Acta vet. scand. 1987, *28*, 361–371.

From the Department of Veterinary Microbiology, Section of Parasitology,
Swedish University of Agricultural Sciences
and the National Veterinary Institute, Uppsala, Sweden.

A Modified IF-test to Demonstrate IgM Antibodies to Babesia Divergens of Cattle

By *Dan A. Christensson*

Christensson, D. A.: A modified IF-test to demonstrate IgM antibodies to Babesia divergens of cattle. Acta vet. scand. 1987, 28, 361–371. – An IF-test modified to increase sensitivity was used to detect the presence of Babesia divergens specific IgM antibodies. It consisted of three steps: (1) the incubation of Babesia antigen with test serum, followed by; (2) its incubation with protein A; and (3) the addition of rabbit anti-bovine IgM bound to protein A, which was bound with FITC.
Compared to the conventional IF-technique, this modified IF-technique detected bovine anti-Babesia-IgM in test serum at a two-fold lower dilution and with a diminished background staining.
This modified IF-technique improves the possibilities for studying class M antibody in the serum of cattle which have been infected with B. divergens. It is possible to determine whether a calf's antibody response is due to maternally transmitted antibodies or actively acquired antibodies by determining the presence of each of the classes of immunoglobulin against B. divergens present in the calf's serum.

FITC-labelled protein A; IF-technique; blocking of antigen receptors.

Introduction

The presence of maternally derived antibodies against Babesia has been demonstrated in calves during their first months of life (*Ross & Löhr* 1970, *Weisman et al.* 1974). Bovine maternal antibodies are selectively transferred with colostrum and consist mainly of IgG (*Murphy et al.* 1964, *Pierce & Feinstein* 1965, *Aalund* 1968, *Kickhöfen et al.* 1968, *Butler* 1974). It is therefore possible, by testing the serum for the presence of Babesia specific antibodies of immunoglobulin classes other than IgG, e.g. IgM, to distinguish between a serological reaction to Babesia caused by passively transmitted antibodies, and a serological reaction caused by antibodies induced actively by a post natal Babesia infection.

The anti-immunoglobulin (anti-Ig) used in the conventional indirect immunofluorescent antibody (IF) test is conjugated directly with fluorescin isothiocyanate (FITC) (*Coons et al.* 1941). The antigen used in the conventional IF test to demonstrate the presence of antibodies to bovine babesiosis is a well rinsed thin film smear of infected erythrocytes (*Ross & Löhr* 1968). When this IF test is used to detect IgM antibodies to B. divergens unspecific reactions and a high, disturbing background fluorescence frequently occur.

A modified IF technique which uses rabbit anti-bovine IgM linked to protein A has been developed. The protein A molecule is labelled with FITC (FITC-protein A) (Pharmacia Fine Chemicals, Uppsala, Sweden). To avoid false reactions due to direct attachment of FITC-protein A to the B. divergens antigen, the receptors for protein A are blocked with protein A. The present study compares this modified IF test with the conventional IF test which uses rabbit anti-bovine IgM conjugated with FITC.

Materials and methods

Protein A

Protein A is produced from the cell surface of some strains of Staphylococcus aureus (*Kronvall* 1973, *Lindmark et al.* 1977). It has an ability to bind immunoglobulins, especially the Fe part of the rabbit IgG molecule (*Forsgren & Sjöquist* 1966). The protein A, and the FITC-protein A used in these tests were commercial products from Pharmacia Fine Chemicals, Uppsala, Sweden. The FITC-protein A was covalently coupled with an average of 6 FITC substituent groups per protein A molecule. The freeze dried protein A powder and FITC-protein A powder were diluted with aq.dest. to a concentration of 1 mg per ml and stored at –20°C until used.

Antisera to immunoglobulins

The antisera to the immunoglobulins were produced in rabbits by the Department of Production, National Veterinary Institute (NVI) and by Mile Laboratories Inc. (Miles) as indicated below. The antibovine IgM sera (anti-IgM) were shown by the manufacturers to be heavy chain specific. The manufacturers' characterization of the antiserum by immunoelectrophoresis was: antiserum versus bovine serum produced one band; antiserum versus bovine IgM produced one band; antiserum versus bovine IgG produced no band. Antiserum specificity by immunodiffusion was: antiserum versus bovine serum showed one band; antiserum versus bovine IgM showed one band; antiserum versus bovine IgG showed no band. The anti-bovine IgG sera (anti-IgG) were shown to be both heavy and light chain specific. They were characterized by immunoelectrophoresis: antiserum versus bovine serum produced IgG band; antiserum versus immunogen produced IgG band. Specificity by immunodiffusion were: antiserum versus bovine serum showed IgG band; antiserum versus bovine IgG showed IgG band.

