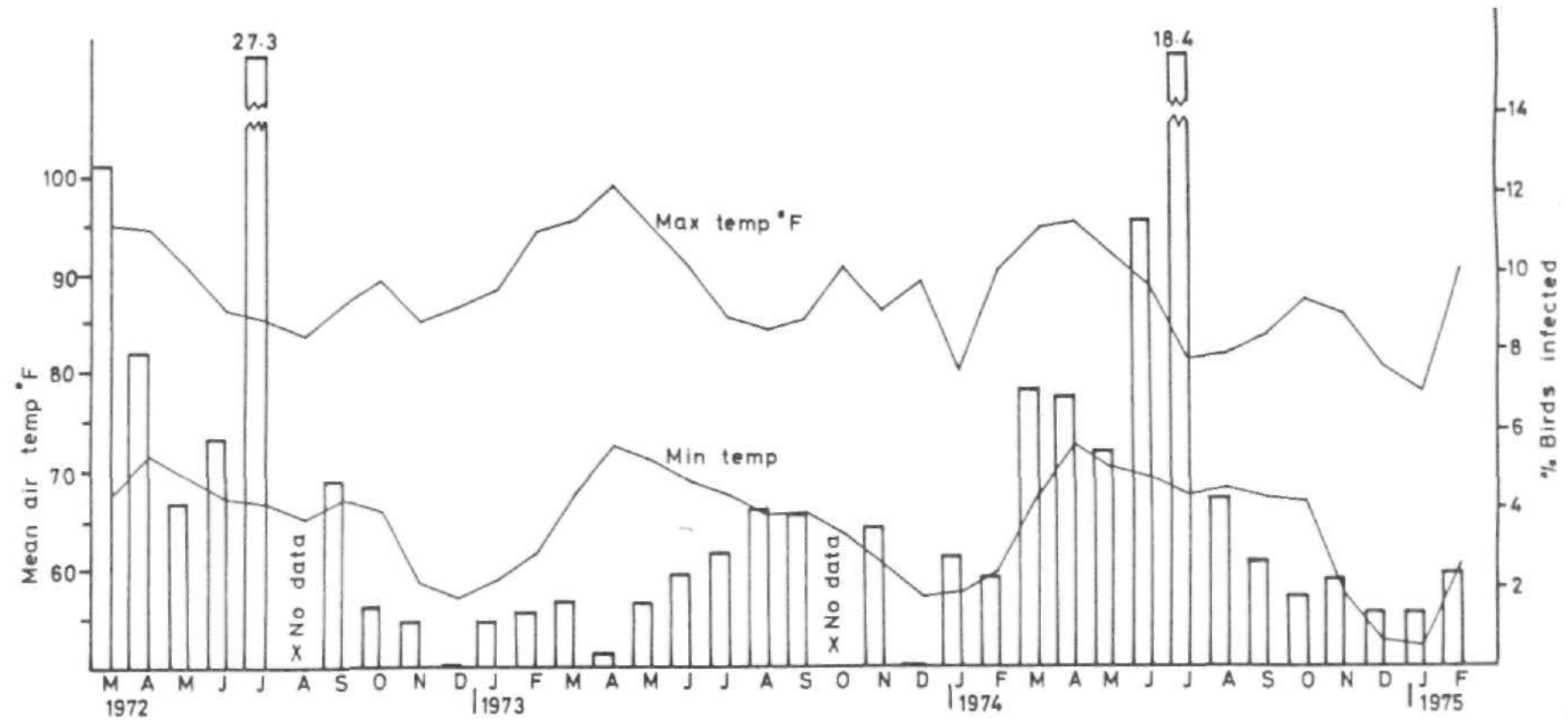
Month	Air Temperatures (°C)									
	1971		1972		1973		1974		Average	
	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
January	13.4	28.0	14.2	30.0	15.2	31.6	14.5	26.8	14.1	30.5
February	16.6	32.7	17.1	32.8	16.7	34.8	15.6	32.3	16.0	32.7
March	20.2	36.4	19.7	35.4	19.8	35.5	19.6	34.8	19.5	35.3
April	21.6	36.7	22.1	35.2	22.5	37.2	23.0	35.6	21.7	35.8
May	21.5	34.4	21.1	32.6	22.0	35.3	21.5	33.2	21.2	33.2
June	19.1	31.8	19.7	30.1	20.4	32.6	20.9	31.4	19.9	30.8
July	19.3	28.7	19.3	29.6	19.5	29.6	19.7	27.4	19.4	28.5
August	18.8	27.7	18.8	28.9	18.6	28.7	20.0	27.8	19.3	27.7
September	19.0	28.9	19.4	30.6	18.8	29.9	19.3	28.6	19.1	29.3
October	17.9	31.3	18.9	31.4	17.8	32.4	19.0	30.7	17.6	31.4
November	16.0	31.2	15.3	29.4	16.0	30.0	14.3	29.9	15.7	31.6
December	13.6	28.6	14.3	30.4	14.6	31.7	12.5	27.1	14.0	30.6
Total/Means	18.6	31.4	18.3	31.4	18.5	32.4	18.3	30.5	18.1	31.5

