Reliability of some simple methods for assessing the immunoglobulin status of new-born calves

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SUMMARY

Blood sera with a wide range of immunoglobulin concentrations were obtained from closely managed beef calves by sampling pre-suckling, at 2 days, and at some intermediate times. Immune globulin status was determined by a zinc sulphate turbidity test, by refractometer, and by the coagulation times with a glutaraldehyde solution. Values for all three tests correlated very significantly ($P<0.001$) with globulin concentrations obtained with a Technicon Autoanalyser, and can be used with confidence for rapid and satisfactory results where elaborate laboratory facilities are unavailable.

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RÉSUMÉ

DJANG-FORDJOUR, K. T., CAMERON, C., FISHWICK, G. & HEMINGWAY, R. G.: *La fiabilité de quelques méthodes simples pour l' évaluation du statut d'immunoglobine des veaux nouveaux-nés.* Le Sera du sang avec une grande variété des concentrations d' immunoglobine était obtenu des veaux de boucherie gérés de prés par le prélèvement d'échantillons de pre-allaitement à 2 jours et à quelques temps intermédiaires. Les déterminations du statut de globuline immunitaire étaient entreprises par un essai de turbidité de sulfate de zinc, par réfractomètre et par les temps de la coagulation avec une solution de glutaraldehyde. Tous les trois essais donnaient des valeurs qui corrélaient très considérablement ($P<0.001$) avec des concentrations de globulines obtenues avec l'Autoanalyseur de Technicon et pourraient être employées avec confiance pour donner des résultats rapides et satisfaisants là où les équipements de laboratoire élaboré ne sont pas disponibles.

Introduction

The death rate in young calves is frequently unnecessarily high. This may be particularly so in the developing savanna regions of Africa where poor body condition and nutrition of cows contribute to low calf birth weights combined with inadequate production of low-quality colostrum and restricted milk yield. Poor management practices and inadequate attention to detail by stock-keepers contribute to the problem. From early morning until late afternoon, new-born calves may be tethered in kraals whilst the cows walk long distances to obtain restricted amounts of feed and water. The critical, early (under 6 h) intake of the maximum possible colostrum is not properly ensured. Osei *et al.* (1993) have reported in Ghana that 26 per cent of calf deaths under 14 days were due to starvation as cows produced inadequate amounts of milk or would not allow the calves to suckle. Frequently, the milk may be consumed by stock-keepers in lieu of wages. In the Northern Region of Ghana, 60 per cent of calf mortality is due to diarrhoea and respiratory diseases, which indicates failure to consume adequate amounts of colostrum at the appropriate time.

For animal welfare and educational purposes, it would be desirable that simple, rapid tests requiring limited apparatus and reagents and laboratory skills be widely used to determine the immunoglobulin status of the serum of young

calves.

This study describes the statistical evaluation of a range of such tests against an accurate, standard method for determining immunoglobulin.

Materials and methods

Animals

Hereford × Friesian beef cows (n = 16) and heifers (n = 7) in-calf to Charolais and Hereford bulls, respectively, were housed in late pregnancy at Glasgow University Veterinary School. All were in good body condition with healthy udders, and received a fully adequate diet (hay, molassed sugar beet pulp, extracted soya bean meal with minerals and vitamins). The expected mean birth weights and daily liveweight gains of the calves to weaning at about 7 months were 45 and 1.1 kg/day, respectively, for Charolais cross and 35 and 0.8 kg/day for Hereford cross. The cows calved in individual pens. Wherever possible, parturition was supervised and the calves were put to the cows as soon as practicable after birth, and were encouraged to suckle the maximum amount of colostrum immediately after birth. Each cow with her calf remained in the pen for about 7 days before returning to housing in small groups.

Blood samples were obtained from 11 calves immediately after birth, but before suckling and again after 2 days. For a further six calves, blood samples could not be obtained before suckling, as they were born in the middle of the night. Consequently, samples from these were only obtained after 2 days. For the remaining six calves, several (2 - 4) samples were obtained at known intervals from a few hours after birth to 2 days after birth to establish how IgG values altered with time (Fig. 1).

