

A Preliminary Study On An Easy And Inexpensive Method For The Detection Of Tuberculosis In Cattle Using The Glutaraldehyde Test

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Throughout the world, particularly in developing countries, the frequency of tuberculosis in live-stock is still very high. The annual economical losses and the human health hazards associated with this disease are impossible to calculate accurately but are generally considered to be immense (Schwabe, 1974).

Except for the lack of experienced laboratory and practising veterinary personnel in many countries one important factor for the relatively slow progress in control programs is no doubt the high cost involved in the necessary survey studies. Particularly due to the latter factor many countries and international organisations as FAO and WHO have to limit the surveys.

With the introduction of the glutaraldehyde and the formol-gel methods for simple semi quantitative determination of immunoglobulins in serum and plasma (BOUVIER, 1936; BENDIXEN, 1954; LIBERG, 1973) and in whole blood (SANDHOLM, 1974) it was logical to test those in a country where the incidence of tuberculosis in cattle was known to be high.

Material and Methods

The study was made on bulls and cows of different breeds and ages. Most observations were done from May to September.

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One of the larger slaughterhouses in Morocco is located in Rabat. Here all slaughtering, except for a few cases takes place twice a week under good veterinary supervision. The animals usually arrive the day before slaughter. Clinical examination was carried out on the day of arrival. In this material no animals with larger skin ulcerations, paratuberculosis, actinomycosis, mastitis, signs of peritonitis or extreme malnutrition were included. However, cows that coughed or on auscultation gave suspicion of pulmonary abnormalities were included in the study even if they were malnourished. Totally 197 animals were subjected to further blood studies and post-mortem examination.

Analyses of blood

Semi-quantitative tests of immunoglobulin on whole blood were made with glutaraldehyde, essentially as described by SANDHOLM (1974). The glutaraldehyde solution was prepared every day of analysis and contained 1.25% glutaraldehyde in 0.9% NaCl. Equal parts of this solution (2.5 ml) and blood with EDTA as anticoagulant were mixed in cylindrical nylon tubes (50x15 mm) with flat base and screw caps. Coagulation was observed every 30 sec. for the first minutes and after that every minute.

Semi quantitative estimation of immunoglobulin was also made with the formol-gel reaction as described by LIBERG (1973) but performed on plasma and with the use of glass tubes (80x6 mm).

As a third semi-quantitative method for immunoglobulins, the turbidometric method, modified and described by McEWAN et al. (1970) was utilised on plasma. As in their method 0.1 ml was used, but in the present study added to 20 ml of the zinc sulphate solution and to 20 ml of distilled water for the blanks. Readings were made on a turbidometer (H.F. Instruments Ltd., Botton, Canada) at 0.10000 Nephelometric Turbidity Units (NTU).

Plasma protein was determined with a refractometer (American Optical, Buffalo, N. Y.). Fibrinogen was calculated according to SCHALM et al. (1975). Thus, plasma was incubated in sealed haematocrit tubes at 57°C. After centrifugation the supernatant was transferred to the refractometer again and the "true" protein read. By subtracting this value from the original plasma protein the amount of fibrinogen was obtained.

Brucellosis was diagnosed by agglutination test (FAO/WHO, 1964; MORGAN, 1967). Dilutions of 1/40 or more were considered as positive when expressed as + + + +.

Specimens from macroscopically affected lymph nodes, pulmonary tissue and liver were collected for histopathological examination. When tuberculosis was suspected the tissues were treated as described by AMAODOUF (1976). The different stages of tuberculosis were classified according to the scheme made by FLACHAT and FAURE (1975).

Results

Macroscopic and histopathological examinations indicated that totally 197 animals included in this study into the scheme of one of the different stages of tuberculosis (FLACHAT and FAURE, 1975). Of those, 27 cases showed tuberculosis as the only post-mortem finding, serum brucellosis agglutination included (Figure 1). In the other cases the tuberculosis was accompanied by brucellosis or hydatid cysts, or by the latter to together.

