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A Comparative Study of the use of Dried Blood on Filter Papers and Serum Samples for Serodiagnosis of Anaplasmosis

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Abstract

A study was conducted to compare the suitability of blood collected on filter papers with corresponding fluid serum samples in serodiagnosis of bovine anaplasmosis using DOT-ELISA, RCAT and Western imunoblotting. Serum was obtained from blood collected by jugular venipuncture of 288 heads of cattle and stored at -70 °C. About 0.2 ml of the corresponding blood sample was applied and dried on filter paper (Whatman No.1) after collection in duplicates. One of the duplicates was stirred at room temperature and the other at 4°C. Subsequently, sera were eluted from the dried blood samples on the filter paper with a solution of 1.8 ml PBS Tween 20% at pH of 7.2 to give a dilution of 1:100. Antigens were prepared as described by Montenegro-James *et al* (1988) and analysed by DOT-ELISA and Western immunoblot at dilutions ranging from 1:25 to 1:200. Results showed that dried blood samples on filter paper were suitable for sero-epidemiological studies of bovine anaplasmosis and storage at room temperature for 6 months did not affect reactivity.

Key words : Dried blood, serodiagnosis, anaplasmosis, filter papers.

Introduction

Bovine anaplasmosis caused by *Anaplasma marginale* is a worldwide tick-borne disease affecting cattle, small ruminants and some wild ruminants such as deer and buffalo. The disease is of great economic importance in tropical and subtropical regions of the world because of losses through mortality, reduced milk production, and poor weight gain especially in the newly introduced highly susceptible exotic dairy cattle.

Currently available diagnostic methods include parasitological examination of blood smears and serological methods such as the Capillary Agglutination Test (CAT) (Ristic, 1962), Rapid Card Agglutination Test (RCAT) (Amerault and Roby, 1976), complement Fixation Test (CFT), Indirect Fluorescent Antibody Test (IFAT), conventional ELISA and more recently DOT-ELISA (Montenegro-James *et al* 1988).

A major problem in the hot tropics is the collection and preservation of biological materials for laboratory diagnosis. These countries lack

cold chain facilities, fast means of transportation, and modern diagnostic laboratories. Consequently, biological samples get spoiled while in transit to the central diagnostic laboratory.

This study was undertaken to evaluate the suitability of blood collected on filter papers in comparison with corresponding fluid serum samples in the serodiagnosis of bovine anaplasmosis using DOT-ELISA, RCAT, and Western immunoblotting.

Materials and Methods

Collection and processing of samples

Ten millilitres of blood were collected from the jugular vein of each of 288 heads of cattle in clean universal bottles. The bottles were left at room temperature for 3-6 hours in order to allow separation of serum. The separated serum was transferred into plastic vials and kept frozen at -70°C until required.

About 0.2 ml of the corresponding blood sample was applied in duplicate on Whatman's filter paper No. 1 immediately after collection from the jugular vein. The filter papers were air-dried and were placed individually in self-sealing plastic bags. One group was stored at room temperature and the other at 4°C.

Elution of serum from dried blood

The samples on the filter papers were cut off the filter paper using a sharp scalpel blade and placed each in a wide mouth plastic centrifuge bottle containing 1.8 ml of PBS 0.05% Tween 20, pH 7.2 to give a dilution of 1:100. The samples were left to elute overnight at room temperature.

Known positive and negative control sera

Sixteen samples of known positive control sera were obtained from cattle experimentally infected with *A. marginale* (Florida strain) and from the naturally infected animals and were parasitologically positive to the infection.

Sixteen samples of negative sera were obtained from cattle known to have been exposed to anaplasmosis and shown to be negative parasitologically and serologically. Filter paper samples were prepared from reference positive and negative sera as described above.

Anaplasma marginale antigens (Florida isolate)

The antigens used in both DOT-ELISA and Western immunoblot were prepared as described by Montenegro-James *et al.* (1988).

All samples were tested in serial dilutions starting at 1:25 through 1:200.

Results

The reactivity in both DOT-ELISA and Western immunoblotting was similar to those obtained with the sera diluted 1:100. Filter paper samples gave lower reactivity in the Rapid Card Agglutination test when compared with the corresponding serum samples.

In the DOT-ELISA and Western immunoblotting, there was 100% agreement in the titres between the eluates from filter papers stored at room temperature and those stored at 4°C. Room temperature storage conditions did not significantly affect reactivity. Eluates from filter papers stored for six months at room temperature continued to give similar reactivity as those from freshly prepared filter papers in both DOT-ELISA and Western blot and in the Rapid Card Agglutination test. Similar polypeptide profiles were demonstrated using conventional sera and filter paper eluates.

Discussion

These data support the suitability of collecting blood samples on filter paper for sero-epidemiological studies of bovine anaplasmosis. There was 100% agreement between the serological results from filter paper samples and those from serum samples in all the tests used. Storage at 4°C and at room temperature for over six months did not affect the reactivity.

Dried blood samples on filter papers have also been used successfully in studies of Eastern Equine Encephalomyelitis (Karstad *et al*, 1957) and Newcastle diseases (Beard and Brugh Jr., 1977), and the technique was also highly recommended and found suitable for ELISA test in the diagnosis of a number of poultry disease (Synder, 1985). This technique offers a number of advantages : (i) only minimum amount of equipment is necessary, (ii) no special technical training is required, (iii) safety and ease of transportation and storage, (iv) long shelf-life (up to six months), and (v) adaptability to small animal blood sampling.

The suitability of blood collected on filter papers in comparison with corresponding fluid serum samples in the diagnosis of bovine anaplasmosis was studied using DOT-ELISA, Western immunoblot and RCAT. Dried blood on Whatman filter paper No.1 was eluted in 1.8 ml of PBS 0.05% Tween 20 given an initial dilution of 1:100. The reactivity in both DOT-ELISA and Western immunoblotting were similar to those obtained with the sera diluted 1:100. Filter paper samples gave lower reactivity in the RCAT when compared with corresponding serum samples. There was no significant difference in the reactivity between the eluates from filter papers stored at room temperature and those stored at 4°C. Storage at room temperature did not even significantly affect reactivity. Eluates from filter papers stored for six months at room temperature continued to give similar reactivity as those from freshly prepared filter papers in both DOT-ELISA and Western blot, and in the RCAT.

It is concluded that collecting blood on filter papers is a suitable technique for large scale screening and for sero-epidemiological studies and offers a lot of advantages especially in developing countries where transport and cold chain facilities are a major constraint.

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