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A survey on human brucellesis "Malta fever" in Iran (Serelogical and bacteriological investigations)

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SUMMARY

A study on human brucellosis in Iran was carried out from 1976 to 1985 at the Razi Institute. A total of 4975 sera and 568 blood cultures have been examined, of which 1497 cases showed laboratory evidence of Brucella infection. From 121 blood cultures, Brucella melitensis biotype 1 was isolated. The comparison of results obtained from this study, showed a trend of increase in the prevelance of the disease in the human in recent years. Since the disease is a zoonosis, efforts in minimising the incidence of the infection in animals would result in the control of human brucellosis.

Introduction

Brucellosis is still one of the major infectious, chronic and zoonotic diseases in many countries of the world, where it is caused by various species of Brucella organisms. The disease is mainly characterised by causing undulant fever in the human and abortion in animals, so it is sanitarilly and economically important.

The disease may be considered as a disease of domestic animals, which infects human beings through consumption of unpasteurised milk and other dairy products, such as fresh cheese, cream or by contact with infected materials. In Iran, cattle, sheep and goats are the principal farm animals and with the prevalence of the disease in such animals, human brucellesis is naturally prevalent too. Because of the lack of the necessary sanitary and hygienic measures the disease is prevalent in both rural and urban areas of the country. Epidemiological studies in areas where the disease is prevalent have reveald that in rural areas both the direct and the indirect transmission is the potential sources of human infection, while in cities the consumption of unpasteursed dairy products is responsible for the infection (Sabbaghian 1975).

The past reports have presented the general scheme of the epidemiology of the human brucellesis (Keyhani and Entessar 1969, Nicoletti and Amini 1971, Feiz, Sohrabi and Sabbaghian 1972, Sabbaghian, Ghiassedin and Abolhassani 1973, Sabbaghian 1974, Sabbaghian and Ndadim 1974, Sabbaghian 1975).

This report presents the serological and bacteriological studies of the suspected human cases referred to this laboratory for diagnosis.

MATERIALS and METHODS

Sera samples

Between 1976 and 1985 a total of 4975 samples were received from hospitals and private practitioners of Tehran and some other provinces. Serum samples were stored at 4°C. without addition of preservatives and were tested on the same day or within two days after collection.

Antigens

The Rose Bangal Plate Test (R.B.P.T.) and Tube Test antigens were prepared and standardized by using either Brucella abortus strain 19 or strain 99 according to method recommended by W.H.O. (Alton et al 1975).

Serological tests

The R.B.P.T. was accomplished by the standard procedure described by Alton et al (1975) and Brinely Morgan (1981). The technic consists of mixing 0.03 ml of the serum with 0.03 ml of antigen, the plate is slowly rotated and the results are recorded after 4 minutes.

In the serum agglutination test (S.A.T), serial dilutions of serum, begining with 1:10 dilution, were made in %5 CLNa phenol saline (in order to minimize the prozon phenomenon) in 0.5 ml volumes. Then 0.5 ml of the antigen was added to the dilutions. The results were recorded after 24 hours of incubation at 37°C. (Alton et al 1975, Brinely Morgan et al 1981).

The complement fixation test (C.F.T.) was performed according to the cold fixation method: serial dilutions of serum begining with 1:5 in veronal buffer in 0.5 ml volumes were distributed in tubes and were inactivated at 60°C. for 30 minu-

tes. After cooling to the room temperature 0.5 ml of antigen and 1 ml of complement were added. The tubes were stored at 4°C. water bath, an aliquot of 0.5 ml of a %2 suspension of sheep red blood cells sensitised with 0.5 ml haemolysin was added to the tubes and again the tubes were incubated at 37°C. for 30 minutes. The degree of haemolysis was then determined in each tube (Alton et al 1975, Brimely Morgan et al 1981).

The disulphide band reduction (mercaptoethanol) test (M.T) is an agglutination test carried out in the presence of 2-mercaptoethanol, which inactivates IgM molecules present in the serum under test, thus the test may be looked upon as an indicator of the amount, if any, of anti-Brucella IgG agglutimin present in the serum. This test is performed by making serial dilutions of serum in 0.1 mol/litre 2-mereapteethanol in saline and adding standard tube test antigen. The tubes were incubated at 37°C, for 24 hours and were recorded in the usual manner for serum agglutination (Alton et al 1975, Brinely Morgan et al 1981).

Serological test interpretations

R.B.P.T-The R.B.P. tests are read as positive with any degree of agglutination and negative when agglutination is negative.

S.A.T-The titre of 40 IU/ml or above was censidered as positive, taking into account the other serological tests as well.

C.F.T-The titre of 1/20 or above was taken as positive.

