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ELECTROPHORETIC PATTERNS OF SERUM PROTEINS IN BLUE FOXES WITH SPECIAL REFERENCE TO CHANGES ASSOCIATED WITH NOSEMATOSIS

By

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MOHN, S. F. and K. NORDSTOGA: *Electrophoretic patterns of serum proteins in blue foxes with special reference to changes associated with nosematosis.* Acta vet. scand. 1975, *16*, 297—306. — The serum proteins in 1 group of healthy breeders, 1 group of healthy pups and 4 groups of foxes suffering from various diseases were separated electrophoretically on cellulose acetate membranes. In most of the sera, the proteins were separated into 1 albumin fraction and 5 globulin fractions designated alpha$_1$, alpha$_2$, alpha$_3$, beta and gamma. The mean concentrations of the total proteins and the various serum fractions in the disease groups were compared statistically with the mean values of the normal groups.

A nosematosis group was characterized by a distinct hypergammaglobulinaemia together with an increase in the total protein — and a decrease in the albumin concentrations. However, in 2 cubs recovering from nosematosis the hypergammaglobulinaemia was shown to be reversible.

In a feed intoxication group the concentration of albumin was found to be lower and the alpha$_3$-globulin higher than the corresponding values in the healthy group. A virus hepatitis group was characterized by a decrease in the concentrations of albumin and beta-globulin and an increase in the alpha$_1$- and alpha$_3$-globulins. In the toxoplasmosis group the total protein and alpha$_1$- and alpha$_3$-globulins showed concentrations below the normal values.

blue fox; serum proteins; nosematosis; hypergammaglobulinaemia.

Paper electrophoretic separations of serum and plasma proteins in healthy blue foxes (Alopex lagopus) have previously been reported by *Deutsch & Goodloe* (1945) and *Balbierz et al.* (1963). As preliminary determinations of the serum proteins in foxes suffering from nosematosis showed an evident decrease in the albumin fraction and a considerable elevation in the gamma-glo-

bulins, it was found to be of considerable interest to investigate sera from a representative number of foxes with this infection. The electrophoretic patterns of sera of foxes suffering from various other diseases were also investigated for comparison, as such studies have been given little attention in the literature.

In order to establish a solid basis for comparison of the results obtained, examinations of a representative population of healthy blue foxes had to be carried out by the same electrophoretic methods.

MATERIAL AND METHODS

Sera prepared from blood samples collected from Vena cephalica of foxes in the following groups were examined:

Normal groups

Breeders: 67 healthy, adult blue foxes representing both sexes and various age groups.

Pups: 13 healthy pups of both sexes, 3—5 months of age.

Experimental groups

The nosematosis group: 63 pups 3—5 months old with clinical signs indicative of nosematosis. The diagnosis was verified by post-mortem examinations and by re-isolation of the parasite by mice inoculation with tissues from pups which were killed in connection with the blood sampling. Two cubs (No. 1 and No. 2) were bled 141 days following the first blood samplings. At the second set of sampling the pups seemed to be clinically healthy, but not thriving. Minor lesions in the tissues characteristic of nosematosis were found at necropsy.

The feed intoxication group: 14 convalescent foxes from a farm where nearly all foxes had suffered from acute feed intoxication *(Nordstoga* 1973).

The virus hepatis group: 11 adult blue foxes from a farm with a serious outbreak of viral hepatitis (*Nordstoga et al.* 1972). The sera which were collected 5—6 weeks after the onset of the disease had positive complement-fixing and virus neutralizing antibody titres for virus hepatitis.

The toxoplasma group: A vixen which had been inoculated during the gestation period with the Toxoplasma gondii RH-strain delivered 8 pups. From 2 of the cubs that died 2 weeks old, toxo-

plasma trophozoites were re-isolated. The vixen and its surviving pups were bled 104 days after the inoculation of the vixen. The 7 sera all showed significant positive dye test titres (i.e. ≥ 50).