Serum samples

Blood samples were obtained from the calves by puncturing the jugular vein, with separation of serum by centrifugation. Plasma cannot be used for tests that rely on precipitation/turbidity or coagulation to determine immunoglobulin due to interference with fibrinogen, especially when low concentrations are expected (McEwan *et al.*, 1970),

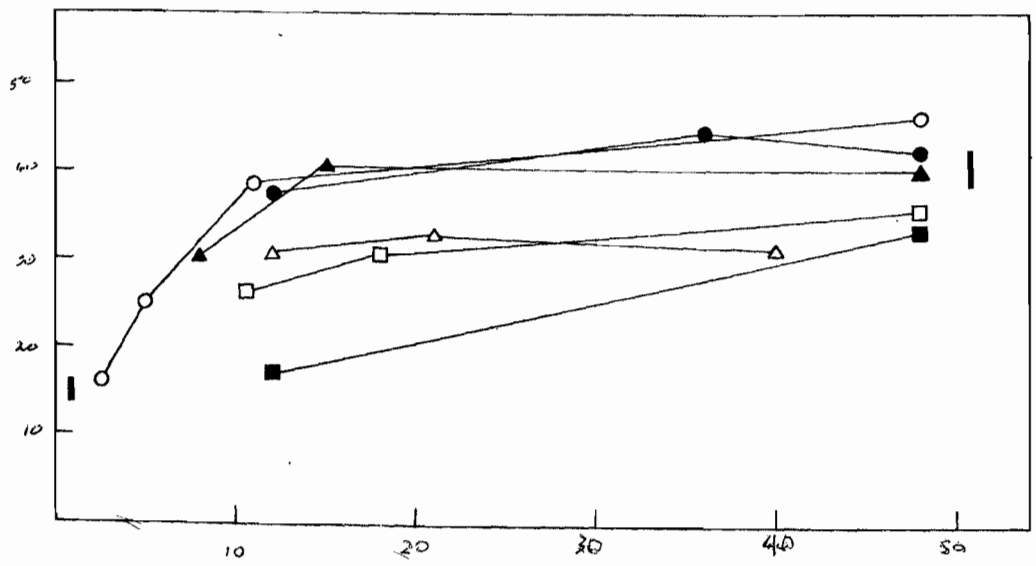

Fig. 1. Serum immunoglobulin concentrations of six calves at intervals from 2.5 to 48 h after birth. Vertical bars indicate mean values (± S E) for 11 calves at both pre-suckling and at 48 h.

Serum samples were frozen for subsequent analysis in batches.

Immunoglobulin determinations

The *standard* method used to determine serum globulin concentration required the use of a Technicon Axon Auto-analyser. Globulin is estimated as the difference between the simultaneous determinations of total serum protein and serum albumin. Such equipment is only found in the largest and best-equipped laboratories.

The zinc sulphate turbidity test (ZST) proposed by McEwan *et al.* (1970) is perhaps the most frequently used *simple* determination, and has the widest supporting literature for calf diseases. Briefly, 0.1 ml serum was added to 6 ml standardized zinc sulphate solution in boiled, distilled water (and which is stored strictly in an aspirator bottle protected with a soda lime tube to prevent carbon dioxide contamination). After shaking, the mixture was allowed to stand for 60 min at 20 °C. After re-shaking, the turbidity was read in a colorimeter. Standing at elevated temperatures can greatly increase ZST values (+ 33 per cent at 25 °C and + 103 per cent at 31 °C (McEwan *et al.*, 1970). To facilitate comparisons with other laboratories, a set of standards prepared by reaction of zinc sulphate with barium chloride solution is required.

The glutaraldehyde coagulation test described by Tennant *et al.* (1979) has received relatively little attention, although good correlations have been obtained in disease situations with young calves between serum coagulation times and immunoglobulin concentrations (Blom, 1982). Essentially, to 0.5- ml serum in a small tube is added 0.05 ml 10 per cent glutaraldehyde solution. The only other apparatus required apart from an accurate pipette is a watch to determine the time (almost immediately to more than 60 min) at which coagulation occurs.

A rapid refractometer test has been described by McBeath, Penhale & Logan (1971) who recorded significant correlations between globulin concentrations in serum with both the direct refractometer reading and the ZST values. Several makes of refractometer are available. That used in this study (Model 20 - 63) was manufactured by Bellingham and Stanley Ltd., North Farm Industrial Estate, Tunbridge Wells, UK TN3 3EY. The refractometer should be set at zero with water at the same temperature as the serum sample. A few drops of serum are placed on the prism and the refractive index is determined directly by reading the scale calibrated as protein percent.

Results

Apart from one cow which required a caesarean section, all the cows calved normally. All the calves suckled promptly and their initial and subsequent growth rates were as expected.

All the serum protein and globulin concentrations are expressed as g l^{-1}, and not as g 100 ml^{-1}. For brevity of presentation, only regression equations, correlation coefficients (r), and probability values (P) are given rather than extensive graphical presentations of data.