M.T-It is advisable to interpret the titres obtained in the mercaptoethanol test alongside those of the S.A.T. It may be assumed that the ordinary agglutination titre indicates the total amount of agglutinins present, the mercaptoethanol test show the amount of IgG agglutinin, and difference between these two titres gives the proportion of IgM agglutinin present in terms of agglutinating activity. IgG is generally associated with the preence of infection and any positive titre in the M.T just described, should be regarded as indicating infection.

Blood culture

For this, standardised Brucella broth medium is to be used. The blood sample, approximately 7-10 ml, is transferred asepti-

cally to a Brucella broth medium bottle in 50-70 ml volume. If blood is not mixed with anticoagulent, it must be transferred just after bleeding, to broth medium and gently mixed with the broth. After inoculation, the bottles are incubated at 37°C.. When there is any possibility of the blood containing Brucella abortus, an incubator with 10% carbon dioxide should be used. Three days later a subculture is made in Brucella agar and then this is repeated daily, keeping cultures in the incubator. Slopes are examined 3 to 4 days later for Brucella colonies. The appearance of colonies from Brucella abortus cases is often delayed to cultures from liquid medium on day 35 to day 50 (Alton et al 1975).

Any colonies resembling those of Brucella that appear on the solid medium slopes should be subcultured for further examinations.

The last subcultures, after being checked for purity and agglutinability with monospecifie anti Brucella abortus, anti Bruella melitensis sera and Brucella negative serum, are also emulsified in sterile normal saline and acriflavine buffer, and biotyped by biotype classification, using the technic recommended by W.H.O (Alton et al 1975, Corbel et al 1978).

In our study 568 blood specimens were cultured for detection of Brucella organisms.

Results

The results of serological and bacteriological examinations on human cases are summarised in Table 1. Out of 4975 suspected cases, 1497 samples (30%) showed evidence of Brucella infection. When samples were positive in R.B.P.T and S.A.T they were checked by C.F. and M.tests as well.

Brucella strains were isolated from 121 out of 568 patients showing fever, joints pain and other clinical symptoms. It must be noted that serological tests were positive in nearly all, with a few exceptions, those patients from whom Brucella organisms were isolated. All the organisms isolated so far were Brucella melitensis biotype 1, which is normally found in domestic animals and in dairy products. Other biotypes have not so far been found. With a view to the seasonal distribution, the cases occurred throughout the year, especially in spring and summer, which

corresponds to the sheep and goats parturitions and large scale production of dairy products.

Discussion

Brucellosis is a disease of public health and economical significance in Iran. The consumption of unpasteurized milk products, such as fresh white cheese, cream, ice-cream ect. are the important source of the human infection.

The results obtained from this investigation suggest an increasing trend in the prevalence of brucellosis in the human in Iran, as evidenced by the comparison of the results of the recent years with those of the past. It is due to the fact that, the extension of animal brucellosis may not only be the source of infection to other animals but to human beings, as well.

In the present study, 1497 of 4975 human cases (Table 1), being mostly in indirect contact with infected animals, were found positive for brucellosis by serological and bacteriological tests. They apparently contracted the infection by eating the unpasteurized dairy products.

Therefore, since brucelosis is a zoonotic disease, efforts made in minimising the incidence of this disease in animals would result in controlling and reducing human infection. And this necessiates the need for involvement of public health authorities and Veterinary Organisation in the control of the disease both in man and animals.

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Table 1-The results of serological and bacteriological studies on human bloods from 1976-1985

Year	No. of sera tested	No. of positive reaction	No. of blood culture	No. of Brucella positive
1976	7	1	—	—
1977	21	11	—	—
1978	14	1	—	—
1979	17	5	3	1
1980	232	59	5	2
1981	176	137	26	3
1982	567	393	100	19
1983	669	290	88	18
1984	2011	314	115	27
1985	1261	286	231	51
Total	4975	1497	568	121

References

Alton, G.G., Jones, L.M. and Pietz, D.E: (1975)-W.H.O Monograph series No: 55, 2nd edn.

Brinley Morgan, W.J., Mackinnon, D.J., Gill, K.P.W., Gower, S.G.M. and Miss Norris, P.I.W. (1981)-Cent. Vet. Lab. New Haw Weybridge 2nd edn. Booklet 2084 RVC 21.

Feiz, J., Sohrabi, F. and Sabbaghian, H. (1972)-Shiraz Med. J. 3, 447

Keyhani, M. and Entessar, F. (1989)-Arch. Inst. Razi 21, 97.

Nicolletti, P. and Amini, H. (1971)-Canadian J. Publ. Hlth. 62, 320.

Sabbaghian, H. (1974)-Iranian J. Publ. Hlth. 2, 103.

Sabbaghian, H. (1975)-Publ. Hlth. Lond. 84, 165.

Sabbaghian, H. Ghiasseddin, M. and Abolhassani, M. (1973)-Iranian J. Publ. Hlth. 1, 239.

Sabbaghian, H. and Nadim, A. (1974)-J. Hyg. Camb. 73.