Optimal dilutions of FITC-conjugated anti-IgM and anti-IgG were found by chessboard titrations according to the procedure described by *Beutner et al.* (1968), using 2 known positive and 2 known negative test sera. Optimal dilutions of non-conjugated anti-IgM and anti-IgG were also found by chessboard titrations modified so that serial dilutions of sera of both anti-immunoglobulin classes and FITC-protein A were tested in combinations against the serial dilutions of the test sera. The optimal dilution used was found to be the second to the last dilution on the plateau made in the chessboard titration. For FITC-protein A the optimum dilution was defined as 0.0625 mg per ml in combination with blocking of the antigen with protein A at an equal dilution. The anti-Ig used and the working dilutions are listed below:

- Rabbit anti-bovine immunoglobulin M fraction. Produced by NVI. Used at a dilution of 1/64. Referred to as anti-IgM no. 14.
- Rabbit anti-bovine immunoglobulin M fraction, conjugated with FITC. The same anti-IgM batch as anti-IgM no. 14. Referred to as anti-IgM no. 14 F. Used at a dilution of 1/64. Molar ratio F/P 1.8.
- Rabbit anti-bovine immunoglobulin M

fraction. Produced by Miles, Lot. no. 19. Referred to as anti-IgM no. 19. Used at a dilution of 1/128.
- Rabbit anti-bovine immunoglobulin M fraction, conjugated with FITC, Miles, Lot. no. 12. Referred to as anti-IgM no. 12 F. Used at a dilution of 1/256. Molar ratio F/P 3.6.
- Rabbit anti-bovine immunoglobulin G fraction, Miles, Lot. no. 20. Referred to as anti-IgG no. 20. Used at a dilution of 1/200. The molar ratio F/P for FITC-protein A + anti-Ig was estimated to be 6, as the average number of fluorescent groups per protein A molecule was 6 (Pharmacia Fine Chemicals) and the molar ratio of protein A bound to rabbit IgG was calculated to be almost 1 (*Lindmark* 1981).

Babesia infection of calves

In order to compare the modified IF test with the conventional test, sera were obtained from calves kept under parasite-free conditions before and after experimental infection with B. divergens. The calves were infected with different isolates of B. divergens. All of the infected animals reacted with a transient parasitaemia within 14 days and serum samples which were drawn 21 days post infection (days p.i.) showed titres of 1/1280–1/2560 for IgM.
The test sera were diluted in doubling dilutions starting at 1/40 or 1/20 as specified below.

Diluent

The bovine sera, anti-immunoglobulins and protein A were diluted in a 0.1 mol/l phosphate buffered saline (PBS) and 0.01 % sodium azide (PBS azide) was added.

Babesia antigen

The antigen consisted of B. divergens organisms either within or free from the erythrocyte membrane fixated to microscope slides (*Garnham & Voller* 1965). The antigen production was a modification of those described by *Ross & Löhr* (1968), and by *Leeflang & Perié* (1972). The antigen used in this experiment was produced from a splenectomized calf infected with a B. divergens isolate, heterologous to the isolates which had been used to infect the donor calf of test sera. When a parasitaemia of about 10 % of the erythrocytes infected with B. divergens was reached, 80 ml blood was drawn into 1 liter of Alsevers solution, pH 6.8. The red blood corpuscles were then washed by 4 separate 500 g centrifugations in PBS, each lasting 20 min. Washing took place within 1 h after the blood was drawn. The sediment of erythrocytes and free Babesia-organisms was resuspended with volumes of PBS 1–2 times that of the sediment. Subsequently it was spread with an applicator in a thin film onto the wells in teflonized slides. Each slide had 10 wells (*Goldman* 1968, *Christensson* 1986). While preparing a solution of the sediment, some antigen slides were stained with acridin-orange (*Winter* 1967) to determine the number of Babesia-organisms and to make sure that no white corpuscles, which interfer with the IF-test, were present. The antigen in the wells was air dried. These antigen slides were wrapped first in special Leitz microscope cleansing tissues and then in aluminium foil, after which they were stored at –70°C in a sealed plastic bag with dry silicagel (*Leeflang & Perié* 1972) until being used within a period of 2 years.
Immediately before use, the antigen was fixated by dipping the antigen slides for 5 min into cold (–20°C) aceton which was kept dry by storage over crystals of $CaCl_2$. The antigen slides were rinsed and washed 3×2.5 min with PBS, which also liberated any haemoglobin from the antigen.

