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With 3tables and 3 figures in the text.

Pathology of Domestic Fowl Spirochaetosis in Different Age Groups of Chicken Experimentally Infected with *Borrelia anserina*

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ملخص البحث

درست العلامات المرضية والصفات التشريحية وتغيرات الانسجة المريضة لمرض زهرى الطيور في ثلأثة اعمار مختلفة من الدجاج (130) كتكوت (110) فروج (110) طيور بالغة. تمت العدوي التجريبية باستخدام عترة محلية. اظهرت الطيور البالغة فترة حضانة تتراوح ما بين 2−3 يوم كما ظهرت اعراض حادة للمرض مع وجود الباكتيريا فى الدم لمدة3−4 أيام مع تضخم و تبقع شديد في الطحال. الطيور صغيرة السن اظهرت أعراض سريرية خفيفة مع وجود أعداد كبيرة من البكتيريا في الدم ولفترة تراوحت بين10−15 يوم مع تضخم خفيف للطحال. أتضح من دراسة الأنسجة المريضة ارتشاحا للخلايا وحيدة النواة وتضخم و نخر في خلايا الطحال و الكبد مع تضخم في جدران الشعيرات الدموية في الطحال .

Summary

Pathological changes in fowl spirochaetosis were studied in three different age groups emphasized 130 chicks, 110 growers and 110 adults experimentally infected with a pathogenic field isolate. A short incubation period (2-3 days) was recorded accompanied by severe clinical signs. spirochaetemia of chickens 3-4 days was encountered. Enlargement and mottling of the spleen were observed in grower and adult chickens. On the other hand, the chicks showed mild clinical signs, extensive prolong duration of spirochetemia (10-15 days) and slight enlargement of the spleen which may be due to maternal immunity. Histopathologically, mononuclear cell infiltrations were observed in the spleen and liver, besides necrosis and thickening of capillary walls in the spleen.

Introduction

Fowl spirochaetosis is a worldwide acute septicaemic tick-borne disease of a variety of avian species caused by the bacterium *Borrelia* (Barnes, 1997). The clinical signs of the spirochaetosis vary according to the virulence of the strain but were characterized by pyrexia, dullness, weight loss, drop in egg production and paleness of comb and wattles. In the Sudan, the disease was reported as early as 1906 (Anon, 1923) and reports from all over the country have indicated that the disease occurred throughout the year. Limited research had so far been done on the pathogenicity of *B. anserine* (Ginawi, 1980).

The majority of the poultry raising systems in the Sudan are the open system farms as well asbackyard system, the latter is considered to be an important and handy source of animal protein (eggs and meat) in some rural areas. Open system and backyard chickens are most likely to being exposed to *B. anserina* due to the enzootic presence of the vector *Argus persicus* (Hoogestral, 1973). Spirochetosis affects wide species including geese, ducks, turkeys, pigeon sand chickens of all ages and breeds (Gross, 1984). Natural outbreaks were recorded in large well designed commercial layer chicken farms in Khartoum State. Therefore, this study was conducted to study the pathological changes of spirochetosis in different age groups of chicken.

Materials and Methods

Experimental birds:

One hundred and thirty, (30-day-old) chicks, 110 growers (30-150 day old) and110 adults (150-270 day old) apparently healthy birds were used in the present study. Chicks were brought as one-day-old from Coral Commercial Poultry Farm and reared in special cages that were designed to prevent exposure of birds to the blood sucking vectors. It was made of fine wire with four long stands. Cages were cleaned, burned before use. Stand of all cages were dipped in

containers filled with oil throughout the experimental period until they reached the required age. Adult chickens were obtained from a well-managed commercial layers farm. Each age group was kept separatelyin metal cages; all experimental birds were fed commercial rations according to their ages.

Preparation of inocula:

A local pathogenic isolate of *B. anserina* was used in this study. It was isolated from naturally infected birds during afield investigation in Khartoum state. Isolates were maintained in 4 to 6–month-old apparently healthy susceptible chickens. Four inocula were used to induce experimental infection namelycitrated blood, serum, plasma and blood clot.

Experimental designs:

Each inoculum was injected by at least four different routes in birds group of the same age, as follows; subcutaneous (S/C), intramuscular (I/M), intravenous (I/V), intranasal (I/N), intraocular (I/O) and orally (O). Inocula were determined according to Wad Dalker and Soni (1982) and Hovin and Hougenk (1995). Types and doses of inocula and routes for the three agegroups are illustrated in Table 1. Each age group was divided into 5 subgroups designated according to the route of infection; one group was kept as uninfected control. All the infected birds were observed daily for clinical signs and level of infection. All birds that showed died during the experimental period were necropsied.