Protein assay

The examinations of the sera and the calculations of the protein concentrations were carried out by the methods described previously (*Mohn & Nordstoga* 1975).

RESULTS

The results of the measurements of total protein and of the electrophoretically separated fractions are given in Fig. 1. The mean absolute concentration values in normal and experimental groups are compared and the significances of the differences are given in Table 1. In 173 out of 175 sera examined (i.e. 99 %), the proteins were separated into 1 albumin- and 5 globulin fractions designated alpha$_1$, alpha$_2$, alpha$_3$, beta and gamma. Fifty-three

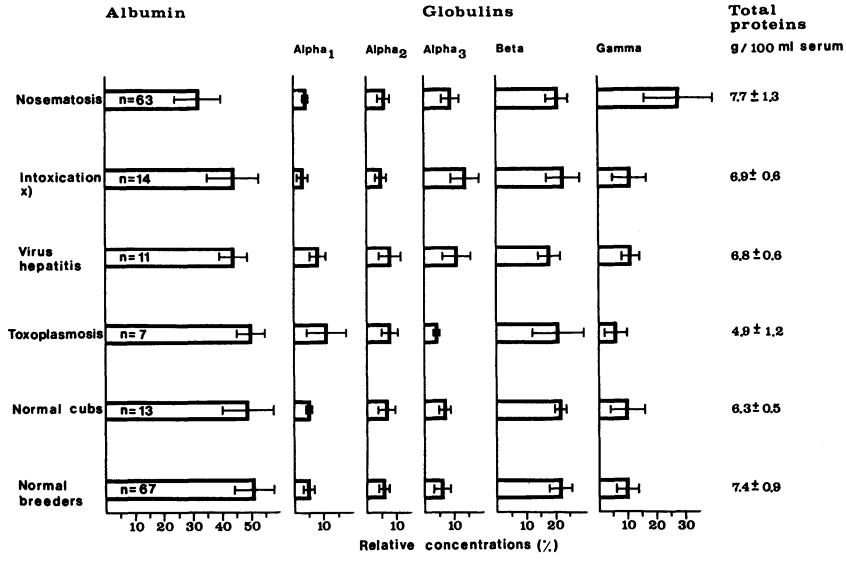

Figure 1. Mean concentration values of serum fractions and total proteins in groups of foxes suffering from nosematosis, feed intoxication, virus hepatitis, toxoplasmosis and in the groups of normal cubs and breeders.

x) Feed intoxication probably caused by Clostridium botulinum type C toxin (*Nordstoga* 1973).

Table 1. Differences between absolute concentrations of total proteins and serum fractions in experimental and normal groups.

Groups compared	Differences between mean absolute concentration values, g/100 ml serum						
	total protein	albumin	alpha$_1$-globulin	alpha$_2$-globulin	alpha$_3$-globulin	beta-globulin	gamma-globulin
Nosematosis — Normal pups	1.4 **	— 0.7 **	0.0 n.s.	0.0 n.s.	0.3 n.s.	0.2 n.s.	1.7 **
Intoxication — Normal breeders	— 0.5 n.s.	— 0.7 **	— 0.2 n.s.	0.1 n.s.	0.4 **	— 0.1 n.s.	0.1 n.s.
Virus hepatitis — Normal breeders	— 0.6 n.s.	— 0.7 **	0.1 **	0.1 n.s.	0.3 **	— 0.4 **	0.0 n.s.
Toxoplasmosis — Normal pups	— 1.4 **	— 0.6 n.s.	0.2 **	— 0.1 n.s.	— 0.2 **	— 0.3 n.s.	— 0.3 n.s.
Normal breeders — Normal pups	1.1 **	0.7 n.s.	0.1 n.s.	— 0.1 n.s.	0.1 n.s.	0.2 n.s.	0.1 n.s.

