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The use of the non-specific immunological factors, conglutinin, immunoconglutinin and heterophile antibody, in the serodiagnosis of bovine brucellosis

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SUMMARY

The changes in the titres of immunoconglutinin (IK), conglutinin and heterophile antibodies were measured in calves vaccinated with *Br. abortus* Strain 19. The IK titres rose rapidly following vaccination but returned to normal within ten weeks.

A survey of the sera of over 300 cattle showed no significant correlation between the Brucella STA titre and any of these 'non-specific' indicators. It is concluded that they are of little assistance in the serodiagnosis of brucellosis.

INTRODUCTION

Immunoconglutinins (IKs) are naturally occurring autoantibodies to fixed complement components. They are produced in response to *in vivo* complement fixation as a result of the generation of new epitopes on fixed C3 or C4 (Lachmann, 1967). Serum IK titres therefore rise in response to the activation of complement components which occurs in bacterial or viral infections (Coombs, Coombs & Ingram, 1961). It is recognized that within populations, the titres of serum IK reflect the prevalence of infectious disease (Lachmann, 1967). In initiating the studies described here it was postulated that elevated IK titres might be associated with Brucella infection in cattle and might therefore be of assistance in the interpretation of equivocal Brucella serology.

In contrast to IKs, conglutinin (K) is a non-immunoglobulin serum protein which is found only in bovidae (Coombs *et al.* 1961), it also binds to new epitopes generated on activated complement components. Not being an antibody, conglutinin does not generally rise in response to infection, but drops (Ingram & Barnum, 1965; Ingram & Mitchell, 1971). This fall may be a consequence of its fixation to complement-containing immune-complexes.

A third group of 'non-specific' factors whose titre may be influenced by infection are the 'heterophile' antibodies. These are naturally occurring antibodies to a variety of unrelated antigens. In cattle, the easiest to measure is the natural anti-Forssman antibody detected by its reaction with sheep erythrocytes (Coombs

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et al. 1961). A rise in the titre of anti-Forssman 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, Brown & Allison, 1969).

The object of this investigation was to determine whether the titres of any of the above factors changed in response to brucella infection in cattle and whether they could be of assistance in the interpretation of bovine brucella serology.

MATERIALS AND METHODS

Animals

Three castrated Holstein bull calves aged seven-and-one-half months and weighing about 100 kg were used for the vaccination experiments.

Vaccination

Each calf was inoculated with 3 ml of live *Brucella abortus* Strain 19 vaccine (Armand Frappier Institute, Montreal) subcutaneously in the neck. The calves were bled six, two and one day before vaccination, daily for one week after vaccination and thrice weekly thereafter for a further nine weeks. The serum was separated after allowing the blood to clot for one hour at 37 °C and stored at −80 °C until tested.

Serum samples

A total of 304 bovine serum samples were obtained from the Animal Pathology Laboratory, Agriculture Canada, Guelph. These samples had been submitted for routine *Brucella* serology. They were not random samples but were selected in order to exhibit a range of brucella STA titres. These samples were stored at −20 °C until tested.

Serology

The standard tube agglutination test (STA) for the detection of Brucella agglutinins was performed according to the method outlined by Brinley-Morgan (1967). The standard tube test antigen was provided by the Animal Diseases Research Institute, Ottawa.

The mercaptoethanol agglutination tests were performed by the method of Alton, Jones & Pietz (1975).

The direct conglutinin and immunoconglutinin tests were performed as described by Coombs *et al.* (1961). After titrating total conglutinating activity the erythrocyte mass was treated with 0·01 M EDTA as described by Lachmann (1967) It was observed that high K titres tended to reduce the observed IK titre if the tubes were read immediately after EDTA treatment. For this reason the tubes were permitted to settle at room temperature for a further two hours before reading the IK titre.