The modified IF technique

1. Dilutions of test sera in a volume of about 0.1 ml were dropped onto the antigen in the wells, which were still wet from being washed. The antigen slides were incubated in moist chambers at 26°C for 30 min and rinsed as described previously.
2. To block the sites on the antigen and on attached serum proteins where protein A could bind, approximately 0.1 ml diluted protein A was dropped into each well. The antigen slides were incubated and rinsed as described previously.
3. After blocking, rabbit anti-IgM, to which FITC-protein A was bound, was added. FITC-protein A was bound to anti-IgM by mixing equal parts of an optimal dilution of anti-IgM with an optimal dilution and FITC-protein A in a test tube, which was subsequently incubated at 26°C for 30 min. This labelled anti-IgM was used within 24 h. Approximately 0.1 ml of the prepared dilution was added to each well and the antigen slide was then incubated and rinsed as described previously.

The slides were mounted under a cover slip using a mountant composed of 9 parts glycerol and 1 part 0.1 mol/l $NaHCO^3$ at pH 8.3 (*Goldman* 1968). The preparations were read within 6 h.

The slides were viewed with a Leitz Orthoplan microscope equipped with incident light exitation, a Ploem-pack 2.1 with a filter block K2 with a blue exitation band pass filter exiting 470–490 nm, and a Hg lamp HBO 50W.

Reactions were graded with 1 of 4 scores: nil,+,++ and +++. A score of +, which was regarded as positive was given when the whole rim of all the parasites was glowing. At higher scores more of the parasites surface was fluorescing. Each day readings were initiated with the same negative and positive sera, always at the same dilutions, 1/40 and 1/640 respectively.

The conventional IF-technique

The conventional IF-test which was performed parallel to the modified IF-test, was similar to the latter except that no protein A was used and the anti-IgM was directly conjugated with FITC.

All of the batches of reagent that were used were tested and found to be free of autofluorescence.

Results

Comparison of the modified and the conventional IF-technique using negative sera

To compare the unspecific reactions occurring with the conventional IF-test for IgM antibodies of Babesia with those of the modified test 10 negative sera from non-splenectomized calves were tested in duplicates, diluted in doubling dilutions starting at 1/20. The 4 anti-IgM batches described above were used.

The results are presented in Table 1. When using modified technique, no unspecific reactions were seen at a dilution of 1/40, but for the conventional technique the sera had to be diluted 2 steps further to give the same negative reaction. This difference of 2 doubling dilution steps was significant ($P < 0.01$, $\chi^2 = 12.1$). The 10 sera reacted uniformly. There was no systematic tendency among the sera, such as e.g. one serum always giving a higher titre than the others.

Comparison of the modified and the conventional IF-technique for positive sera

To compare the specific reactions occurring with the conventional IF-test for IgM antibodies to Babesia with those of the modified test 10 positive test sera were used. They were obtained from the same calves which previously had been used to give the negative sera. Positive sera were obtained 8–22 days p.i. and the 4 anti-IgM batches described before were used.

Table 1. A comparison of the performance on negative control serum of a modified IF-technique which uses anti-IgM bound with FITC-protein A as the anti-immunoglobulin, with the conventional IF-technique which uses FITC-conjugated anti-IgM. B. divergens was the antigen used. Sera were obtained from 10 non-infected calves. Each serum was tested in duplicates. Two different anti-IgM preparations were used for each IF-technique. Titres are given as reciprocal values.

	No. of sera with a positive reading at a titre of (reciprocal values)				
Anti-Ig used	20	40	80	160	320
Modified technique					
anti-IgM no. 14	8	0	0	0	0
anti-IgM no. 19	8	0	0	0	0
Conventional technique					
anti-Igm no. 14F	10	10	8	0	0
anti-IgM no. 12F	10	10	10	2	0

The results showed that the titres varied from 1/80 to 1/5120 depending on how many days p.i. the samples were drawn. There was no discrepancy between the modified tests performed with the 2 anti-IgM batches described, nor was there any difference between conventional tests performed with the 2 FITC-conjugated anti-IgM batches. Comparing the 2 IF-techniques the modified technique gave an endpoint titre 1 dilution step lower in 6 of 10 cases. The endpoint reading was more distinct with the modified technique.

