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Abstract: Serum samples from apparently healthy wild populations of moose and caribou in the province of Quebec, Canada were screened for the presence of conglutinin (K), immunoconglutinins (IKS) and heterophile antibodies. The conglutinating factor in moose and caribou sera was characterized utilizing the necessity of calcium ions for its reaction with sensitized sheep erythrocytes which had been alexinated with horse complement. The conglutinating substance in these animals did not require calcium ions for its activity. The conglutinating activity in both moose and caribou sera was characterized due to IKS as those present in sheep, dog and rabbit sera. Both moose and caribou had non-agglutinating type of heterophile antibodies. Their titres varied from 0 to 80. None of the animals tested had K in their blood. The titre of IKS varied from 0 to 640 with a mean value of 41 in moose, whereas it varied from 0 to 80 with a mean value of 18 in caribou. About 75% of all the animals in both the groups were positive for IKS. The specificity of IKS was demonstrated by the total removal of its activity on absorption with alexinated cells. Presence of IKS in these animals is suggestive of latent infection(s) possibly of bacterial, viral or parasitic origin.

INTRODUCTION

Conglutinin (K) is a naturally occurring serum protein (beta-globulin) of cattle and certain other species which causes clumping or conglutination of particles or antigen-antibody complexes that have been coated with complement components. Immunoconglutinins (IKS) are immunoglobulins which react in the same way as naturally occurring K. IKS are produced in the sera of animals as a result of various natural infections or experimental procedures (Lachmann, 1962). Bovine K reacts exclusively with fixed C_3 and C_1 (Lachmann, 1962). Bovine K also differs from IKS in that calcium ions apparently participate in the reaction between K and complement (Lachmann, 1962). K has been found to occur exclusively in bovidae but not in sheep, goats, pigs, fowl and humans (Mittal and Jaiswal, 1973).

IKS are produced in response to *in vivo* complement fixation as a result of the generation of new epitopes on fixed C_3 and C_1 (Lachmann, 1967). Thus IKS are naturally occurring auto-antibodies to fixed complement components. Serum titres of IKS therefore rise in response to the activation of complement components which occurs in various bacterial, viral or parasitic infections (Coombs et al., 1961). It is recognized that within a population, the titres of serum IKS reflect the prevalence of infectious disease (Lachmann, 1967). In contrast to IKS, K does not rise in response to infection, but drops (Ingram and Mitchell, 1971).

The other factors whose titres may be influenced by infection are the heterophile antibodies. These are naturally occurring antibodies to a variety of unrelated antigens. A rise in the

titre of these antibodies may be due to cross-reactivity between invading micro-organisms and sheep erythrocytes or alternatively due to non-specific polyclonal B cell stimulation such as occurs in trypanosomiasis (Houba et al., 1969).

Literature on prevalence of K, IKS and heterophile antibodies in wild animals is scant; that in moose and caribou is non-existent. Thus these studies were carried out to gain information on the health status of these wild animals.

MATERIALS AND METHODS

Serum samples

A total of 141 moose and 46 caribou serum samples were obtained during the 1979 hunting season in the province of Quebec. These animals were 1-2 yr of age and had never been artificially infected or vaccinated. Blood samples were collected from the heart or the thoracic cavity immediately after death and allowed to clot. Serum was harvested 4-6 hr later and stored at -20 C until tested.

Assay of total conglutinating activity

The titrations for conglutinating activity were performed in tubes according to the centrifugation-suspension technique (Coombs et al., 1961). Sheep red blood cells sensitized with heterophile antibodies occurring naturally in cattle serum were alexinated with fresh horse serum. All the test sera were heat-inactivated at 56 C for 30 min and absorbed with sheep red blood cells prior to testing.

Positive controls consisting of bovine serum containing conglutinin, sheep and dog sera containing auto-stimulated IKS, rabbit sera containing hetero-stimulated IKS as well as negative control sera from rabbits, dogs and cattle were always kept. A cell suspension prepared with heat-inactivated horse serum was also kept as control.

Treatment with ethylene diamenotetraacetate (EDTA)

The titrations for total conglutinating activity were performed as described earlier. A 0.15 M EDTA saline was added to tubes containing the conglutinated cells as described by Bienenstock and Bloch (1966), and mixed well. The results were recorded for conglutination 30 min after mixing of EDTA with incubation at room temperature.