Bovine hetrophile anti-Forssman antibodies were titrated by the direct

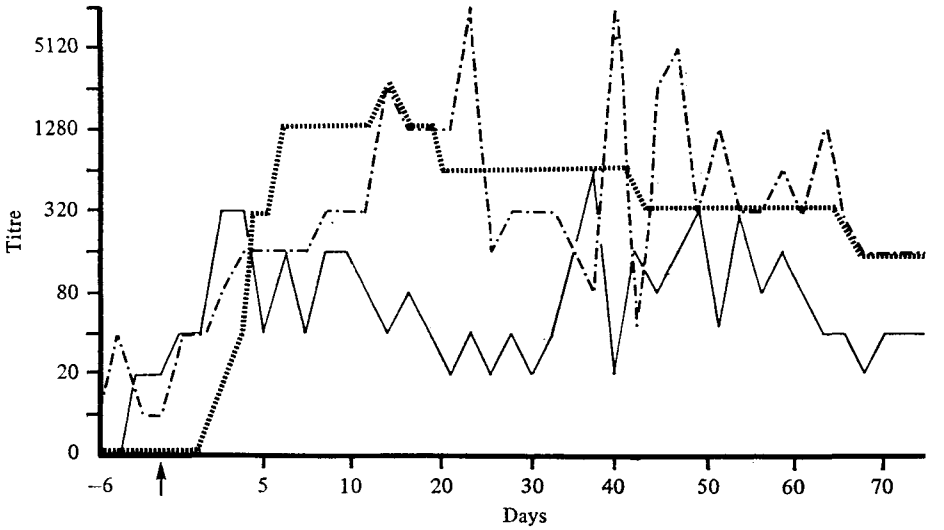

Fig. 1. The serum agglutinin, heterophile antibody and serum immunoconglutinin levels in a calf vaccinated with *Brucella abortus* Strain 19 vaccine. The other calves showed almost identical responses. ||||||, STA titres; ——, heterophile antibodies; –·–·–, immunoconglutinin.

conglutination method of Coombs *et al.* (1961). The source of complement in this technique was horse serum diluted in normal saline 1/10. The source of immunoconglutinin was heterostimulated rabbit serum containing 4 MKD.

RESULTS

Response to S19 Vaccination

In all three vaccinated calves the immune response followed a consistent pattern. Brucella agglutinins were first detected five days after vaccination. They rose rapidly to peak between 10 and 20 days at 1/2560–5120 and declined gradually thereafter, reaching 1/160 by 75 days after vaccination (Fig. 1).

Prevaccination IK titres were between 1/10 and 1/40. They dropped precipitously following vaccination. They reappeared by five to six days and climbed to reach maximum titres around day 20 when titres of 1/640–1280 were reached. They declined relatively rapidly thereafter returning to the normal range by day 30. However, the IK responses were marked by extreme fluctuations in titre. The periodicity of these fluctuations was approximately seven days in all three animals.

K titres in these calves were exremely low, never exceeding 1/10.

Heterophile antibody fluctuated around a titre of 1/80. Although there was no obvious immediate response to vaccination, titres did tend to rise slightly over the course of the experiment.

Serologic survey on bovine sera

The brucella STA, K, IK and heterophile antibody in 304 bovine sera were measured. STA titres varied from 1/10 to 1/10240 as did IK titres. There was no significant correlation between brucella SAT and any of the other factors tested

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Table 1. *The correlation coefficients and their significance between the STA, IK, K and heterophile antibody levels in* 304 *bovine sera*

Factors	Correlation	Significance
STA and IK	−0·024	⩽0·66
STA and total K	0·111	⩽ 0·05
STA and heterophile antibodies	0·272	⩽ 0·01