Total serum protein, globulin, and albumin concentrations

When assessed by the *standard* Technicon Autoanalyser method, the very significant relationship between immunoglobulin and total serum protein for all 46 samples was Immunoglobulin (g l^{-1}) = 1.07 × protein - 27.0 (r = 0.98, P<0.001). The mean albumin concentration was 22.9 ± 0.42 g l^{-1}. The mean albumin/globulin ratio for the 11 calves sampled before suckling was 1.72 ± 0.05 ; that for the same 11 calves after 2 days was 0.56 ± 0.05. Immunoglobulin concentrations increased rapidly after consumption of colostrum from birth for the six calves sampled at intervals over the first 2 days (Fig.1). Fig. 1 also shows that the mean (± S E) values for the 11 calves from which serum samples were obtained pre-suckling was 14.9 ± 0.62 g l^{-1} and 40.2 ± 2.15 g l^{-1} after 2 days.

Globulin concentration and zinc sulphate turbidity values

The mean ZST value for the sera of the 11 calves obtained pre-suckling was 4.3 ± 1.12. In contrast, the mean value for the same 11 calves obtained at 2 days had increased to 24.1 ± 1.53. For all 46 samples, a significant ($P<0.001$) relationship was calculated: Immunoglobulin (g l^{-1}) = 1.07 × ZST + 14.0, with $r = 0.79$.

Globulin concentration and glutaraldehyde coagulation time

The sera obtained from the 11 calves pre-suckling all had coagulation times greater than 60 min, and were still uncoagulated after 24 h (Fig. 2). In marked contrast, the mean coagulation time for the same calves after 2 days was 2.5 ± 0.66 min. A further 22 sera obtained from the other calves between 8 h and 2 days after suckling had IgG concentrations between 30 and 62 g l^{-1}, and coagulated in less than 8 min. One calf sampled at 2.5 and 5 h after birth had IgG concentrations of 16 and 25 g l^{-1}, respectively, and both had coagulation times greater than 60 min (Fig. 2). Only one of the 46 sera coagulated between 8 and over 60 min. That was after 33 min for a sample obtained 10 h after birth and which had 23 g IgG l^{-1}.

For all 46 sera, a significant ($P<0.001$) negative relationship was established: Immunoglobulin (g l^{-1}) = 42.1 - 0.444 × coagulation time (min), with $r = -0.87$.

Refractometer assessment of protein concentration in serum

The mean serum protein concentration for the 11 samples obtained pre-suckling was 40.3 ± 0.65 g l^{-1}. The corresponding mean concentration after 2 days was 66.4 ± 3.37 g l^{-1}. For all 46 sera, there was a significant ($P<0.001$, $r=0.93$) relationship: Immunoglobulin (g l^{-1}) = 0.922 × refractometer protein (g l^{-1}) -19.5.

Discussion

The mean serum immunoglobulin concentration for 11 calves sampled pre-suckling was 14.9 ± 0.62 g l^{-1} relative to a mean value for all 23 calves after 2 days of 42.4 ± 1.80 g l^{-1}. This compares favourably with a mean value of 14.2 ± 1.25 g l^{-1}

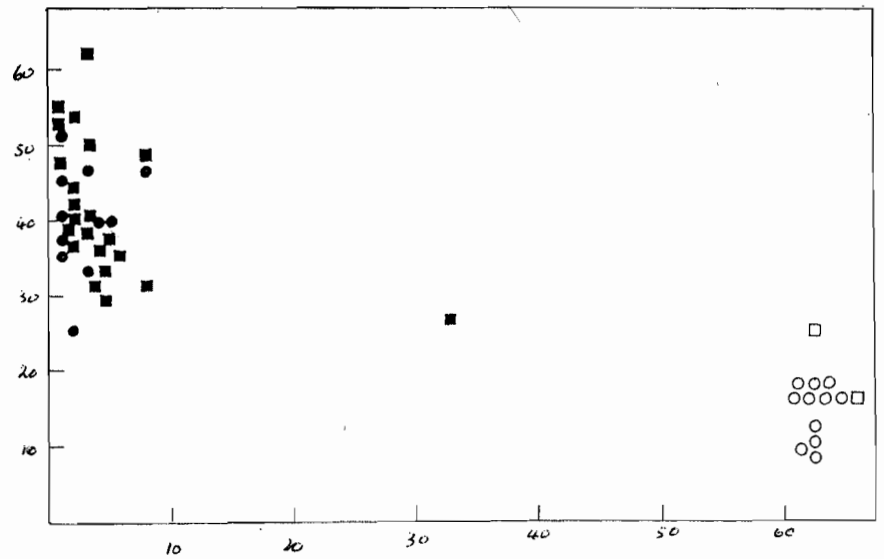

Fig. 2. The relationship between immunoglobulin concentrations in serum (g l^{-1}) and the glutaraldehyde coagulation time (min).
Values for 11 calves sampled pre-suckling (●) and again at 2 days (○); 22 samples obtained from calves between birth and 2 days (□); One calf sampled at 2.5 and 8 h after suckling (■).