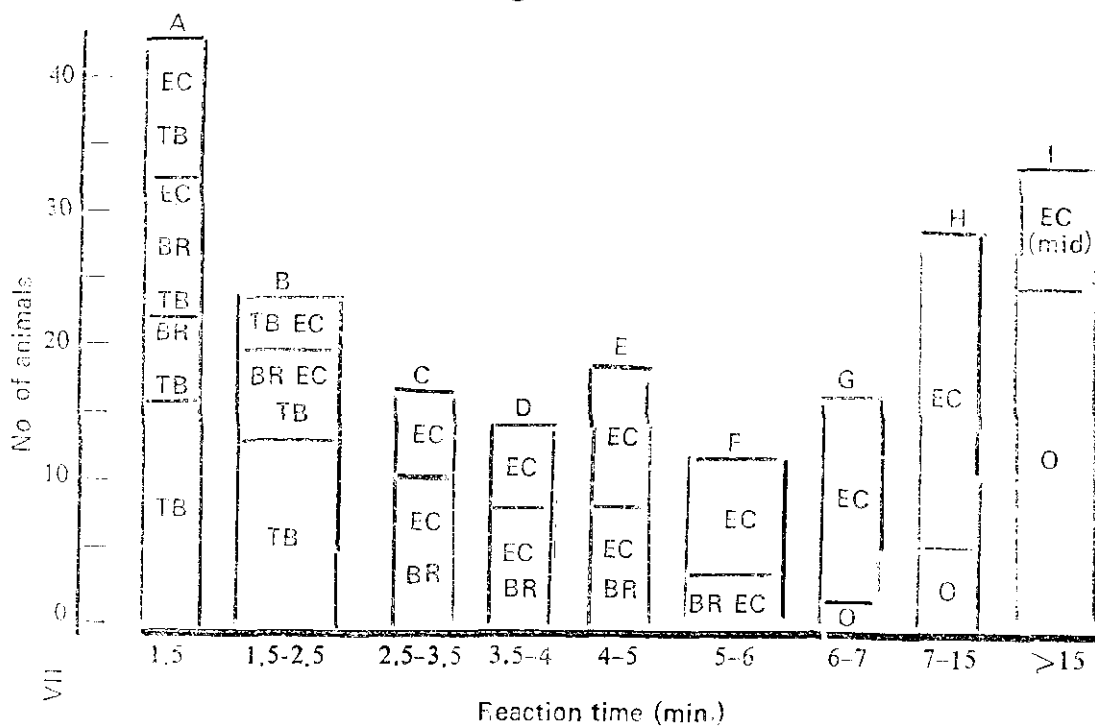
Brucellosis was found in 52 cows, always associated with tuberculosis and/or hydatid cysts (Figure 1).

Hydatid cysts of different size and distribution were found in 168 animals (84%), in some cases as the only post-mortem finding (Figure 1).

Glutaraldehyde test

The results from this test, where the gelatination time of the blood is divided in different groups (Figure 1, A-I), indicate that in all cases with a 'coagulation' time less than 2.5 minutes (A & B) tuberculosis could be detected at the post-mortem examination. In no single case any evidence could be found of tuberculosis, when the reaction time was more than 2.5 minutes. In 63% of the animals with tuberculosis the blood coagulated with glutaraldehyde in less than 1.5 minutes (Figure 1).

Figure- 1



At post-mortem examination it was found that in the large majority of the cases (90%) the tuberculosis involved the respiratory organs. Brucellosis was not found without concomitant tuberculosis and/or hydatid cysts (Figure 1).

In some cases the animals in the present material could be traced back to the owners. It was then found that some of the animals were brought to the slaughterhouse because of positive tuberculin reaction (only bovine tuberculin had been used). Some other animals were slaughtered because of positive brucellosis test. The number of animals in those cases and the post-mortem findings in relation to the results of the slaughterhouse test (the group classification is given in Figure 1) may be seen from Table I. The glutaraldehyde test shows that of 16 animals with positive tuberculin reaction only 12 were tuberculous at post-mortem examination. In 6 animals brought to the slaughterhouse because of positive brucellosis agglutination test, retesting verified brucellosis and the autopsy also showed one case of additional tuberculosis (Table I, B). In the five other cases of brucellosis hydatid cysts were also present.

Table- I

Classification of animals after the groups given in Figure 1, with positive tuberculin reaction and/or positive brucellosis test.

Group	A	B	C	D	E	F	G	H	I
No. of positive tuberculin reactions	7	5		2				2	
No. of positive brucellosis tests		1	4	1					

Reaction time in minutes of the glutaraldehyde test divided after reaction times in 9 groups, A-I, with the number of animals given in each group with post-mortem and serological findings.