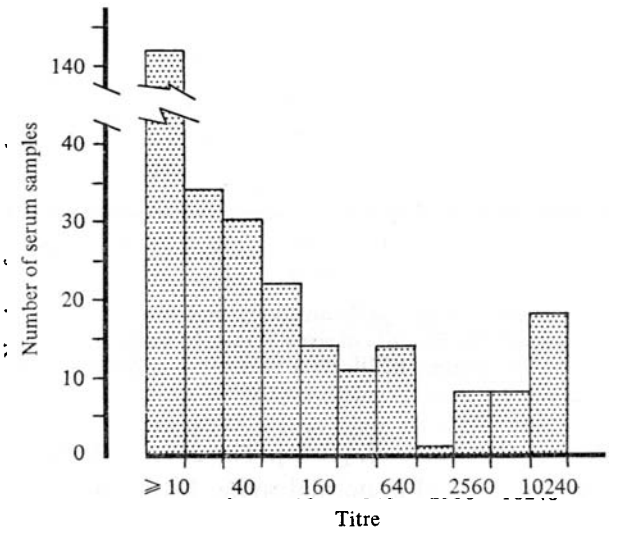

Fig. 2. The distribution of immunconglutinin titres in 304 bovine sera.

(Table 1). On examining the IK responses it was apparent that these titres showed a bimodal distribution (Figure 2). Thus, most animals tended to have very low titres while a subpopulation had titres in excess of 1/2560. The relative sizes of these populations was unrelated to the Brucella STA titre.

DISCUSSION

Immunoconglutinins may be produced in animals as a result of *in vivo* complement fixation and thus reflect the general degree of infection within an animal population. In the case of infection of calves with *B. abortus* strain 19 it is clear that an IK response does occur but that it is relatively transient since IK titres return to normal fairly rapidly. When the relation between the Brucella STA titre and IK titre was investigated in cattle of unknown Brucella infection status, it was apparent that they were unrelated. While it might be argued that high IK titres may reflect recent Brucella infection, this was not reflected in the survey results. On the contrary, the proportion of high titred IK samples was essentially the same irrespective of the titre of Brucella agglutinins. It is apparent that measurement of serum IK is of little use in the serodiagnosis of brucellosis.

The rapid fluctuations in serum IK titres in vaccinated animals and the bimodal distribution in the serum samples were unexpected findings for which we have no explanation.

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Both K and heterophile antibody titres were unrelated to Brucella agglutinins, an observation which was expected in view of the absence of any effect of strain 19 on these titres in vaccinated calves. Conglutinin, not being an antibody, tends to drop rather than increase in bacterial infections (Lachmann, 1967) probably as a result of consumption.

The factors which influence heterophile anti-Forssman antibody titres are not well understood. They tend to rise in situations in which B cells are non-specifically stimulated, for example, trypanosomiasis (Houba *et al.* 1969) but have never been consistently reported to change in other infectious diseases. In cattle it has been shown that anti-Forssman antibodies show seasonal fluctuation (Ingram & Barnum, 1971) and these authors also failed to demonstrate any change in the titre of these antibodies occurring as a result of infection.

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REFERENCES

Alton, G. G., Jones L. M. & Pietz D. E. (1975). *Laboratory techniques in Brucellosis.* W.H.O. Geneva.

Brinley-Morgan, J. W. (1967). The serological diagnosis of bovine Brucellosis. *Veterinary Record* **80**, 612–20.

Coombs, R. R. A., Coombs, A. M. & Ingram, D. G. (1961). *The Serology of Conglutination.* Blackwell, Oxford.

Houba, V., Brown, K. N. & Allison, A. C. (1969). Heterophile antibodies. M-antiglobulins and immunoglobulins in experimental trypanosomiasis. *Clinical and Experimental Immunology* **4**, 113–23.

Ingram, D. G. & Barnum, D. A. (1965). Fluctuations in the level of Conglutinin in Bovine Serum. *Canadian Veterinary Journal* **6**, 162–9.

Ingram, D. G. & Mitchell, W. R. (1971). Conglutinin level in Dairy Cattle: Changes associated with Disease. *American Journal of Veterinary Research* **32**, 875–8.

Lachmann, P. J. (1967). Conglutinin and Immunoconglutinins. *Advances in Immunology* **6**, 479–527.