Assay of heterophile antibodies

a) Direct agglutination method

The level of heterophile antibodies were measured in 0.1 ml volume of serially two-fold diluted serum and 0.1 ml of 0.5% suspension of sheep red blood cells (SRBC) in saline was added to each tube. The reaction mixture was shaken and incubated at room temperature for 2 hr. The reciprocal of the highest dilution showing positive agglutination was scored as the end point.

b) Direct conglutination method

The heterophile antibodies were titrated by the direct conglutination method (Coombs et al., 1961). The source of complement in this technique was fresh horse serum diluted to contain four minimal conglutinating doses. The source of IKS was hetero-stimulated rabbit serum containing four minimum conglutinating doses.

Production of hetero-stimulated immunoconglutinins (H.S. IKS) in rabbits

Kaolin coated with fresh horse serum was used for preparing H.S. IKS in rabbits (Mittal and Ingram, 1969).

Sheep, dog and bovine sera

Five apparently healthy adult cows, and 39 sheep positive for antibodies to *Toxoplasma gondii* were bled by jugular puncture. Blood samples from 27 dogs showing various clinical signs were drawn from the cephalic vein and allowed to clot at room temperature. The serum was harvested and stored at -20 C until tested.

Absorption of conglutinating activity in caribou and moose sera with alexinated cell suspension

The washed, packed SRBC, sensitized SRBC or alexinated SRBC were used to absorb conglutinating activity from caribou and moose sera according to Mittal and Ingram (1974). The absorption was carried out utilizing one part of particles to nine parts of serum at 37 C for 1 hr. The suspensions were centrifuged and the clear supernatants were tested for conglutinating activity.

RESULTS

The distribution of conglutinating activity and heterophile antibodies in moose and caribou sera are shown in Tables 1 and 2. About 25% of the total sera of both moose and caribou had no conglutinating activity at all. The majority of the moose sera had a titre of 10 to 80. The highest titre of conglutinating activity observed was 640 (Table 1).

The range of conglutinating activity in caribou sera varied from 0 to 80 (Table 2).

The serum samples of moose and caribou were titrated for heterophile antibodies by both direct agglutination and direct conglutination methods. The results are shown in Tables 1 and 2. It was not possible to titrate the heterophile antibodies by direct agglutination test. The majority of the sera showed the presence of heterophile antibodies only when tested by the direct conglutination method.

The conglutinating factor in moose and caribou sera was characterized utilizing its requirement of calcium ions for its activity by treating the conglutinated mass with EDTA. The results are shown in Table 3. The conglutinating activity in both moose and caribou sera was neither destroyed nor reduced after treatment with EDTA and it followed the pattern exactly like conglutinating substance present in sheep, dog and rabbit

TABLE 1. Levels of conglutinating factor and heterophile antibodies in 141 serum samples of apparently healthy moose.

Serum titre	No. of animals showing	
	Total conglutinating activity	Heterophile antibody
0	36	26
10	25	47
20	26	42
40	29	14
80	17	12
160	5	0
320	1	0
640	2	0

TABLE 2. Levels of conglutinating activity and heterophile antibodies in 46 serum samples of apparently healthy caribou.

Serum titre	No. of animals showing	
	Total conglutinating activity	Heterophile antibody
0	11	12
10	10	5
20	16	18
40	8	9
80	1	2

sera; however, conglutination due to bovine K was reversed immediately when the conglutinated mass was treated with EDTA solution (Table 3). Results of absorption of moose and caribou sera with alexinated or non-alexinated cell preparations are given in Table 4. The conglutination due to moose and caribou conglutinating substance was removed only on absorption with

TABLE 3. Effect of EDTA treatment on the level of conglutinating activity in sera of moose, caribou and other animal species.

Animal species	No. of sera samples	Mean titre treatment with EDTA	
		Before treatment	After treatment
Moose	141	41	41
Caribou	46	18	18
Rabbit H.S.			
IKS[a]	2	1280	1280
Cattle[b]	5	300	40
Sheep[c]	39	52	52
Dog[d]	27	33	33

[a]Hetero-stimulated immunoconglutinins.
[b]Apparently healthy cattle.
[c]Sheep positive for toxoplasmosis by indirect haemagglutination test.
[d]Dogs with various clinical signs.