Counterstaining with Evans Blue compared to the conventional IF-test

Parallel with the conventional IF-tests of negative and positive test sera, IF-tests using counterstaining with 0.01 % Evans Blue added to the antiglobulin solution were performed (*Goldman* 1968). In 2 cases with positive sera, counterstaining with Evans Blue gave an endpoint titre 1 dilution step higher than cases not using Evans Blue, and in 1 case such counterstaining gave an endpoint titre 1 dilution step lower.

Table 2. A comparison of the affinities for bovine Ig of: protein A as used in the test, and rabbit IgG anti-bovine IgG bound with FITC-protein A as used in a modified IF-test. Sera were obtained from 10 calves before and at intervals after infection with B. divergens (end point titres are given as reciprocal values).

		End point titre with conjugate	
Days p.i.	No. of sera	FITC-protein A	FITC-protein A + rabbit anti-IgG
0	10	< 40	< 40
9	10	< 40	160–640
24	10	40	1280–2560

The specificity of the modified IF-test

The specificity, i.e. the ability of the test to differentiate between infected and non-infected animals, was investigated by using positive and negative test sera diluted in doubling dilutions starting at 1/40. This dilution was shown to be a possible breaking point for the modified test (Table 1). In conventional tests performed by others (*Ross & Löhr* 1968, *Leeflang & Perié* 1972) this dilution was critical. Test sera were obtained from 43 splenectomized calves kept indoors at the NVI. Blood samples were drawn 1 week before and 21 days after the experimental infection with B. divergens. Sera were tested with the anti-IgM described previously.

At a dilution of 1/40, 42 of 43 (97.7 %) of serum samples from the non-infected animals showed no reaction in the test for IgM. One had an endpoint titre at 1/40. After infection, all 43 animals (100 %) showed positive reactions at 1/40. Endpoint titres were 1/640 to 1/1280.

The reproducibility of the modified IF-test

The reproducibility of the modified IF-test was investigated with a within-test (*Chisholm et al.* 1978), where duplicate dilutions of the same test serum were made daily for 5 consecutive days and titrated to their endpoints. The difference in dilution steps within each daily duplicate was a measure of the reproducibility. All of the endpoint titres were also tabulated together in a test-to-test comparison in which all of the results from 1 serum were compared. Anti-IgM no. 14 was used with 10 positive and 5 negative test sera obtained from non-splenectomized animals.

The result of the within test of positive sera showed that the reproducibility within 1 doubling dilution step was 96 %. There was no difference more than 2 doubling dilution steps. The result of the test-to-test comparison of positive sera showed that the reproducibility was 90 % within 1 doubling dilution step and 100 % within two. The reproducibility for negative sera was 100 % regarding both the within test and the test-to-test comparison.

The blocking capacity of protein A in the modified IF-test

In order to verify that the blocking of protein A binding sites was total, sera known to react strongly to anti-IgM, were tested. Sera were drawn from each of 5 non-splenectomized calves of 14, 21 and 28 days p.i. and diluted 1/80. The modified IF-test was performed as described previously with the exception that instead of anti-Ig anti-IgM no. 14 bound with protein A without FITC was used. After the slides were washed a solution containing 0.625 mg/ml of FITC-protein A was dropped onto the wells and incubated for 30 min. The antigen slides were washed and mounted as previously described.

No fluorescence was observed. This indicates that there was no disturbing exchange of FITC-protein A and protein A bound to antigens or antiglobulins and no disturbing exchange of FITC-molecules from FITC-protein A to protein A.

A comparison of the affinity of FITC-protein A to bovine Ig with its affinity to rabbit IgG anti-bovine immunoglobulin

Protein A binds to different classes of immunoglobulins of many animal species, but with different affinities (reviewed by *Lindmark* 1981). The test was made to compare the practical effect of the direct binding of FITC-protein A to bovine Ig used in the standard test with the indirect binding of FITC-protein A to rabbit Ig anti-bovine immunoglobulin used in the modified IF-test. The rabbit anti-bovine Ig used was

anti-IgG no. 20, which was prepared by the manufacturer as a rabbit IgG.

