Acta vet. scand. 1975, *16*, 236—243.

From the Research Station of the Veterinary Institute, Skara, Sweden and the Department of Medicine, College of Veterinary Medicine, Helsinki, Finland.

THE VALUE OF THE GLUTARALDEHYDE AND FORMALDEHYDE TESTS IN EVALUATION OF THE GLOBULIN LEVEL IN BOVINE BLOOD*

By

P. Liberg, B. Pehrson and *M. Sandholm*

LIBERG, P., B. PEHRSON and M. SANDHOLM: *The value of the glutaraldehyde and formaldehyde tests in evaluation of the globulin level in bovine blood.* Acta vet. scand. 1975, *16*, 236—243. — So-called lability reactions are based on the gelification of blood globulins when reacting with, i.e., glutaraldehyde or formaldehyde. Solidifying time is inversely proportional to the globulin concentration of the blood. In the present investigation glutaraldehyde and formaline-gelification tests were compared concerning their diagnostic and prognostic value in dairy cows with internal diseases.

The investigation confirmed earlier results. The correlation between globulin concentration and gelification time was significant, and both tests give very good possibilities to reveal hyper-γ-globulinaemias.

The 2 tests were relatively equal as regards the reliability. Even though the formol-gel reaction allows a somewhat better safety and semi-quantitative differentiation than the glutaraldehyde test, the latter has obvious practical advantages. It can be done on whole blood and with a short observation time. Thus, the blood globulin status can be evaluated during the visit on the farm.

glutaraldehyde test; formaldehyde test; formol-gel reaction; gammaglobulin; chronic inflammations; cattle.

A great deal of diseases in cattle, with unfavourable prognosis, are associated with chronic inflammatory conditions (e.g. traumatic peritonitis with complications, infections of the urinary tract, latent abscesses, pneumonia, arthritis). The extent of the processes and the prognosis for the illness can be evaluated using quantitative determinations of certain blood proteins. This, however, requires laboratory facilities, which generally are not

* The investigation was supported by the Swedish Council for Forestry and Agricultural Research.

at the disposal of a practising veterinarian. In chronic inflammatory conditions the most marked change in the blood is hyperglobulinaemia and therefore it is often sufficient to determine the blood globulin fraction. For this a number of relatively simple semi-quantitative methods have been described. The most suitable method so far for adult cattle seems to be the formol-gel test, which is based on a so-called lability reaction. This means that blood globulins, when mixed with formaline, are converted from a soluble state to a gel. Solidifying time is inversely proportional to the globulin content of the sera. The formol-gel test has been evaluated by several authors. The advantage of the formol-gel reaction has recently been established by *Liberg* (1973), who found a very good correlation between the amount of globulin in serum and the solidifying time.

The formol-gel reaction is a valuable diagnostic and prognostic test in practice. However, the method requires serum, and therefore centrifuging must be performed. Furthermore, the observation period is relatively long.

In order to avoid these practical drawbacks, *Sandholm* (1974) launched a coagulation test on whole blood based on the same principle as the formol-gel test. Glutaraldehyde was used to cross-link basic globulins at their $-NH_2$ groups. Glutaraldehyde has better cross-linking properties than formaldehyde as it contains 2 aldehyde groups per molecule.

The present study was undertaken to compare the glutaraldehyde and formaline-gelification tests in evaluation of the protein pattern in the blood as well as their diagnostic and prognostic value.

MATERIAL AND METHODS

The material consisted of 91 dairy cows with internal disorders which were subjected to ambulatory care and examination at the Research Station of the Veterinary Institute, Skara, during the period from December 1973 to May 1974. The majority of the cows were of the Swedish red-and-white breed. The patients were examined clinically and routine laboratory analyses were performed. Blood samples were drawn for determination of the glutaraldehyde test (GLA) on whole blood and formol-gel reaction (FR) and protein determinations on serum. The dominating diagnoses were traumatic peritonitis with and without complications (17 cows), laminitis-polyarthritis (14), pneumo-

nia-pleuritis (8), left-side abomasal displacement (6), urinary tract disorders (5), ketosis (5), abscesses (4), poisoning (4) and metritis (3). Isolated cases of mastitis, leucosis, fatty degeneration of the liver, endocarditis, panaritium, urticaria, recurrent tympanism and traumatic muscle injuries occurred. In 27 cases the diagnosis was confirmed by post-mortem examination or laparotomy. In 14 cases no definite clinical diagnosis could be established.

Formol-gel reaction

The formol-gel test was performed according to *Liberg* (1973). One ml of serum was mixed with 3 drops (0.1 ml) of neutral 35 % formaline solution in a test tube, after which the time for complete gelling was recorded: this was done during the 24 hr. incubation period at room temperature. The samples remaining liquid after 24 hrs. were considered negative.