**: Significant difference ($P \leq 0.01$)

n.s.: No significant difference ($P > 0.01$)

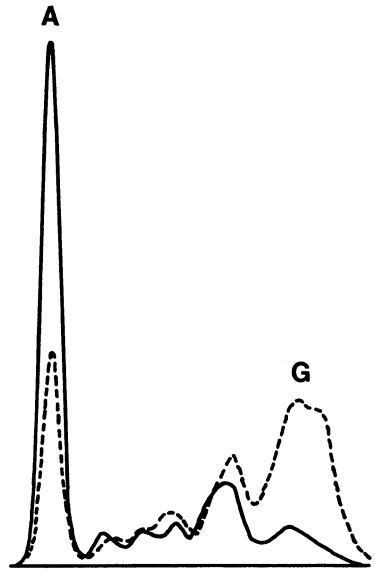

Figure 2. Electrophoretic patterns of sera from a healthy pup (drawn line) and from a pup with nosematosis (broken line). A = albumin fraction. G = gamma-globulin fraction. The globulin fractions between A and G are alpha$_1$, alpha$_2$, alpha$_3$ and beta.

of the sera (i.e. 24 %) showed incomplete resolution either between alpha$_1$ and alpha$_2$, between alpha$_2$ and alpha$_3$, or between alpha$_3$ and beta. In these sera, and in 2 sera which separated into only 4 globulin fractions (alpha-, alpha-, beta- and gamma-globulins), 5 globulin fractions were calculated in order to gain uniformity of the results, although this caused some degree of inaccuracy in the calculation of the concentrations of the poorly resolved fractions.

Fig. 2 shows typical electrophoretic diagrams of serum samples from a healthy cub and from a cub suffering from nosematosis. In the nosematosis group the mean concentration of total protein was 7.7 ± 1.3 g/100 ml, albumin 32 ± 8 %, alpha$_1$-globulin 4 ± 1 %, alpha$_2$-globulin 6 ± 2 %, alpha$_3$-globulin 9 ± 3 %, beta-globulin 21 ± 4 % and of gamma-globulin 28 ± 12 %. The highest concentrations of total protein and gamma-globulin (i.e. total protein 12.0 g/100 ml, gamma-globulin 53 %) were found in a pup with advanced nosematosis, while pups showing moderate clinical signs had less pronounced alterations in the serum proteins.

Fig. 3 shows the electrophoretic diagrams of sera collected from pup No. 1 and pup No. 2 during the disease stage of the

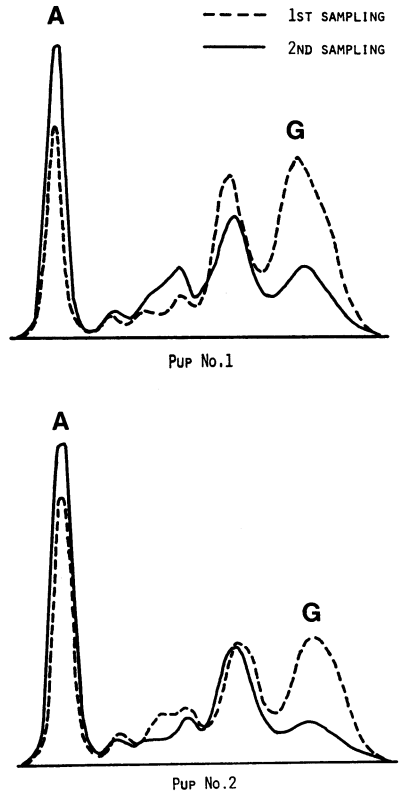

Figure 3. Electrophoretic patterns of serum samples from 2 pups with nosematosis (No. 1 and No. 2). The first set of samples was taken on the 22nd of August 1973 during the course of the disease. The second set was collected 141 days later (11th of January 1974). A = albumin fraction. G = gamma-globulin fraction. The globulin fractions between A and G are $alpha_1$, $alpha_2$, $alpha_3$ and beta.

nosematosis and during the convalescence period. From the first to the second sampling the total protein concentration in pup No. 1 decreased from 9.5 to 6.2 g/100 ml serum and in pup No. 2 from 7.5 to 6.0 g/100 ml serum. The relative albumin concentrations increased from 21 to 35 % in pup No. 1 and from 27 to 43 % in pup No. 2. The beta-globulin concentrations increased from 22 to 36 % in pup No. 1 and from 24 to 29 % in pup No. 2. The gamma-globulins decreased from 40 to 17 % in pup No. 1 and from 31 to 12 % in pup No. 2. The relative concentrations of the alpha-globulins varied within normal ranges.