Sera from 10 non-splenectomized calves were obtained: before experiment infection with B. divergens (negative serum), 9 days p.i. (weak positive serum), and 24 days p.i. (strong positive serum). The modified IF-test was performed on these sera as described before. FITC-protein A was used in a conventional IF-test as the "antiglobulin" at a dilution of 0.0625 mg/ml.

The results are summarized in Table 2. FITC-protein A could be used to detect bovine Ig only in strong positive serum. The indirect, modified IF-test was more sensitive.

The modified IF-test with anti-IgG

The modified IF-test was performed with an anti-IgG as described for the use of anti-IgM. Comparable results were obtained, except that no difference was registered with regards to unspecific reactions, probably because unspecific reactions were more uncommon with FITC-conjugated anti-IgG than with FITC-conjugated anti-IgM. The specificity of the IF-test was excellent. 161 of the 162 non-infected animals tested (99.9 %) showed no reaction, while 100 % of the infected animals showed a positive reaction. The reproducibility of the within test was 98 % within 1 doubling dilution step and 100 % within 2. Test-to-test reproducibility was 100 % within 1 doubling dilution.

Every batch of antiserum, anti-Ig dilution, and serum, as well as the antigen were checked to be free of autofluorescence.

Discussion

The first descriptions of the usage of an IF-test to detect antibodies in animals infected with Babesia were published by *Madden & Holbrook* (1968), *Ross & Löhr* (1968) and *Zwart et al.* (1968). It is now the most widely used serological technique to demonstrate the presence of Babesia antibodies (*Todorovic & Carson* 1981). However, the test has been regarded as a less suitable tool for the investigation of the Babesia infection in calves less than 6 months old (*Aragon* 1976, *Todorovic & Carson* 1981), because maternally derived antibodies against this parasite have been demonstrated in calves up to 6 months of age (*Ross & Löhr* 1970, *Weisman et al.* 1974). The FITC-conjugated antiglobulin used in these tests were, however, either anti-bovine serum (*Ross & Löhr* 1970) or anti-bovine IgG (*Weisman et al.* 1974). The maternal Ig is mainly IgG_1 (*Murphy et al.* 1964, *Pierce & Feinstein* 1965, *Aalund* 1968, *Kickhöfen et al.* 1968, *Butler* 1974). Although all of the different immunoglobulin subclasses are absorbed by the newborn calf (*Butler* 1974), only a relatively small percentage are IgM immunoglobulins. It is therefore possible to use the presence of IgM antibodies specific for Babesia as an indication that the animal being tested has acquired antibodies actively due to Babesia infection. The presence of IgG_2 antibodies could also be used as an indicator that antibodies have been actively acquired, but this subclass of the bovine immunoglobulins was reported to be completely absent in 1–2 % of a group of Danish cattle and present only in low levels in another 15 % (*Mansa* 1965, *Nansen* 1970).

Disturbing background fluorescence and difficulty in distinguishing between low positive reacting sera and known negative sera are two of the problems that arise when using FITC-conjugated anti-IgM immunoglobulins in the conventional IF-test (*Goldman* 1968). Comparable problems have been described by *Callow et al.* (1975) in tests for equine IgG of Babesia equi.

With the modified technique, infected animals could be separated from non-infected at a serum dilution of 1/40 by demonstrating the presence of IgM or IgG antibodies specific for Babesia divergens. This dilution was also found by others to be the lowest positive titre when using conventional IF-tests with anti-IgG or antiserum to demonstrate the presence of antibodies against B. divergens (*Leeflang & Perié* 1972, *Joyner et al.* 1972) and against other bovine Babesia species (*Ross & Löhr* 1968, *Leeflang & Perié* 1972, *Callow et al.* 1974). Others, have found that serum dilutions of 1/16 (*Goldman et al.* 1972) or 1/64 (*Johnston et al.* 1973a) distinguish false positive from true positive sera. The specificity and the reproducibility were tested and found to be high.

The present study is presumably the first attempt to demonstrate the presence of B. divergens specific IgM antibodies. Recently *O'Donoghue et al.* (1985) published the results of an experiment in which they used the ELISA technique to investigate the kinetics of IgM and IgG antibodies in splenectomized calves after they were infected with B. bigemina. The presence of IgM antibodies in cattle after infection with B. bovis has also been demonstrated by using a gel filtration technique (*Mahoney* 1972) and by using a gel separation technique which revealed both IgM and IgG activity in serum obtained after infection (*Goff et al.* 1982). *Cox & Turner* (1970) demonstrated the presence of IgM antibodies in mice after a Babesia infection by using a conventional IF-technique and they reported no problems with background fluorescence. Both the modified IF-technique and the ELISA technique seem to be suitable for testing bovine sera to investigate the IgM response to a Babesia infection.