TABLE 4. Absorption of conglutinating activity from sera of various animal species with alexinated sheep red blood cells.

Animal species tested	Number of serum	I.K. titre absorption of serum with			
		NIL	SRBC	Sensitized SRBC	Alexinated SRBC
Moose	1	80	80	80	0
	2	40	40	40	0
	3	160	160	160	0
	4	10	10	10	0
Caribou	1	10	10	10	0
	2	20	20	20	0
	3	40	40	40	0
	4	80	80	80	0
Rabbit H.S.	1	2560	2560	2560	40
IK serum[a]	2	1280	1280	1280	40
Sheep[b]	1	80	80	80	0
	2	80	80	80	0
Dog[c]	1	160	160	160	0
	2	40	40	40	0

[a]Hetero-stimulated immunoconglutinins.
[b]Auto-stimulated immunoconglutinins in sheep.
[c]Auto-stimulated immunoconglutinins in dog.

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alexinated cells. The conglutinating factor thus behaved exactly like that present in rabbit, sheep or dog sera.

DISCUSSION

Ingram and Barnum (1965) studied the levels of conglutinin and heterophile antibodies in cattle sera. They reported that conglutinating activity of the serum drops markedly in most cows at calving time; however, there was no consistent variation in the titre of heterophile antibodies. Conglutinin has been found in cattle and buffalo but not in sheep, goats, pigs, fowl and humans (Mittal and Jaiswal, 1973). Thus a feature of conglutinin is that it is found in certain mammalian species and not in others. Conglutinin has been found not only in cattle but also in a number of African ruminants like African buffalo, water buck, unganda-kob, kenya-kob, dik-dik, topi and Jackson's hartebeest (Lachmann, 1967). To our knowledge, there is no information regarding the presence of conglutinating activity and its nature in moose and caribou which in our studies was found to be due to IKS. Results shown in Tables 1, 2 and 3 indicate that moose sera had a higher level of IKS than sera from caribou. However, none of the animals tested had any K activity in their sera. This may be due to species characteristics or to some other environmental effect. Conglutinin, not being an antibody, does not generally rise in response to infection but drops (Ingram and Mitchell (1971). Since none of the moose or caribou sera was found to contain EDTA reversible conglutinating factor, it seems plausible that the absence of K in these animals may be a species characteristic.

Immunoconglutinins may be produced in animals as a result of *in vivo* complement fixation and these reflect the general degree of infection within an animal population. The activities of K and/or IKS are specific for adsorbed complement but non-specific for the antigenic characteristics of the infecting organism. Thus increased levels of IKS indicate that an antigenic stimulation has occurred (Coombs et al., 1961). Presence of IKS in the sera of clinically healthy animals is a manifestation of the immunizing process involving their own complement which then plays the role of an auto-antigen. These processes are probably associated with various latent infections possibly of bacterial, viral, protozoal or parasitic origin. It also seems logical that higher levels of IKS in certain individuals, may be due to more frequent subclinical or clinical infections in the population. Since moose were found to have significantly higher titres of IKS than caribou, it is suggestive of the fact that moose may be more likely to be exposed to certain viral, bacterial or more possibly parasitic infections than caribou. Antibodies to bovine viral diarrhea, bovine adenovirus 3, infectious bovine rhinotracheitis virus and coronavirus have been demonstrated in caribou herds of northern Quebec (ElAzhary et al., 1982).

The factors which influence heterophile antibody titres are not well understood. They tend to rise in situations in which B cells are non-specifically stimulated, for example, in trypanosomiasis (Houba et al., 1969). In cattle, it has been shown that anti-Forssman antibodies show seasonal fluctuations (Ingram and Barnum, 1965). Mittal et al. (1980) also failed to show any correlation between *Brucella* infection and heterophile antibody titre. The heterophile antibodies in moose and caribou sera were found to be of non-agglutinating type like those present in cattle and buffalo (Maurya et al., 1977) but capable of fixing complement.

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