Acta vet. scand. 1977, *18*, 290—292.

Brief Communication

THE INDIRECT FLUORESCENT ANTIBODY TEST (IFAT) FOR THE DETECTION OF NOSEMA CUNICULI ANTIBODIES IN THE BLUE FOX (ALOPEX LAGOPUS)

During recent years nosematosis has been a major problem in the breeding of blue fox in the Scandinavian countries, causing heavy losses among growing pups (*Nordstoga* 1972, *Nordstoga et al.* 1974). The lack of reliable methods for diagnosing the infection in live foxes has so far made epizootiologic studies of the disease very difficult. However, reports on the IFAT in rabbits with nosematosis (*Cox et al.* 1972, *Chalupsky et al.* 1971, 1973, 1974), encouraged the search for a method of detecting Nosema antibodies in fox sera.

Materials and methods

Conjugate. Rabbits were immunized with pooled normal fox gammaglobulin according to the methods by *Hijmans et al.* (1969). The anti-fox-gammaglobulin levels appearing in the rabbits were measured by a standardized double agar diffusion test (*Brandtzaeg et al.* 1970). The gammaglobulins were prepared and specificity tested by immunoelectrophoresis. Pooled gammaglobulin fractions were conjugated with fluorescein isothiocyanate (FITC) (Baltimore Biological Laboratories), filtered through a column of Sephadex G-25 gel and characterized by the methods of *Brandtzaeg* (1973). The conjugate with an average protein concentration of 17 mg/ml and a FITC/protein ratio of 3.2 was diluted 1:80 in 0.1 M-PBS pH 7.1.

Antigen. Nosema spores isolated from a spontaneously infected fox and propagated in monolayer cultures of ovine choroid plexus cells were harvested by centrifugation of the supernatant fluid and resuspended in PBS to a cell density yielding about 50 spores per high power microscopic field. Separate drops, each 25 µl, were placed on slides, air dried, fixed in acetone for 20 min. and stored at —20°C until used.

Sera. Samples from 17 foxes suffering from nosematosis (*Mohn & Nordstoga* 1975) and from 10 healthy foxes were examined.

Procedure. The antigens covered with reagents were incubated in a humidity chamber at 37°C for 1 hr., rinsed in PBS, and finally counterstained with Evans blue 1:10,000 for 10 min. (*Chalupsky et al.* 1973). The stained samples were examined in a Leitz Ortolux microscope with an Osram HBO 200 w lamp, UG 1 and BG 38 primary filters and a K 430 as secondary filter. Controls for the various reagents were included. The working magnification was 10×100 with immersion oil.

Results

In positive reactions the spores showed a bright yellow-green fluorescence uniformly distributed on the surface or on the periphery of the cells and on the extruded polar filaments. There was no fluorescence in negative reactions, the spores showing a purple-red colour. The titer of each serum was defined as the reciprocal value of the highest serum dilution showing fluorescence on the periphery and/or on the filaments of the spores. The results are listed in Table 1. The values definitely indicate that the IFAT is a sensitive test suitable for the detection of nosematosis in blue foxes.

Table 1. The indirect fluorescent antibody test (IFAT). Serum antibody titers against Nosema cuniculi of 17 blue foxes with nosematosis and of 10 healthy animals. The titers are expressed as the reciprocal value of the highest serum dilution showing fluorescence.

	Reagents used									
	Antigen		Sera of foxes		PBS	Unconjugated rabbit-anti-fox-gamma-globulin	Conjugated rabbit-anti-fox-gamma-globulin	PBS	Evans blue	IFAT-titers
	Inoculated cultures	Uninoculated cultures	17 infected foxes	10 healthy foxes						
Samples	X[a]		X		X		X	X	X	800—12,800
	X			X	X		X	X	X	≦10
Controls	X		X		X	X	X	X	X	<2
	X			X	X	X	X	X	X	<2
	X				X		X	X	X	<2
	X				X			X	X	<2
	X								X	<2
		X	X		X		X	X	X	<2
		X		X	X		X	X	X	<2

[a] Reagents used as read from left to right in the table.

S. F. Mohn and *Ø. A. Ødegaard,*
The National Veterinary Institute, Oslo, Norway.

REFERENCES

Brandtzaeg, P.: Conjugates of immunoglobulin G with different fluorochromes. 1. Characterization by anionic-exchange chromatography. Scand. J. Immunol. 1973, *2*, 273—290.

Brandtzaeg, P., I. Fjellanger & S. T. Gjeruldsen: Human secretory immunoglobulins. 1. Salivary secretions from individuals with normal or low serum immunoglobulins. Scand. J. Haemat. Suppl. 12, 1970.

Chalupsky, J., P. Bedrnik & J. Vavra: The indirect fluorescent antibody test for Nosema cuniculi. J. Protozool. 1971, *18*, Suppl. Abstract 177, p. 47.

Chalupsky, J., J. Vavra & P. Bedrnik: Detection of antibodies to Encephalitozoon cuniculi in rabbits by the indirect immunofluorescent antibody test. Folia Parasit. (Praha) 1973, *20*, 281—284.

Chalupsky, J., J. Vavra & P. Bedrnik: The persistence of antibodies to Encephalitozoon cuniculi in rabbits as revealed by immunofluorescent antibody test (IFAT). III Int. Congr. Parasit., Munich 1974, *1*, 158.

Cox, J. C., N. B. Walden & R. C. Nairn: Presumptive diagnosis of Nosema cuniculi in rabbits by immunofluorescence. Res. Vet. Sci. 1972, *13*, 595—597.

Hijmans, W., H. R. E. Schuit & F. Klein: An immunofluorescence procedure for the detection of intracellular immunoglobulins. Clin. exp. Immunol. 1969, *4*, 457—472.

Mohn, S. F. & 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.

Nordstoga, K.: Nosematosis in blue foxes. Nord. Vet.-Med. 1972, *24*, 21—24.

Nordstoga, K., S. F. Mohn & G. Loftsgård: Nosematose hos blårev. (Nosematosis in blue foxes). Proc. 12th Nord. Vet. Congr., Reykjavik 1974, pp. 183—186.

(Received May 4, 1977).

Reprints may be requested from: Øivind A. Ødegaard, the National Veterinary Institute, P. O. Box 8156, Oslo Dep., Oslo 1, Norway.