Blocking was necessary, since the test could be disturbed by a direct binding of FITC-protein A to bovine Ig (Table 2). Blocking also eliminated the disturbing background fluorescence better than counterstanding with Evans Blue. The endpoint titres for both IgM and IgG were easier to determine when using the modified technique since it produced less background staining and gave an increased fluorescein/protein (F/P) molar ratio.

Disturbing background fluorescence was also eliminated with an anticomplement IF method developed to detect antibodies to B. bovis (*Johnston et al.* 1973b). The improved results in both the modified IF-test and the anticomplement IF-test may, to some extent, have depended upon the fact that with both techniques the FITC-molecules were indirectly bound to the antiserum and thereby not easily attached to other proteins.

Molar ratio F/P gives a good reflection of the number of FITC molecules bound to an anti-Ig (*Beutner* 1971). The modified IF-test produces a higher molar ratio F/P (about 6) than is considered optimal (about 1.5–4) for conventional FITC conjugates, since this induces non-specific staining (*Goldman* 1968).

Protein A has been shown to bind to the immunoglobulins of many different animal species (*Goudswaard et al.* 1978) and to varying degrees to the different classes and subclasses of the Ig as listed by *Lindmark* (1981). Only 26 % of bovine IgG_1 antibodies showed reactivity to protein A, while 98 % of the IgG_2 antibodies did so (*Goudswaard et al.* 1978). The relative affinity of protein A to rabbit IgG was, however, estimated to be 50 times greater than to bovine Ig (*Goudswaard et al.* 1978). This stronger affinity to rabbit IgG was also demonstrated with a modified IF-test compared with FITC-protein A as the "only antiglobulin" (Table 2).

The reactivity of protein A to bovine IgM seems to be unknown, but is poor to IgM of other ruminant species (*Lindmark* 1981).

FITC-protein A has been used as an alternative to monospecific anti-IgG (*Bieberfeldt et al.* 1975). It has also been used as an indirect marker of specific anti-Ig in tests in which animal antibodies have a low affinity for protein A (*Haaijman & Slingerland-Teunissen* 1978, *Matzku & Zöller* 1979, *Ghetie & Mota* 1980). These authors also report that the test using FITC-protein A bound with an anti-Ig gives an increased sensitivity compared to the test using a directly FITC conjugated anti-Ig.

The capacity of protein A to interact exclusively with rabbit IgG (*Lindmark* 1981) had the advantage of making all of the rabbit antiglobulins used in the modified IF-test more comparable since serum components of the rabbit anti-Ig other than rabbit IgG, would not be bound with FITC-protein A. This may explain the discrepancy between the conventional and the modified IF-tests: in the conventional FITC-conjugated anti-Ig residues of non-specific protein might have become FITC-labelled and caused non-specific reactions.

Other advantages that the labelling of rabbit anti-Ig with a standardized FITC-protein A has over the conventional FITC-conjugation of anti-Ig are: technical simplicity, the rapidity of the labelling method and the fact that no anti-Ig is lost. For these reasons even small batches of anti-Ig could be used.

This modified IF-technique was previously used to improve the IF-test for Babesia motasi (*Christensson & Thunegard* 1981). During that experiment the unspecific background fluorescence was also eliminated.

Acknowledgements

I wish to express my gratitude to Dr. L. Prage for introducing me into the potential uses of protein A, to Dr. T. Morén for taking care of the animals and collecting serum samples, to Ms. K. Candelöf, Ms. M. Väisänen and Ms. E. Tidblom for technical assistance and to Ms. G. Wikne for typing the manuscript.

References

Aalund O: Heterogeneity of ruminant immunoglobulins. Thesis. Munksgaard, Copenhagen 1968.

Aragon RS: Bovine babesiosis: A review. Vet. Bull. 1976, *46,* 903–917.

Bieberfeldt P, Ghetie V, Sjöquist J: Demonstration and assaying of IgG antibodies in tissues and on cells by labelled staphylococcal protein A. J. Immunol. Meth. 1975, *6,* 249–259.

Beutner EH, Sepulveda MR, Barnett EV: Quantitative studies of immunofluorescent staining. Bull. Wld. Hlth. Org. 1968, *39,* 587–606.