Monthly Prevalence of Borrelia anserina Infection at Zaria, Nigeria

Infection in Market Fowls March, 1972 - February, 1975

Month	1972	1973	1974	1975	Total
	%	%	%	%	%
January	-	5/408(1.2)	3/111(2.7)	3/230(1.3)	11/749(1.5)
February	-	5/368(1.4)	6/275(2.2)	4/174(2.3)	15/817(2.0)
March	17/133(12.8)	7/436(1.6)	18/258(7.0)	-	42/827(5.1)
April	14/176(8.0)	1/297(.3)	13/191(6.8)	-	28/664(4.2)
May	7/170(4.1)	6/366(1.6)	20/363(5.5)	-	33/899(3.7)
June	2/35 (5.7)	5/214(2.3)	21/185(11.4)	-	28/434(6.5)
July	9/33 (27.3)	4/139(2.9)	45/244(18.4)	-	58/416(13.9)
August	-	4/135(4.0)	10/232(4.3)	-	14/367(3.8)
September	4/85 (4.7)	3/79 (3.8)	5/193(2.6)	-	12/357(3.4)
October	4/274(1.5)	-	3/175(1.7)	-	7/449(1.6)
November	3/251(1.2)	6/171(3.5)	5/240(2.1)	-	14/662(2.1)
December	0/197(0)	0/48 (0)	3/210(1.4)	-	3/455(.7)
Total	60/1354(4.4)	46/2661(1.7)	144/2450(5.9)	11/524(2.1)	265/7096(3.7)

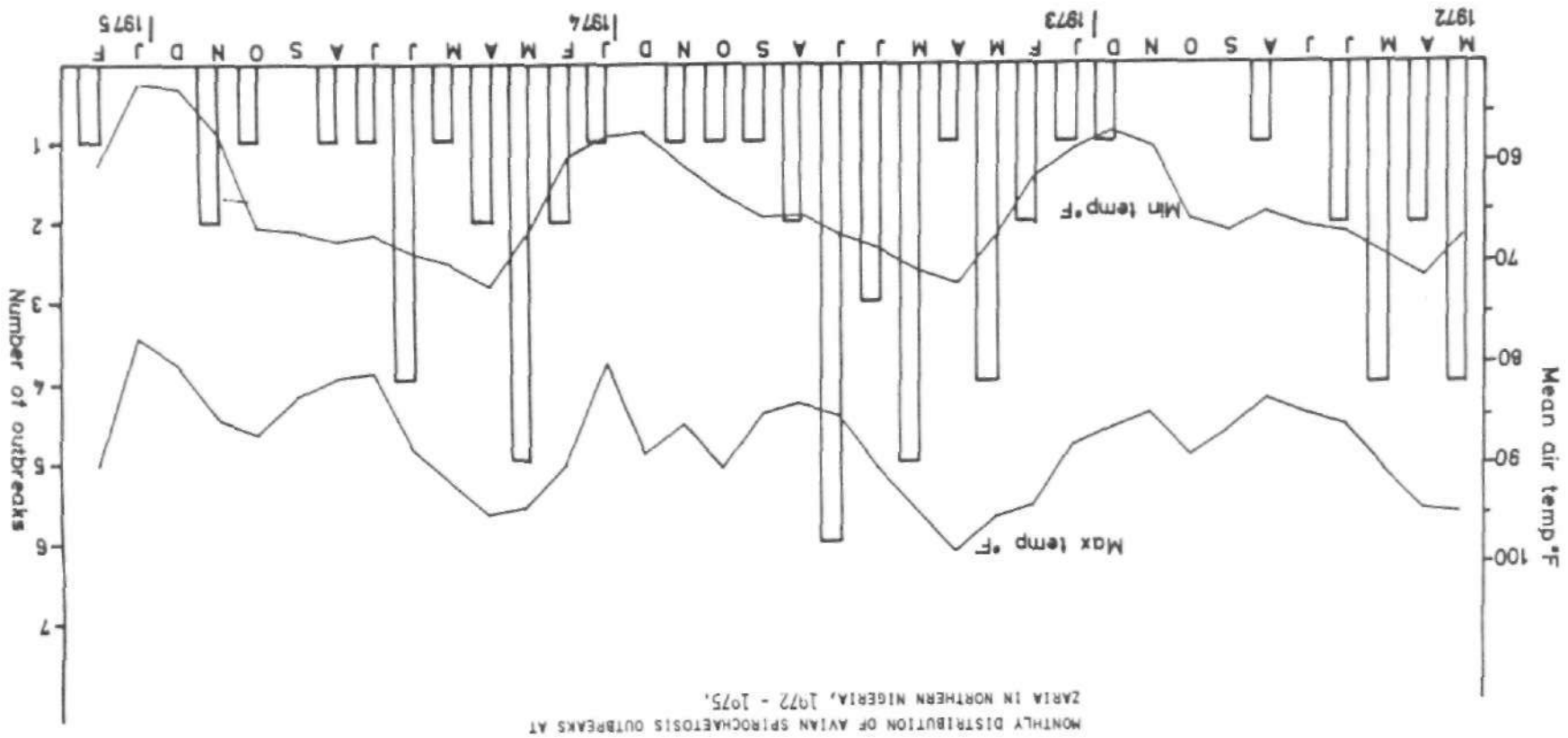
MONTHLY VARIATIONS IN THE PREVALENCE OF BORRELIA ANSERINA INFECTION IN MARKET FOWLS IN NORTHERN NIGERIA, 1972 - 1975.