DISCUSSION

The mean protein concentration in the group of healthy breeders was, as expected, at a higher level than in the group of healthy cubs. However, there was no significant difference in the relative distribution of the proteins between the 2 age groups. The results of the measurements of total proteins in normal foxes are in accordance with the results reported by *Balbierz et al.* (1963).

The electrophoretic separations of blue fox serum carried out on cellulose acetate membranes revealed the same patterns that were obtained on paper electrophoresis of 35 sera from normal foxes showing 5 globulin fractions in 26 sera and 4 fractions in 9 sera (*Balbierz et al.*). These authors, however, designated the fractions alpha$_1$, alpha$_2$, beta$_1$, beta$_2$ and gamma. Corresponding components were previously found by paper electrophoresis of plasma samples from cats, monkeys and guinea pigs, while fox plasma was separated into only 4 globulin fractions, excluding the fibrinogen fraction (*Deutsch & Goodloe* 1945).

The mean relative concentration values of the protein fractions in normal foxes recorded in the present paper (i.e. albumin 50, alpha$_1$ 5, alpha$_2$ 6, alpha$_3$ 7, beta 22 and gamma 10 %) show considerable discrepancies when compared with the results reported by *Balbierz et al.* (i.e. albumin 35.26, alpha$_1$ 13.63, alpha$_2$ 10.43, beta$_1$ 7.43, beta$_2$ 20.45 and gamma 15.19 %). The reason for the discrepancies may be different separation methods, although a real difference in the composition of sera from foxes in various populations cannot be excluded.

Nosematosis group

The nosematosis group is characterized by a total protein concentration of 7.7 ± 1.3 g/100 ml serum, which amounts to 1.4 g/100 ml above the mean level of normal pups. The mean relative albumin concentration is 32 ± 8 % or 2.3 ± 0.5 g/100 ml in mean absolute concentration amounting to 0.7 g/100 ml below the mean normal level. The mean gamma-globulin concentration is 28 ± 12 % and its mean absolute concentration 2.3 ± 1.3 g/100 ml or 1.7 g/100 ml above the mean values for healthy cubs.

The results of the examination of sera from pups suffering from nosematosis show distinct hypergammaglobulinaemia and significant elevation of the mean total protein concentration. The increase in the total protein concentration is mainly due to the

increase in the absolute concentration of the gamma-globulin fraction.

In nosematosis in blue foxes, polyarteritis nodosa is a very common finding (*Nordstoga* 1972, *Nordstoga et al.* 1974), indicating hypersensitivity. The changes in the serum proteins in foxes suffering from nosematosis exhibit similarities with those occurring in infectious plasmacytosis in mink. In contrast to the latter disease which is a progressive lethal disease, the hypergammaglobulinaemia in nosematosis is obviously, at least partly, a reversible condition where the normalization of the serum proteins parallels a corresponding regression in the tissue lesions (Fig. 3).

A major part of the increase of the gamma-globulins is probably produced as result of a reaction to a hypersensitive or autoimmune condition. Specific circulating antibodies against the causative protozoon are demonstrated in other species of animals suffering from nosematosis and suggested to be mostly incorporated in the gamma fraction (*Chalupsky et al.* 1971, *Cox et al.* 1972, *Hübner & Uhlikova* 1973). One may assume that foxes suffering from nosematosis also produce specific antibodies against Nosema cuniculi. It is, however, unlikely that these antibodies are produced in such large quantities that they alone account for the pronounced hypergammaglobulinaemia. Antibodies of autoimmune character should, therefore, be expected to be incorporated in the gamma fractions. However, further immunological examinations ought to be carried out to characterize the proteins responsible for the hypergammaglobulinaemia in fox nosematosis.