Beutner EH: Defined immunofluorescent staining in present status, and future prospects for defined conjugates, In: "Defined immunofluorescent staining" (ed. E. H. Beutner). New York Acad. Sci. 1971, 506–526.

Butler JE: Immunoglobulins of the mammary secretions. In: "Lactation, comprehensive treatise" vol. III (ed. B. L. Larsson & V. Smith) Academic Press, N. Y. 1974, 217–255.

Callow LL, McGregor W, Parker RJ, Dalgliesh RJ: The immunity of cattle to Babesia argentina after drug sterilisation of infections of varying duration. Aust. vet. J. 1974, *50,* 6–11.

Callow LL, McGregor W, Rodwell BJ, Rogers RJ, Frazer GC, Mahoney DF, Robertson GM: Evaluation of an indirect fluorescent antibody test to diagnose Babesia equi infection in horses. Aust. vet. J. 1975, *55,* 555–559.

Chisholm ES, Ruebush II TK, Sulzer AJ, Healy GR: Babesia microti infection in man: Evaluation of an indirect immunofluorescent antibody test. Amer. J. trop. Med. Hyg. 1978, *27,* 14–19.

Christensson D: Improvement of the teflonized slides used in the immunofluorescent antibody technique. Acta vet. scand. 1986, *27,* 296–298.

Christensson DA, Thunegard E: Babesia motasi in sheep on the island of Gotland in Sweden. Vet. Parasit. 1981, *9*, 99–106.

Coons AH, Creech HJ, Jones RN: Immunological properties of an antibody containing a fluorescent group. Proc. Soc. exp. Biol. Med. 1941, *47*, 200–202.

Cox FEG, Turner SA: Antibodies in mice infected with Babesia microti. Ann. Trop. Med. Parasitol. 1970, *64*, 164–173.

Forsgren A, Sjöquist J: Protein A from S. aureus. J. Immunol. 1966, *97*, 822–827.

Garnham PCC, Voller A: Experimental studies on Babesia divergens in rhesus monkeys. Acta Protozool. Warsaw, 1965, *3*, 183–187.

Ghetie V, Mota G: Multivalent hybrid antibody. Molecular Immunology, 1980, *17*, 395–401.

Geoff LW, Wagner GG, Craig TM, Long RF: The bovine immune response to tick-derived Babesia bovis infection: Serological studies of isolated immunoglobulins. Vet. Parasit. 1982, *11*, 109–120.

Goldman M: Fluorescent antibody methods. Academic Press, N.Y. 1968.

Goldman M, Pipano E, Rosenberg AS: Fluorescent antibody test for Babesia bigemina and B. berbera. Res. vet. Sci. 1972, *13*, 77–81.

Goudswaard J, v. d. Donk JA, Noordzij A, v. Dam RH, Vaerman J-P: Protein A reactivity of various mammalian immunoglobulins. Scand. J. Immunol. 1978, *8*, 21–28.

Haaijman JJ, Slingerland-Teunissen J: Equipment and preparative procedures in immunofluorescent microscopy: Quantitative studies. In: "Immunofluorescence and related staining techniques" (ed. W. Knapp, K. Holubar & G. Wick) 1978, 11–29.

Joyner LP, Donelly J, Payne R, Brocklesby DW: The indirect fluorecsent antibody test for the differentiation of infections with Babesia major. Res. vet. Sci. 1972, *13*, 515–518.

Johnston LAY, Pearson RD, Leatch G: Evaluation of an indirect fluorescent antibody test for detecting Babesia argentina infection in cattle. Aust. vet. J. 1973a, *49*, 373–377.

Johnston LAY, Pearson RD, Leatch G: A comparison of indirect, direct inhibition and labelled anticomplement fluorescent antibody test in the detection of Babesia argentina infection of cattle. Aust. vet. J. 1973b, *49*, 421–423.

Kickhöfen B, Hammer DK, Scheel D: Isolation and characterization of gamma-G type immunoglobulins from bovine serum and colostrum. Hoppe-Seyler's. Z. phys. Chem. 1968, *349*, 1755–1773.

Kronvall, G: A surface component in group A, C and G streptococci with non-immune reactivity for immunoglobulin G. J. Immunol. 1973, *111*, 1401–1406.

Leeflang P, Perié NM: Comparative immunofluorescent studies on 4 Babesia species of cattle. Res. vet. Sci. 1972, *13*, 342–346.