Table 1: Doses of inocula materials given via different routes in chicken

Route / Materials	S/C	I/M	I/N	Ocular	I/V	Blood clot
Chicks:					-	-
Citrated blood	0.2ml	0.2ml	1 Drop	1 Drop		
serum	0.4ml	0.4ml	2 Drop	2 Drop	-	-
plasma	0.4ml	0.4ml	2 Drop	2 Drop		-
Growers:			-	-		-
Citrated blood	0.2ml	0.2ml			0.1ml	
serum	0.5ml	0.5ml	-	-	0.2ml	-
plasma	0.5ml	0.5ml	-	-	0.2ml	-
Blood clot	-	-	-	-	-	0.5g
Adults:			-	-		-
Citrated blood	0.2ml	0.2ml			0.1ml	
serum	1ml	1ml	-	-	1ml	-
plasma	0.5ml	0.5ml	-	-	0.2ml	-
Blood clot	-	-	-	-	-	0.5g

Results

Clinical signs:

The prominent clinical signs were seen on day 2 and day 3 post infection (pi). Chicks were the least that showing clinical signs. Fifteen out of 120 chicks (12.5%) showed drowsiness and ruffled feathers, while 72 out of 100 (72%) of growers and 80 out of 100 (80%) adults showed similar clinical signs accompanied by pyrexia that increased gradually to reach the peak (45°C) on the 5^{th}-6^{th} day (pi), dullness, drowsiness, ruffled feathers, greenish diarrhoea, paleness of combs, paralysis of wings and lateral recumbancy that observed in the late stage of the disease. Clinical signs in the three age groups are listed in table 2.

Table 2: Clinical Signs in birds of different age groups

Clinial signs	2-5 days post infection			5-6 days post infection		
	chicks	rowers	adult	chicks	growers	adults
dullness	N	+	+	N	N	N
ruffled feathers	N	+	N	+	+	N
pyrexia	N	+	+	N	N	N
paralysis of wing	N	N	N	N	+	+
dairrhea	N	N	N	N	N	N
anorexia	N	+	+	N	N	N
recumbancy	N	N	N	N	+	+
Pale comb and wattle	N	N	+	N	N	+
lacrimation	N	N	N	+	N	N

N=Nil +=+ve

Table 3: Lesions in chickens of different age groups

Lesions	3-5 days post infection			6-9days post infection		
	chick	grower	adult	chicks	grower	adults
Pale carcasses	N	+	+	+	+	N
Enlarged fragile liver	N	+	+	+	+	N
Enlarged mottled spleen	N	+	+	+	+	+
Pale kidneys	N	N	N	N	N	+
Heamrrahgic lungs	N	N	N	N	N	+
Catarrahal intestinal	N	N	N	N	+	+
Greenish diaharria	N	N	N	N	N	+

N=nil += +ve

Macro and Microscopic appearance:

The most striking lesions in all infected birds were; enlargement and mottling of the spleen. Histopatholgically, spleen showed haemorrhage and necrotic foci in the reticular cells with active proliferation of lymphatic cells and thickening of the capillary walls. Changes in liver included accumulation of lymphocytes in the interlobular connective tissue, congestion and thickening of the portal veins.

Spirochetaemia:

Chickens showed spirochetaemia even in apparently healthy ones. *B. anserine* appeared in the peripheral blood 48 hr pi. Spirochetemia peaked on day 3 pi and persisted for 4-6 days. Clumping began from day 8 pi onwards but a few actively motile organisms remained in the circulation up to the10th day. However, complete disappearance of the organisms from the peripheral blood occurred on day 15 pi. Grower and adult had pyrexia during spirochetaemia and *B. anserina* appeared in peripheral blood on day 2 pi. The number of organisms increased gradually to peak on 3-4 days. This phase lasted for1-2 days .Clumping appeared 5-6 day pi. Lyses and complete disappearance of *B. anserina* from the circulation occurred on day 6-7pi. Mortalities occurred after this phase. *B. anserina* in peripheral blood of thedifferent age groups is shown in Fig.1. The *B. anserina* was observed in Giemsa stain impression smears from hearts, spleens, livers and lungs.

Lymphocytic and other mononuclear cells infiltrations were evident in the spleen and hepatocytes accompanied by with necrosis and thickening of capillary walls of the spleen (Fig.2; 3).