Glutaraldehyde test

The glutaraldehyde test was performed according to *Sandholm* (1974), using ready made test kits containing 2.5 ml of 1.2% glutaraldehyde solution in physiological saline containing 1 mg/ml $Na_2EDTA \times 2H_2O$*. An equal volume of whole blood (2.5 ml) was drawn directly from the jugular vein. The tube was corked and mixed by gentle invertions for 10 sec. (avoiding foaming). Then the tube was left in a vertical position at room temperature. The reading was done by inverting the tube once a minute for 15 min. The test was regarded positive when the blood did not move from the bottom when tilted. If the blood was still liquid after 15 min., the test was regarded negative. Parallel GLA analyses were performed on heparinized blood in the laboratory.

Protein determination

The serum albumin was determined according to *Doumas et al.* (1971) with the application described by *Liberg*. Total serum protein was determined by the biuret method. The globulin concentration and albumin/globulin ratio in serum were calculated using total protein and albumin values.

---

* From Orion Diagnostica, Helsinki, Finland.

Table 1. Total protein, albumin, globulin (g/100 ml) and albumin/globulin quotient in serum at different gelling time intervals for GLA-test.

GLA-test	n	Total protein $\bar{x} \pm s$	Albumin $\bar{x} \pm s$	Globulin $\bar{x} \pm s$	Alb/Glob.-quotient $\bar{x} \pm s$	Number of cases grouped according to FR			
						0-60 min.	1-8 hrs.	8-24 hrs.	> 24 hrs.
0—3 min.	32	8.74 ± 1.05	3.82 ± 1.01	4.93 ± 1.11	0.83 ± 0.32	15	9	7	1
3—6 min.	12	7.55 ± 1.70	3.58 ± 0.61	3.98 ± 0.62	0.92 ± 0.23	0	5	5	1
6—15 min.	9	7.59 ± 0.84	4.12 ± 0.58	3.47 ± 0.69	1.22 ± 0.25	0	1	4	4
> 15 min.	38	7.25 ± 0.71	4.18 ± 0.66	3.07 ± 0.53	1.42 ± 0.39	0	0	5	33
Variance quotient (F)		(17.20) ***	$(2.45)^{n.s.}$	(31.11) ***	(33.60) ***				

RESULTS

No difference in the glutaraldehyde coagulability was found between the specimens taken directly from the blood vessel and those analyzed from a heparinized sample later. It made no difference whether the heparinized blood was stored at 4 or 20°C for 24 hrs.

Based on the glutaraldehyde coagulation time the material was divided into 3-minute groups. However, due to limited number of samples in the 6—9-, 9—12- and 12—15-min. groups, these were put together before statistical analysis (Table 1). As shown in Table 1, there was a significant difference in serum protein pattern between the groups formed according to different coagulation times. There were no significant variations for albumin. The changes in the total protein and A/G quotient can thus be concluded to be due to the differences in the globulin content.

When the material was grouped according to the formol-gelification time (Table 2), corresponding differences in the globulin pattern could be found. The range between the highest and lowest group mean value was somewhat higher for the FR than for the GLA test.

DISCUSSION

The clear correlation between serum globulin and gelification time, when glutaraldehyde is mixed with whole blood, shows that the GLA test according to Sandholm gives very good possi-

Table 2. Total protein, albumin, globulin (g/100 ml) and albumin/globulin quotient in serum at different gelling time intervals for FR.

FR	n	Total protein $\bar{x} \pm s$	Albumin $\bar{x} \pm s$	Globulin $\bar{x} \pm s$	Alb/Glob.-quotient $\bar{x} \pm s$	Number of cases grouped according to GLA-test 0-3 min.	3-6 min.	6-15 min.	> 15 min.
0—60 min.	13	9.58 ± 0.91	3.81 ± 1.35	5.78 ± 0.98	0.70 ± 0.32	13	0	0	0
1—8 hrs.	17	8.34 ± 0.68	3.84 ± 0.72	4.51 ± 0.74	0.89 ± 0.28	11	5	1	0
8—24 hrs.	21	7.80 ± 0.62	3.91 ± 0.68	3.90 ± 0.50	1.03 ± 0.27	7	5	4	5
> 24 hrs.	39	7.10 ± 0.73	4.10 ± 0.72	2.99 ± 0.49	1.42 ± 0.38	1	1	4	33
Variance quotient (F)		(41.12) ***	$(0.67)^{n.s.}$	(70.13) ***	(21.09) ***				

bilities to reveal hyper γ-globulinaemia in the cow. Earlier, *Liberg* (1973) showed that the same applies to FR. The present study fully confirms the fact that using either method the gelification time is inversely proportional to the globulin content in the sample.

According to Tables 1 and 2, FR gives a somewhat better correlation to the globulin level than GLA. This may, however, be due to the way of constructing time groups. For instance, the first FR group includes more samples than the corresponding GLA group. If the first and second FR groups are put together, the number of samples will be nearly the same as in the first GLA group and the mean globulin value close to the mean value given for this group in Table 1.

