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EPIDEMIOLOGICAL STUDIES ON HUMAN BRUCELLOSIS IN IRAN AND THE IDENTIFICTION BY BACTERIOPHAGE

by:

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Introduction

Epidemiological studies on human brucellosis in Iran have been carried out at Razi Institute. A survey of brucellosis shows that certain areas of the country are seriously affected with a high incidence of Brucella infection. These areas are Isfahan, Tehran and Khorassan province. The highest incidence of human brucellosis in Iran appears to be in Isfahan. According to Sotoodeh's thesis (1960), There were 883 cases in 1959. The population in Isfahan was reported to be 240,000 with a total of 1,600,000 in the entire province. The incidence may therefore be more than 1 per 10,000. Most of the cases occured in the Spring and Summer and about 25 per cent were under the age of 15. It has been suggested that the main source of infection was from cheese and ice cream which are made from unpasteurized milk of sheep and goats. The disease exists in some other areas of the country although not many reports have been received.

The present paper deals with epidemiological studies on human brucellosis in Iran. Brucella strains isolated from blood culture of human cases in different provinces of Iran were typed by the standard biochemical and serological methods. These strains were also studied from the point of view of geographic origin, host origin and phage typing.

Materials and Methods

The culture of Brucella were isolated from blood culture of human cases of brucellosis from different areas of Iran. A numerical listing of the cultures and their geographical and host origin are included in the table of data in the section on results. Each culture has been isolated from a seperate host. None of the cultures represent repeated isolations from the same individuals. The standard biochemical and serological methods recommended by the World Health Organization (1953, 1958) were followed for species differentation. Glycerine dextrose agar was used throughout the study. All cultures were tested in the presence of two concentrations of basic fuchsin and thionine (1:50,000 and 1:100,000).

The Brucella phage was propagated for phage typing by double-agar-layer technique. The propagating culture *Br. abortus* strain 19 was grown on glycerine dextrose agar for 24 hours and the growth was suspended in peptone water and

adjusted so to contain 10^{10} cells per ml. The semi solid agar was melted and cooled to 44° C. in water bath and 0.25 ml of the bacterial suspension and bacteriophage was added. The inoculted semi solid agar was well mixed and poored over a plate containing glycerine dextrose agar and incubated for 24 hours at 37° C. The amount of phage necessary to cause nearly complete lysis was titrated in a preliminary test before making the main batch of phage. The phage was harvested in peptone water and left for 48 hours at 4° C.

The bacterial lysate was centrifuged at 3000 RPM for 15 minutes to deposit the agar and the cells. The supernatant was then filtered through a millipore filter (type HA, 0.45 micron pore size). The assessment of susceptiblity to lysis by Brucella bacteriophage was performed by phage typing method (Adams, 1959; Keyhani and Entessar, 1968; Keyhani, 1968) using Brucella bacteriophage type abortus, strain 3. The RTD dilution was determined by making serial ten fold dilution of phage in peptone water. Glycerine-dextrose-agar plates were inoculated with suspention of a 24 hours growth of *Br. abortus* S. 19 and allowed to dry for 2 hours at 37° C. One drop of each phage dilution was placed on the inoculated plates. The plates were incubated for 48 hours at 37° C. and then the results were read. The RTD dilution was determined as the highest dilution that causes complete lysis of the culture. For phage typing of Brucella strains one tenth of ml of a suspention of a 24 hours growth of Brucella in peptone water containing 10^9 organisms per ml was inoculated on glycerine-dextrose-agar. A spreader was used to distribute this suspention evently over the surface of the medium. One drop of phage dilution at RTD and 10,000 X RTD was spoted on the dried bacterial mat by means of a fine pasteur pipette. After inoculation has dried, the petri dishes were incubated at 37° C, and the results of phage typing was read after 36 hours.

Results

Studies on human Brucella infection in Iran indicate that the practice of eating fresh cheese and ice cream made from unpasteurized sheep and goats milk contribute greatly to the high incidence of Brucella infection in the months following the lambing season. The experiment on viability of *Br. melitensis* in cheese made from unpasteurized milk has shown that *Br. melitensis* remained viable up to 10 weeks in salted cheese stored at 4° C. However, the vialibity of *Br. melitensis* in unsulted cheese was much longer than 10 weeks. The distribution of Brucella strains isolated from man and also the results of identification by bacteriophage, biochemical and serological methods are presented in table I.