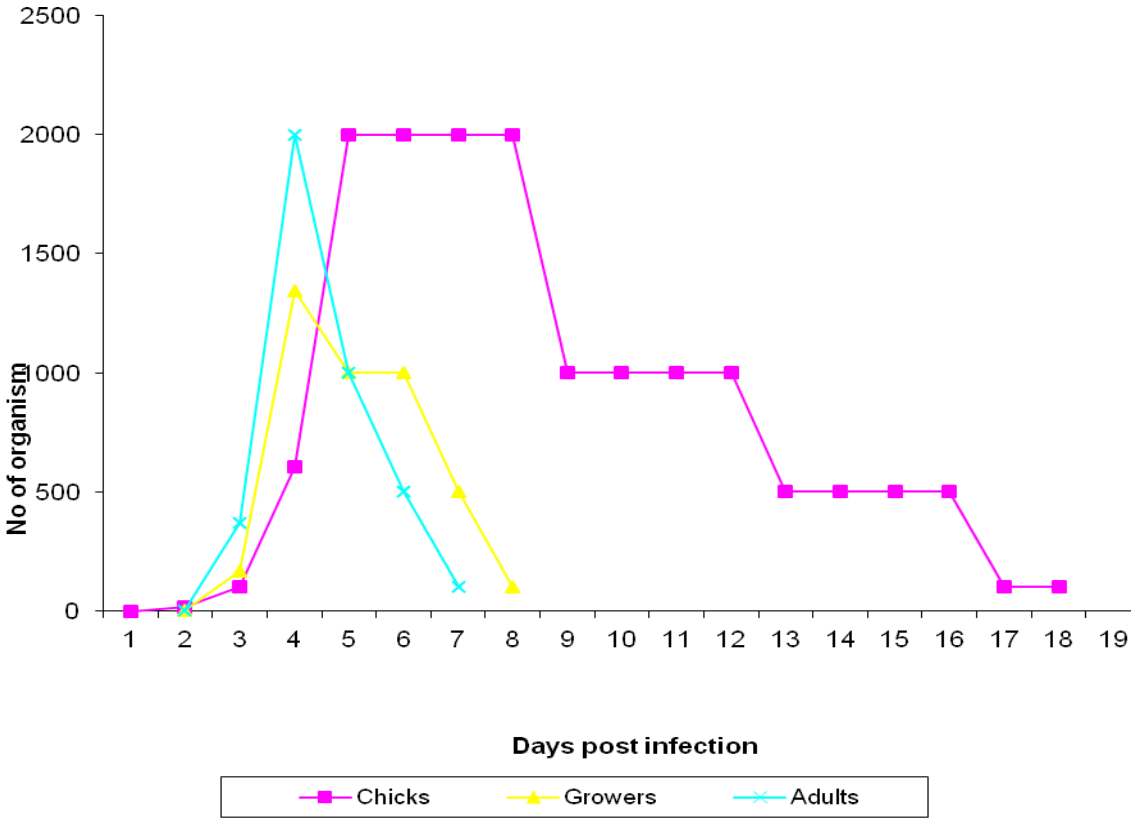

Fig1: *Borrelia anserina* in the peripheral blood of different age groups of infected Brids with Soba1 isolate during spirochetemia

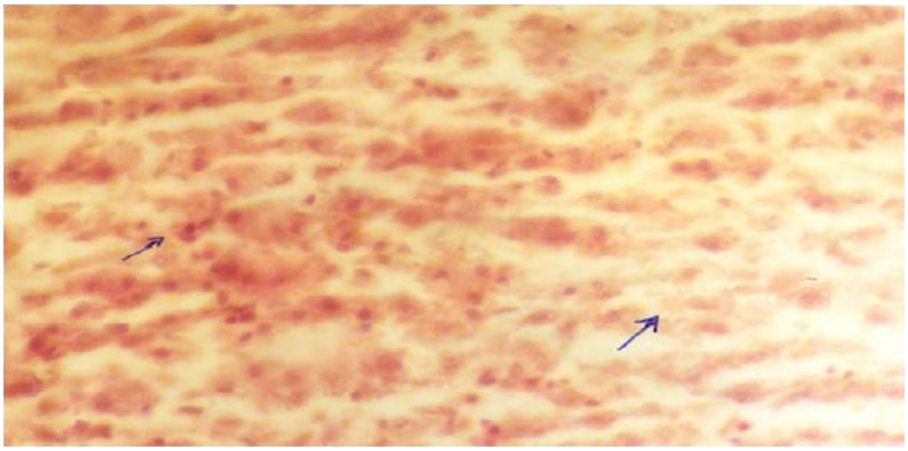

Fig 2: Spleen of an experimentally infected chicken with *Borrelia anserine* showing lymphocitic infltration and necrosis see arrows (H&E×10)