Other groups

The feed intoxication group shows a mean relative albumin concentration of 44 ± 9 % amounting to 0.7 g/100 ml serum below the corresponding concentration in normal breeders. The mean relative concentration of the $alpha_3$-globulin is 14 ± 5 % amounting to 0.4 g/100 ml serum above the mean absolute value of normal breeders.

The mean relative concentration of albumin in the virus hepatitis group is 44 ± 5 %, while the beta-globulin concentration is 18 ± 4 %. The absolute concentrations for these 2 components are, respectively, 0.7 and 0.4 g/100 ml serum below the levels for healthy breeders. The concentrations of $alpha_1$- and

alpha$_3$-globulins are 8 ± 3 and 11 ± 5 % which amount to 0.1 and 0.4 g/100 ml serum above the normal levels, respectively.

The toxoplasmosis group is characterized by a total protein concentration of 4.9 ± 1.2 g/100 ml serum which is 1.4 g/100 ml below the normal levels for cubs. The mean relative concentration of alpha$_1$-globulin is 11 ± 7 % and of alpha$_3$-globulin 4 ± 1 %, concentrations which are, respectively, 0.2 g/100 ml serum above and 0.2 g/100 ml below the concentrations of the corresponding components in healthy pups.

Significant differences in the concentration of gamma-globulins between the normal groups and the feed intoxication, the virus hepatitis and the toxoplasmosis groups were not demonstrated. The electrophoretic diagrams of sera from the disease groups do not show conspicuous variations from those of normal sera, although the differences in the concentrations mentioned above are statistically significant ($P \leq 0.01$).

The sera in the feed intoxication, the virus hepatitis and the toxoplasmosis groups did not reveal the same electrophoretic patterns as those found in the nosematosis group, where the observed hypergammaglobulinaemia seems to be a characteristic finding associated with Nosema cuniculi infections.

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SAMMENDRAG

Elektroforetisk separasjon av serumproteiner hos blårev spesielt med henblikk på forandringene ved nosematose.

Serumproteinene i én gruppe av klinisk friske voksne blårever, én gruppe av klinisk friske valper og 4 grupper av blårever med forskjellige sykdommer er undersøkt elektroforetisk på celluloseacetatmembraner. Proteinene i de fleste sera er separert i én albuminfraksjon og 5 globulinfraksjoner betegnet alfa$_1$, alfa$_2$, alfa$_3$, beta og gamma. De kalkulerte gjennomsnittsverdier for totalprotein og de enkelte serumfraksjoner i sykdomsgruppene er sammenlignet statistisk med de tilsvarende gjennomsnittskonsentrasjoner i normalgruppene.

Gruppen av valper med nosematose er karakterisert med en uttalt hypergammaglobulinemi sammen med forøket totalproteinkonsentrasjon og nedsatt albuminkonsentrasjon i forhold til normale valper. Hypergammaglobulinemien synes imidlertid å være reversibel idet det skjer en normalisering i serumproteinene samtidig med at de kliniske symptomer gradvis forsvinner og at de typiske patologisk-anatomiske forandringer i organene blir mindre uttalt.

I forgiftningsgruppen er albuminverdien funnet lavere og alfa$_3$-globulinverdien høyere enn de tilsvarende verdier i normalgruppen. Virushepatittgruppen er karakterisert med mindre albumin og beta-globulin i forhold til normalverdiene, mens alfa$_1$- og alfa$_3$-fraksjonene er større enn normalt. Gruppen av rever med toxoplasmose viser lavere konsentrasjoner av totalprotein, alfa$_1$- og alfa$_3$-globulin enn normale valper. Tilsvarende forandringer som er funnet i serumproteinene hos nosematosegruppen er ikke påvist i de tre andre sykdomsgruppene.

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