Monthly Prevalence of Borrelia anserina Infection at Zaria, Nigeria

Outbreaks of Avian Spirochaetosis January, 1971 - Februay, 1975

Month	1971	1972	1973	1974	1975	Total
January	0	0	1	1	0	2(.4)
February	2	4	2	2	1	11(2.2)
March	3	4	4	5	0	16(4.0)
April	0	2	1	2		5(1.3)
May	1	4	5	1		11(2.8)
June	2	2	3	4		11(2.8)
July	1	0	6	1		8(2.0)
August	0	1	2	1		4(1.0)
September	1	0	1	0		2(.5)
October	2	0	1	1		4(1.0)
November	4	0	1	2		7(1.8)
December	2	1	0	0		3(.8)
Total	18	18	27	20	1	84(1.6)



MONTHLY DISTRIBUTION OF AVIAN SPIROCHAETOSIS OUTBREAKS AT ZARIA IN NORTHERN NIGERIA, 1972 - 1975.

Borrelia anserina and Aegyptianella pullorum infections in chicks from a tick and spirochaete-free flock compared with chicks hatched and being reared in a tick-infested environment.

Introduced Chicks	Date 1/12 (10)*	4/12 (13)*	11/12 (20)*	15/12 (24)*	18/12 (27)*	22/12 (31)*	25/12 (34)*	29/12 (38)*	2/1 (42)*	5/1 (45)*	8/1 (48)*	12/1 (52)*	22/1 (62)*	29/1 (69)*
RN	Neg	Neg	Neg	Neg	Neg	Neg	S	A	A	A	Neg	Neg	Neg	Neg
RNT	Neg	Neg	Neg	Neg	Neg	S	S	Neg	?					
WW	Neg	Neg	S	+										
WWT	+													
LW	Neg	Neg	Neg	S	?									
LWT	Neg	Neg	Neg	S	S	Neg	Neg	Neg	Neg	A	A	Neg	Neg	Neg
N	Neg	Neg	Neg	Neg	Neg	Neg	Neg	S	A	Neg	Neg	Neg	Neg	Neg
T	Neg	Neg	S	Died										
Total	0/7	0/7	2/7S	2/5S	1/4S	1/4S	2/4S	1/4S 1/4A	2/3A	2/3A	1/3A	0/3	0/3	0/3
Resident Chicks														
3 Black	0/3	0/3	1/3A	0/3	1/3A	1/3A	0/3	ND	1/3S	1/3A	1/3A	1/3A	1/3S	1/3S
6 White	0/5 +	0/5	0/5	3/5A	3/5A	4/5A	2/5A	ND	1/5S 3/5A	1/5S	1/5S	1/5S 1/5A	2/5S	1/5S
Total	0/8	0/8	1/8A	3/8A	4/8A	5/8A	2/8A		3/8A 2/8S	1/8A 1/8S	1/8A 1/8S	2/8A 1/8S	3/8S	2/8S

+Killed by kites
?Disappeared. Presumed taken by hawk or dead.
S B. anserina found in blood.
A Aegyptianella found in blood.
(0)* Day after hatching. Introduced chicks were added on day 6 therefore 1/12 would be the 10th day of age and 4th day after groups were placed together.

AVIAN SPIROCHAETOSIS IN NORTHERN NIGERIA

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Avian spirochaetosis was first reported from Nigeria over 60 years ago. Subsequently its wide distribution and importance in the country were established in various veterinary divisional and departmental reports. In spite of this, there has been very little investigation of the disease in Nigeria with the exception of the original report. From clinical and diagnostic work, avian spirochaetosis was recognized as a common disease causing high mortality in small units of indigenous and exotic poultry in the Zaria area. Numerous questions arose concerning diagnosis, epizootiology and pathogenesis which could not be answered from the scant information on the disease in Nigeria or conflicting reports in the world literature. As a result, these studies were undertaken to investigate avian spirochaetosis in northern Nigeria.