for immunoglobulin determined by a radial diffusion technique for 24 calves under one week old born to out-wintered beef cows (McBeath *et al.*, 1971). The mean ZST value for the 23 calves after 2 days was 24.3 ± 0.96 units, whilst that for 11 calves pre-suckling was only 4.3 ± 1.12 units. Petrie Acres & McCartney (1984) recorded a mean value of 29.9 ± 2.7 ZST units for 40 calves born to a herd of beef cows which were closely supervised at calving to encourage early suckling. In contrast, in a less adequately supervised herd, Petrie *et al.* (1984) found a significantly ($P < 0.001$) lower mean ZST value of 19.2 which was more variable (SE 7.5). Five of the 42 calves studied by those workers had serum with below 10 ZST units. Close supervision of beef cows, well fed and in good body condition, results in higher immunoglobulin concentrations in the serum of their calves.

Values for assessment of serum protein by refractometer were well correlated ($r = 0.93$) with immunoglobulin. McBeath *et al.* (1971) similarly found a significant ($P<0.001$) correlation ($r = 0.72$) for 105 calves purchased from markets at 2-3 to 14 days old, which had serum values over the approximate ranges 36 - 72 g protein l^{-1} and 1 - 25 g IgG l^{-1}. McBeath *et al.* (1971) concluded that refractometer assessment of immunoglobulin could be used by the veterinary surgeon in the field. However, they cautioned that, as with ZST deter-minations, refractometer readings are affected by haemoconcentration, and that the test is of little value in assessing the immunological status of moribund or dehydrated calves.

Fig. 2 indicates that 30 out of 31 sera with glutaraldehyde coagulation times less than 10 min had immunoglobulin concentrations greater than 30 g l^{-1}. In contrast, 12 out of 13 sera which had not coagulated after more than 60 min had immunoglobulin values less than 18 g l^{-1}. A similarly divided distribution of serum values obtained at 1 - 7 days of age from 75 Holstein-Friesian dairy calves was recorded by Tennant *et al.* (1979). Whilst the overall immunoglobulin concentrations were lower than those for the beef calves in this study, 16 out of 17 sera with coagulation times greater than 60 min had values below 6 g l^{-1}. In contrast, 50 out of 52 calves with concentrations between 10 and 30 g l^{-1} had glutaraldehyde coagulation times less than 10 min. Only six out of the 75 sera samples had coagulation times between 15 and 45 min. Blom (1982) established a significant negative correlation ($P<0.001$, $r = -0.93$) between immunoglobulin concentration and glutaraldehyde coagulation time for 15 dairy-bred calves. Six of these had coagulation times between 20 and 50 min which allowed a significant linear regression to be established.

The grouping of values for individual results in Fig. 2 and in the similar data presented by Tennant *et al.* (1979) suggests that a linear relationship is inappropriate. Nevertheless, glutaraldehyde coagulation times less than 10 min and greater than 60 min clearly separate two groups reflecting high and low immunoglobulin concentrations, respectively. To that extent, this relatively simple and rapid test has a good diagnostic value in assessments of the status of calves with respiratory and/or enteric diseases as described by Tennant *et al.* (1979) and Blom (1982).

The highly significant ($P<0.001$, $r=0.79$) relationship, Immunoglobulin (g l^{-1}) = 1.07 × ZST + 14.0 for the current sera of beef calves (n = 46), has the same regression coefficient (1.07) as that established by McEwan *et al.* (1970) for 53 dairy-bred calves, relating ZST values with those determined by an immunodiffusion technique. The difference in the constant terms (+14.0 *v* -2.2) in the two equations reflects the much higher immune status of calves born to beef cows which were actively encouraged to suckle immediately from birth, compared with purchased dairy calves where the early management needed may not have been fully adequate. Similarly, McBeath *et al.* (1971) also established a significant correlation ($r = 0.99$, $P< 0.001$) between serum immunoglobulin and ZST values for many calves of differing husbandry backgrounds.

It is concluded that assessments of the immune status of young calves by using the zinc sulphate turbidity test, the refractometer, and a glutaraldehyde coagulation technique give rapid and reliable results across a wide range of values. Nevertheless, to apply these methods in differing and sometimes challenging and adverse husbandry situations, appropriate numerical values for each test indicative of the clinical health status of calves need first to be reliably established with calves of known individual management history, particularly in the amounts and timing of colostrum intake and environmental circumstances.

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