As it has been demonstrated in table I, all of the bacteriologically proved cases of brucellosis in Iran were due to *Br. melitensis*. This is in contrast to the reports (Spink, 1959) in other countries like the U.S.A, Puerto Rico, England and Scotland in which most cases of brucellosis are due to *Br. abortus* and *Br. suis*. This difference in the type of infection in Iran is most probably due to the fact that in Iran sheep and goats and its milk products are used most

Table 1

The distribution and identification of Brucella strains isolated from human cases in Iran

Description of strains	Origin of cultures		Co2 requirement	H2S Production	Growth on 1/100,000:a		Agglutination with monospecific sera		Lysis by phage
	Hosts	Provinces			Basic fuchsin	Thionine	A	M	
Br. melitensis strains									
76	Human	Mashad	-	x	x (x)	x (x)	-	x	-
163	"	Esfahan	-	-	x (x)	x (x)	-	x	-
168	"	Tehran	-	-	x (x)	x (x)	-	x	-
171	"	Mashad	-	x	x (x)	x (x)	-	x	-
173	"	Mashad	-	x	x (x)	x (x)	-	x	-
190	"	Hessarak	-	-	x (x)	x (x)	-	x	-
417	"	Mashad	-	-	x (x)	x (x)	-	x	-
436	"	Mashad	-	-	x (x)	x (x)	-	x	-
537	"	Mashad	-	-	x (x)	x (x)	-	x	-
594	"	Hessarak	-	-	x (x)	x (x)	-	x	-
615	"	Mashad	-	x	x (x)	x (x)	-	x	-
616	"	Mashad	-	-	x (x)	x (x)	-	x	-
617	"	Mashad	-	-	x (x)	x (x)	-	x	-
648	"	Karaj	-	-	x (x)	x (x)	x	-	-
695	"	Mashad	-	-	x (x)	x (x)	-	x	-
712	"	Hessarak	-	x	x (x)	x (x)	-	x	-
788	"	Tehran	-	-	x (x)	x (x)	x	-	-
790	"	Tehran	-	-	x (x)	x (x)	-	x	-
792	"	Tehran	-	-	x (x)	x (x)	-	x	-
833	"	Tehran	-	-	x (x)	x (x)	-	x	-
834	"	Tehran	-	x	x (x)	x (x)	-	x	-
835	"	Tehran	-	-	x (x)	x (x)	-	x	-
852	"	Tehran	-	-	x (x)	x (x)	-	x	-
853	"	Tehran	-	-	x (x)	x (x)	-	x	-
855	"	Tehran	-	-	x (x)	x (x)	-	x	-
860	"	Tehran	-	-	x (x)	x (x)	-	x	-
885	"	Hessarak	-	-	x (x)	x (x)	-	x	-
961	"	Tehran	-	-	x (x)	x (x)	-	x	-
768	"	Hessarak	-	-	x (x)	x (x)	-	x	-
854	"	Tehran	-	-	x (x)	x (x)	-	x	-

a) Results obtained from plates incubated in air and in 10% CO2 are recorded with the latter in parantheses.

x = Positive

- = Negative

extensively unlike other countries. Although the incidence of *Br. abortus* is very high in cattle in Iran. Fortunately, the risk of bovine brucellosis to the public health was very small. It must be mentioned that we have not found any cases of human brucellosis due to *Br. abortus* in the Tehran area where the population of cattle is very dense. In view of this observation *Br. abortus* appear to be less pathogenic for human than *Br. melitensis.* However, it should be mentioned that milk from cattle is sent to the pasteurization plant in Tehran. Up to present time *Br. suis* has not been isolated from swine in Iran and human infection by this organism has not been reported.

Discussion

The results of this investigation indicate that the main source of human brucellosis in Iran appears to be fresh cheese and ice cream made from unpasteurized milk. Milk from sheep and goats is seldom pasteurized and is used much more extensively than cow's milk in producing fresh white cheese. The incidence of human brucellosis due to the custom of eating cheese fresh is very high, especially in the Spring and Summer months. Etiological studies shows that human brucellosis in Iran is due to *Br. melitensis* infection in sheep and goats. Until the incidence of the disease in these animals can be reduced by vaccination, the risk of infection for man remain high. Since 1963 Elberg's Rev. 1 vaccine has been effectively used in Iran for vaccination of sheep and goats against *Br. melitensis.* Animals vaccinetd with Rev. 1 vaccine produced a solid immunity against *Br. melitensis* infection. Fortunately, vaccination of sheep and goats with Rev. 1 vaccine has shown good results in infected areas and as a result the number of Brucella incidence in man has been reduced.

Summary

Epidemiological studies of human brucellosis in Iran indicate that the main source of human brucellosis is unpasteurized milk of sheep and goats and its products such as cheese and ice cream. The incidence of human brucellosis is especially high in the Spring and Summer months. All of the bacteriologically proved cases of brucellosis in this investigation were due to *Br. melitensis.*

Resumé

Les recherches épidemiologique de brucellose humaine en Iran indiquent que l'origin de l'infection de la fièvre de Malte est due par la consommation du lait et des produits laitiers, en particulier la crême glacée et des fromages fraichement préparés à partir du lait cru des chèvres et des moutons. L'incidence de l'infection de brucellique chez l'homme est spécialement très élevée pendant le printemps et l'été. Tous les cas de l'infection examinée dans cette expérence sont dûs bactériologiquement au *Br. melitensis.*

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