TB=tuberculosis; BR=brucellosis; EC=echinococcus cysts

Formol-gel test

In Table II the results of formol-gel tests are given according to the grouping after the glutaraldehyde reaction times seen in Figure 1. It may be seen that there is a fairly good correlation with the different groups, particularly with A and B, where also the tuberculous cases were found. In the other groups there is a tendency of over-lapping which makes it difficult to draw any conclusions.

Turbidometry

Table II also shows the results of the turbidometric measurements for the different groups. Due to the wide ranges in NT-units in those no conclusions can be drawn.

Plasma protein

The values for the plasma protein do not indicate any difference between the different groups (Table II).

Fibrinogen

When fibrinogen is expressed in absolute terms (g/l) or as a ratio between free plasma and fibrinogen, one may find a tendency for higher fibrinogen values along the groups, being highest in A and B (Table II). Due to the number of animals in the different groups there are no statistical differences.

Table- II

Certain blood parameters grouped after the glutaraldehyde reaction times given in Figure I.

Groups	A	B	C	D	E	F	G	H	I	
Formol-gel reaction time (min.)	0.6-1.0	0.5-2.0	3-5	3-30	4-420	60-360	too long reaction time			Range
ZST (NTU)	192	190	189	185	163	161	125	92	93	Mean
Plasma protein (g/100 ml)	8.3	8.4	8.1	8.1	7.8	7.7	7.6	7.4	7.5	Mean
Fibrinogen (g/l)	18	19	16	14	14	12	10	10	7	Mean
PP/F	3.9	3.4	4.1	5.2	4.8	5.4	6.8	6.8	10.0	Mean

Discussion

The study at the slaughterhouse in Rabat seems to indicate that the simple and inexpensive glutaraldehyde method is accurate enough for making a tentative diagnosis of tuberculosis, as gelatinisation in the majority of cases with this disease takes place in less than $1\frac{1}{2}$ min. In the remaining 37% of the cases with tuberculosis the reaction time was between $1\frac{1}{2}$ and $2\frac{1}{2}$ minutes. Provided a clinical examination is included, it seems as the glutaraldehyde method under the experimental conditions in this study is more accurate than the conventional comparative tuberculin test. In the latter test various factors have been found to interfere (PATERSON et al., 1958;

FAO/WHO, 1967; ALHAJI, 1976; HALL, 1977). The uncertainty with the evaluation of the tuberculin test was also found in a study done in Malawi at two slaughterhouses. (BERGGREN, 1978). The basic conditions of that study and the one reported here were rather much alike. One slaughterhouse in the Malawi study received animals from a region with known low incidence of tuberculosis. In 185 animals where no tuberculosis was found at post-mortem examination, the tuberculin tests indicated positive reactions in about 2% of the cases, interpreted as allergic reactions caused by atypical mycobacteria. At the other slaughterhouse the animals were collected from a district obviously similar to that in the present study, with known high incidence of tuberculosis. In 186 cattle, 59 animals (32%) were found to be infected with tuberculosis at post-mortem examination. It is, however, of interest to note that the tuberculin test with bovine and avian tuberculin only gave positive reaction in 63% of the verified cases, while the corresponding figures with mammalian and avian tuberculin gave positive reaction in 83%. On the other hand it was shown that this test indicated a high number of positive reactions (2.2% positive and 2.7% suspicious) where no visible tuberculosis was found at bovine-avian tuberculin testing, whereas the corresponding numbers when mammalian-avian tuberculin was used, gave positive results in 4.8% and 11.3%, respectively. Judged from the reactions of the present study and that referred above (BERGGREN, 1978) it seems at the tuberculin test in cattle from areas with high incidence of tuberculosis is not an ideal method in field survey programs. This is particularly true when the economical aspects are taken into account. In the "Rabat" material clinical examination of the animals was done, but tuberculin testing should also be done. As mentioned before, and seen from Table I and II, and Figure 1, the glutaraldehyde test gave surprisingly reliable results. Table I gives the classification of cattle with positive bovine tuberculin reaction. In only 12 out of 16 animals which were positive, tuberculosis could be confirmed at post-mortem examination. Although the material is very small, it confirms the uncertainty of the conventional tuberculin tests, as shown by BERGGREN (1978), and supports the other finding in the present study.