For routine diagnosis of avian spirochaetosis, direct examination of wet, blood or tissue smears by darkfield microscopy was found to be the most useful method. Spirochaetes were found to remain demonstrable in carcasses for at least 5, 12 and 15 days at incubator, room and refrigerator temperatures respectively, which greatly facilitated postmortem diagnosis. For detecting low spirochaetemias, smears made from the buffy coat of centrifuged blood were found to be more sensitive than direct wet smears. A modified direct complement-fixation test using an antigen prepared from sonicated Borrelia anserina was developed to detect antibodies. It was found that B. anserina could be separated from avian blood for use in preparing antigen by using DEAE-cellulose.

Avian spirochaetosis appears to be more common in the northern, particulary north-western, areas of Nigeria. The known distribution of the disease was determined by collating scattered reports which were augmented with observations during these studies. An overall prevalence rate for B. anserina infection of 3.7% was found in 7096 market fowls examined during a 3 year period. Highest prevalence rates occurred during July, June and March, while lowest rates were

found during December and January. Reported outbreaks of avian spirochaetosis in the Zaria area averaged 1.6 per month and the distribution of prevalence rates for infected market fowls. These patterns were associated with climatic conditions that would affect the tick vector.

As elsewhere, Argas persicus was found to be the reservior and principal vector of avian spirochaetosis. New outbreaks most commonly resulted when tick free and tick infested fowls were mixed together. Market fowls were frequently a source of infection. Other arthropods, including the dog tick (Rhipicephalus Sp.) and ornithophilic mosquitoes may also act as vectors. Direct transmission of avian spirochaetosis by several routes was experimentally shown. Cannibalism and scavenging were direct methods by which infection was suspected to spread in infected flocks. Susceptible birds could not be infected by exposing them to faeces from spirochaetemic fowls.

Natural infections in birds less than 10 weeks of age were uncommon. However, birds of all ages were found to be fully susceptible to experimental and natural B. enserina infection if they came from spirochaete and tick free flocks. Chicks that hatched from immunized or tick infested flocks were resistant for at least 6 weeks after hatching. Experimental studies suggested passively acquired maternal antibody as the probable protective factor. When chicks from a tick free flock were mixed with chicks in a tick infested environment where both Aegyptianella pullorum and B. anserina infections were occurring, two distinct patterns of infection resulted. In the introduced chicks B. anserina preceded A. pullorum infection whereas in the resident chicks, A. pullorum preceded B. anserina infection.

Embryos between 6 and 18 days of incubation could be infected with B. anserina. Embryos infected between 6 and 12 days of incubation invariably died 4-8 days later. Prominent liver changes, splenomegaly and swollen kidneys were consistent postmortem findings in these

embryos. Penicillin in amounts of 100-2,000 units/embryo suppressed but did not cure the infection. When infected at 15 or 18 days, the chicks were spirochaetemic at or just after hatching. The course of infection in these chicks was similar to infected day old chicks except mortality was considerably lower.

In experimentally infected adult fowls, splenomegaly occurred early during the disease while hepatomegaly was more prominent terminally. Weight loss was found to be significantly greater in infected fowls (up to 28.8%) than starved fowls, which was attributed to dehydration in the former. Two patterns of body temperature, one continously elevated and the other biphasic, were found to be typical of fowls that recovered or died respectively. Anaemia was apparent during the later stages of the infection. Borrelia anserina infection in day old chicks differed considerably from that in adults being longer, less severe and resulting in high mortality. Possible explanations for these differences were discussed.

Water soluble tetracycline was effective in curing avian spirochaetosis if given for three days. Tick control was possible by using insecticides and improving husbandry but required considerable owner interest to be completely successful. Tick eradication was extremely difficult and accomplished only with the most dire methods of depopulating the flock and burning the infested house. Immunity resulted following recovery and could be actively induced by experimentally infecting fowls and then treating them with tetracycline. In the absence of tick exposure, immunity to reinfection with the homologous isolate of B. anserina persisted for at least 4-5 months. Field and clinical observations suggested the presence of two or more types of B. anserina existing in northern Nigeria.

It is concluded that avian spirochaetosis is still an important disease of poultry in northern Nigeria. Awareness and application of the findings of these studies will provide a greater understanding of the disease as it affects poultry in northern Nigeria and should

lead to improved diagnosis, treatment and control thereby reducing mortality. Although much has been learned, a great deal remains unknown and further investigations are indicated. Certain similarities between avian spirochaetosis and acute borrelioses in man and other animals suggest that avian spirochaetosis could be a useful model for studying basic mechanisms of Borrelia infections.