Lindmark R, Mowitz J, Sjöquist J: Extracellular protein A from a methicillin-resistant strain of Staphylococcus aureus. Eur. J. Biochem. 1977, *74*, 623–628.

Lindmark R: Studies on protein A from Staphylococcus aureus. Abs. of Uppsala Diss. from the faculty of Medicine 412, Uppsala, Sweden, 1981.

Madden PA, Holbrook AA: Equine piroplasmosis: Indirect fluorescent antibody test for Babesia caballi. Amer. J. vet. Res. 1968, *29*, 117–123.

Mahoney DF: Immune response to haemoprotozoa II: Babesia spp. In: "Immunity to animal parasites" (ed. E. J. L. Soulsby). Academic Press, N.Y. 1972, 301–341.

Mansa B: Hypo-7S-gamma-globulinaemia in mature cattle. Acta path. microbiol. scand. 1965, *63*, 153–158.

Matzku S, Zöller M: A sandwich isotopic antiglobulin assay. Application to the detection of antibodies in non-SPF bearing spontaneous tumors. Immunobiol. 1979, *156*, 483–497.

Murphy FA, Aalund O, Osebold JW, Carrol EJ: Gamma globulins of bovine lacteal secretion. Archs. Biochem. Biophys. 1964, *108*, 230–239.

Nansen, P: metabolism of bovine immunoglobulin-G. Thesis. Munksgaard, Copenhagen 1970.

O'Donoghue PJ, Friedhoff KT, Kizcaino OG, Weyreter H: The detection of IgM and IgG antibodies against Babesia bigemina in bovine sera using semi-defined antigens in enzyme immunoassays. Vet. Parasitol. 1985, *18*, 1–12.

Pierce AE, Feinstein A: Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. Immunology 1965, *8*, 106–123.

Ross JP, Löhr KF: Serological diagnosis of Babesia bigemina infection in cattle by the indirect fluorescent antibody test. Res. vet. Sci. 1968, *9*, 557–562.

Ross JP, Löhr KF: Übertragung und Verweildauer von kolostral erworbenen Babesia bigemina- und Anaplasma marginale-Anti-Körpern. (Transmission and persistance of colostral acquired antibodies of Babesia bigemina and Anaplasma marginale). Zschr. Tropenmed. 1970, *21*, 401–411.

Todorovic RA, Carson CA: Methods for measuring the immunological response to Babesia. In: Babesiosis (ed. M. Ristic & J. P. Kreier) Academic Press, N.Y. 1981, 394.

Weisman J, Goldman M, Mayer E, Pipano E: Passive transfer to newborn calves of maternal antibodies against Babesia bigemina and Babesia berbera. Refuah vet. 1974, *31*, 108–113.

Winter H: Staining with acridin-orange. Res. vet. Sci. 1967, *8*, 170.

Zwart D, van den Ende MC, Koowenhoven B, Buys J: The difference between B. bigemina and Dutch strain of B. major. Tijdschr. Diergeneesk. 1968, *93*, 126–140.

Sammannfattning

Modifierad indirekt immunofluorescensteknik för påvisande av IgM-antikroppar mot Babesia divergens infektion hos nötkreatur.

Jämförelse av ett konventionellt utfört IF-test med ett modifierat test i vilket utnyttjas FITC-protein A konjugerat antiglobulin samt blockering av protein A receptorer på antigenet.

För att öka den indirekta immunofluorescens(IF)-teknikens känslighet och därmed kunna påvisa närvaro av IgM-antikroppar specifika mot B. divergens utfördes testet på följande sätt: 1) inkubering av Babesia-antigen med testserum, 2) följt av inkubering med protein A och därefter 3) tillsattes kanin anti-nöt IgM till vilket bundits protein A bundet med FITC.

Med den modifierade IF-tekniken kan IgM-antikroppar påvisas tidigt efter en infektion med B. divergens ty reaktionen kan avläsas vid en låg serumspädning tack vare en låg bakgrund. Genom att bestämma förekomst av immunoglobuliner av olika klasser kan man avgöra om ett antikroppssvar beror av maternalt överförda antikroppar eller är resultat av en egen, förvärvad infektion.

(Received February 2, 1987).

Reprints may be requested from: Dan Christensson, Dept. of Parasitology, National Veterinary Institute, P. O. Box 7073, S-750 07 Uppsala, Sweden.