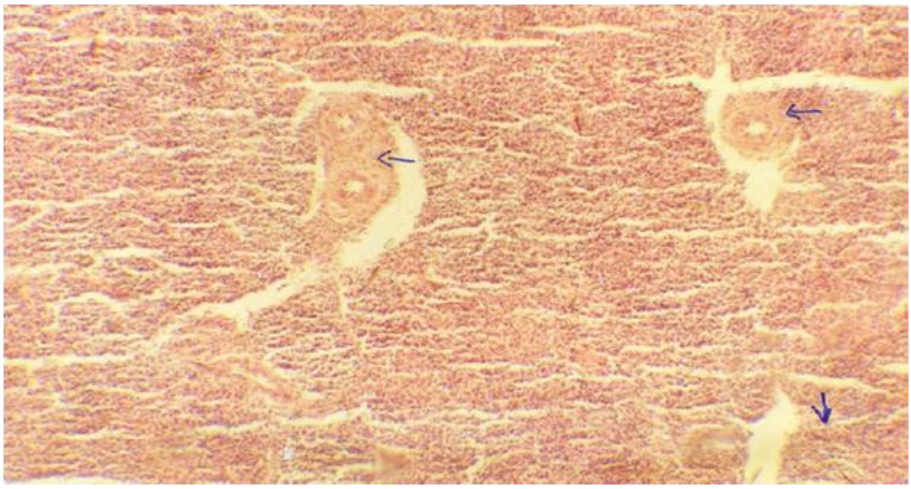

Fig 3: Spleen of an experimentally infected adult chicken showing thickness of capillary walls of the spleen accompanied with mononuclear cells infiltration see arrows (H&E×10)

Discussion

During afield investigation, the clinical of fowl spirochetosis was markedly observed **i**n grower and adult chickens only. The clinical picture of the disease was markedly different between groups. Prolonged spirochetemia was recorded in chicks. The delay in clumps formation as well as the disappearance of *B. anserine* from the blood circulation suggests reduce antibody production and probably the titers antibodies available were too low to remove and clear rapidly multiplying *B. anserina*. These functions appeared to have been improved with the advancement of age. The characteristic gross lesions were the enlargement and mottling of the spleen that were detected even in apparently healthy chicks. The course of the disease was 11-15 days . Our findings support that of Choudhary and Roa (1985) who showed that chicks were more susceptible to the disease. The present data contradict with Djankov*et al* (1974) in Bulgaria in one-day-old white leg horn birds chicks and also with Damassa and adler (1979) who reported that, birds under three weeks of age are most sensitive to spirochetosis than older one in USA. Soliman *et al(*1965) reported considerable losses in young chicks in Eygpt. Similar results were reported by William (1955) in USA who claimed that the response of chicks to *B. anserina* infection was variable ranging from severe symptoms and mortality to only transient spirochetemia. He suggested that chicks from vaccinated hens carry passive immunity that is protective at early age and it decline gradually with age. This could be the possible reason for the absence of natural outbreaks in chicks or could be due to age susceptibility or misdiagnosis of the disease in the field. In addition, to the misuse of antibiotics as prophylaxis by poultry keepers, especially in early chick life, that possibly controls the organism which is sensitive to a wide range of antibiotics (Phulan *et al*, 1988).

The clinical picture of the disease in both grower and adult was almost similar and nervous signs were frequently seen. Birds showed typical clinical signs of spirochetosis .In other relevant studies, many gross lesions in both growers and adults were described which were not observed during this investigation. This included petecial heamorrage on the liver and kidney, enlargement of gall bladder and linear heamorrhage in the inter glandular space of proventriculus, ictrus and muscle heamorrhage (Barnes and Swayne ,1998; Choudhary and Roa,1985). Splenomegaly as well as mottling is characteristic of spirochetosis caused by highly virulent strains of *Borrelia anserina* which may not be evident when birds were infected with

low virulent strain and during the early stages of the disease (Barnes, 1997; Cooper and Bickford, 1993). In this study, Spleens of all infected chickens showed varying degree of enlargement and mottling. This finding is in agreement with Bandopadhayay and Vegad (1983) who described the same clinical signs and lesions in birds infected with a virulent strain of *B. anserina*. During this study *B. anserina* was demonstrated in Giemsa's stain impression smears of the heart, spleen, liver and typifying that reported by Gross and Ball (1964).

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