In the present investigation the test tubes were not controlled more frequently than once a minute. It seems likely that an improved differentiation is possible for the GLA test by making more frequent readings at the onset. Of the 32 blood samples, which proved positive within 3 min., none had gelled in 1 min., 20 in 2 min. and 12 in 3 min. The mean serum globulin content was higher for those specimens which proved positive after 2 min. than for those positive after 3 min. (5.2 cf. 4.5 g/100 ml). Even if the difference does not reach a significant level, more frequent reading intervals during the first 3 min. can be suggested, eg. every 30 sec. In this context it may be mentioned that differentiation over a greater range than used in the present

series is also possible for FR by more frequent reading during the first 60 min. In fact, such a division within 60 min. is most valuable for differentiation between highly elevated γ-globulin values (*Liberg*).

The increase of globulin in conjunction with positive formol-gel reactions is due almost exclusively to the increase of the γ-globulin fraction. When the GLA test is performed on whole blood the glutaraldehyde has been shown to polymerize mainly γ-globulins and fibrinogen discriminating albumin and α-globulins (*Sandholm* 1974). Discrepancies between the FR and GLA results may partly be due to the fact that they do not measure exactly the same parameters.

Even though FR allows a somewhat greater differentiation and safety than the GLA test, the latter has obvious practical advantages. It can be done on whole blood and with such a short incubation time that the blood globulin status can be established with a sufficient degree of accuracy during the veterinarian's visit to the patient. If a strong hyperglobulinaemia occurs, the result is obtained within 2 min. from blood collection. Although the GLA concentration used has been selected on the basis that blood samples with a normal globulin level do not coagulate at all, the results presented give cause for a certain caution in interpretation of positive tests with longer coagulation times because such can occur in samples having only an insignificantly elevated serum globulin content. Negative results, on the other hand, seem to exclude a significantly elevated globulin content.

When the globulin analysis is done on whole blood, attention should be paid to the red cell content of the blood (fluid balance). However, the PCV in dairy cows is relatively constant, and changes of similar magnitude which occur in the globulin level are unusual. Thus, such calculations have not been considered necessary in the present material.

No attempt has been made to distribute the material according to clinical diagnosis, even though the type, duration and extent of the illness have had a bearing on the γ-globulin content. Cases of acute traumatic peritonitis, ketosis, leucosis or abomasal displacement have generally fallen in the negative GLA and FR groups, and the cases of chronic traumatic peritonitis, chronic polyarthritis or chronic pneumonia have fallen in the most rapidly positive groups. During acute inflammatory processes, e.g. acute traumatic peritonitis, it seems plausible that GLA will

give positive results earlier than FR, because fibrinogen as an acute phase reactant is included in the GLA test when performed on whole blood.

The GLA and FR tests used above have been developed for dairy cows. A simple test for demonstration of hypogammaglobulinaemia in neonatal calves is also required, since the quantity of immunoglobulins absorbed has been showed to have bearing on the susceptibility to infections. Using the above concentrations of GLA or FR most calves having inflammatory diseases show negative results which can be explained by the fact that the serum globulin content is so low that, even if elevated, the amount does not reach the level of normal adult cattle. This also means that FR has a very limited diagnostic value in calf diseases (*Jönsson & Liberg* 1974), as the formalin reagent cannot be obtained in a more concentrated form. On the other hand, it is possible to use more concentrated GLA solutions for calves. Such a reagent is under development at the moment.

REFERENCES

Doumas, B. T., W. A. Watson & H. G. Biggs: Albumin standards and the measurement of serum albumin with bromcresol green. Clin. chim. Acta 1971, *31*, 87—96.

Jönsson, G. & P. Liberg: Liver abscesses in intensively fed cattle. Serial investigations of serum proteins. Acta vet. scand. 1974, *15*, 264—273.

Liberg, P.: The formol-gel reaction in cattle. Acta vet. scand. 1973, *14*, 712—722.

Sandholm, M.: A preliminary report of a rapid method for the demonstration of abnormal gammaglobulin levels in bovine whole blood. Res. vet. Sci. 1974, *17*, 32—35.

SAMMANFATTNING

Glutaraldehydtest och formaldehydtest för bestämning av globulinstatus i bovint blod.

Så kallade labilitetsreaktioner bygger på att blodets globuliner vid reaktion med t. ex. glutaraldehyd eller formaldehyd omvandlas till gel. Stelningstiden är omvänt proportionell till blodets globulinkoncentration.

I föreliggande arbete jämfördes glutaraldehydtester och formolgelreaktioner, med speciell inriktning på testernas diagnostiska och prognostiska värde vid internmedicinska sjukdomar hos mjölkkor.

Undersökningen bekräftade tidigare resultat. Korrelationen mel-

lan globulinkoncentrationen och gelbildningstid ger för båda testerna mycket goda möjligheter att avslöja hyperglobulinemier.

Testerna visade tämligen likvärdig tillförlitlighet. Även om formolgelreaktionen ger möjlighet till något större semikvantitativ differentiering och säkerhet än glutaraldehydtestet har det sistnämnda uppenbara praktiska fördelar. Det kan användas på helblod och kräver endast kort observationstid. Blodglobulinstatus kan därför fastställas redan vid besöket på gården.

(Received November 18, 1974).

Reprints may be requested from: P. Liberg, The Research Station of the Veterinary Institute, Box 180, S-532 00 Skara, Sweden.