The tubercular lesions in the "Rabat" material were predominantly found in the respiratory organs and thorax, which is in agreement with earlier studies in Morocco (AMAQDOUF, 1976) and in other countries (STAMP and WIGSON, 1946; ALHAJI, 1976; HALL, 1977; BERGGREN, 1978).

It should also be pointed out that the present results have been obtained under conditions prevailing in Morocco, and on animals arriving largely from the northern parts of the country. The frequency of tuberculosis in cattle from this area is lowest during the period of study, May-August (AMAQDOUF, 1976).

From Figure 1 it may be seen that the shortest reaction times between blood and glutaraldehyde were always associated with the post-mortem findings of tuberculosis, either as only finding or associated brucellosis and/or hydatid cysts. As brucellosis is very common in Morocco (DAKKAK, 1973), although no reliable new

data exist for this region of the country, as well as hydatid cysts, in cattle, about 14% (Abattoirs municipaux de Rabat, 1979), it is not surprising that the tuberculous animals often are affected with one or both of these disease. It seems, however, that the short glutaraldehyde reaction times in groups A and B are determined by tuberculosis.

The major cause of tuberculosis in cattle is *Mycobacterium bovis* (SCAWABE, 1974) but it is well known that cattle may become infected by man (FAO/WHO, 1967). It has been suggested that dilute glutaraldehyde solutions form intramolecular bridges with albumin and intermolecular bridges with globulin and fibrinogen (SANDHOLM, 1974). In low concentrations glutaraldehyde has been found to polymerize the most basic blood proteins. It is therefore assumed that the glutaraldehyde method is relatively specific for gammaglobulin in bovine blood (SANDHOLM, 1974), but can also be used in sheep blood (CABERNET and LARSSON, 1980). The formol-gel test supposedly gives rise to the same reaction with serum as does glutaraldehyde (LIBERG, 1973; LIBERG et al., 1975). In the present study with narrow test tubes used for the formol reaction the solidifying time was very short in cattle with tuberculosis and no doubt related to this disease, while for the other groups the reaction times overlapped. In earlier studies the formol-gel reaction was made on serum (LIBERG et al., 1975), but in this study plasma was used in order to include a possible portion of fibrinogen in the reaction (SANDHOLM, 1974). Plasma was also used in the zinc-sulphate test. The plasma fibrinogen level in cattle is influenced by many physiological factors, and disease have a marked influence on the circulating fibrinogen level (SCHALM, 1970; McSHERRY et al., 1970). Vaccination against *Brucella abortus* can also temporarily elevate the plasma fibrinogen level (SCHALM et al., 1975). Generally, high plasma fibrinogen values are considered to be more indicative of the severity of an infectious disease process in cattle than the total leucocyte count during the initial stages of acute diseases and in many chronic inflammatory diseases (SCHALM et al., 1965). As haemoconcentration produces a relative elevation of fibrinogen, the plasma protein/fibrinogen (PP/F) ratio is considered more relevant in interpretation of disease in the presence of dehydration (SCHALM et al., 1975). The ratio is obtained by subtracting the fibrinogen from the total plasma protein concentration and then dividing the remainder by the fibrinogen figure. The absolute values of fibrinogen and the PP/F ratios in this study are so pronounced in the various groups that at present no conclusions can be drawn, even if the ratios seem to follow the results of the glutaraldehyde test.

The reason for the fast gel formation in bovine tuberculosis can not be explained by the present results without direct measurements of the circulating immunoglobulins, fibrinogen and perhaps are substances elevated at the same time. It is well known that *Mycobacterium tuberculosis* in man produces a complex spectrum of immune responses. The cellular mechanisms involved in the immunopathogenesis in human tuberculosis have been reviewed recently in an excellent way (SETHI and SINGH, 1987). In cattle it has been shown that abscesses and necroses give short reaction times between glutaraldehyde and blood, as well as of the formol-gel test (LIBERG,

1982). In view of what is known about the process of granuloma formation in tuberculosis where macrophage derived mediators might play an important role together with B-lymphocytic antibody production those factors could be responsible for the very short glutaraldehyde reaction time (SETH and SINGH, 1987).

Summary

A study from a slaughterhouse material indicates that the glutaraldehyde test seemingly can be used as a screening method for tuberculosis in cattle. The method is fast and inexpensive, and does not require revisiting the animals if they do not react to the test.

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