Case study on human brucellosis of bovine-origin in Taiwan

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SUMMARY Between 1979 and 1981, a total of 16 persons were infected with *Brucella abortus* as a result of direct contact with infected cattle or contaminated materials. Thirty-eight serum samples were collected from persons who had direct comtact with infected cattle or contaminated materials, and 42 % of them (16/38) had an antibody against *B. abortus*. All 25 serum samples from persons who had no contact with the infected cattle or contaminated materials tested negative to the *B. abortus* antibody. The symptoms of patients were fever, lassitude, anorexia, poor libido, orchitis, and pain in muscles and joints. Two isolates of *B. abortus* were recovered from the blood of two patients. They were identified as biotypes 1 and 2 *B. abortus* by bilolgical, biochemical and serological examinations and bacteriophage typing. The status of the *B. abortus* antibody response of four patients was followed for ten years (1981～1990). Two of these patients maintained the 1 : 40 agglutination antibody titers and 1 : 5 complement-fixation antibody titers ten years after infection. [* Lu TS, Lee YL, Lin DF, Tsai HJ. Case study on human brucellosis in Taiwan. J Chim Soc Vet Sci 20 (1) : 1—8, 1994. * Corresponding author TEL (02) 621-2111, FAX (02) 622-5345]

Key words: Human brucellosis, B. abortus, Agglutination antibody, Complement-fixation antibody

INTRODUCTION

In Taiwan, Liu et al.[3] first reported the isolation of *B. abortus* from infected dairy cattle. Then, an intensive eradication program was conducted in Taiwan. Cattle were serologically tested twice a year by serumplate agglutination test, tube agglutination test and complement fixation test. Antibody-positive cows were sent to an abattoir immediately and the remaining cows in the contaminated farms were then tested monthly. By this effort, the infection rate of bovine brucellosis in Taiwan decreased from 4.99 % in 1962 to 0.06 % in 1979[7]. In 1979 some infected cows were illegally moved from Pintung county to Taiwan and Taipei counties and the disease spread to these two counties. During the period Oct. 1979～1980, in total 141 cattle from 14 dairy farms in these three counties were serologically positive to *B. abortus* and the total infection rate in Taiwan in 1980 climbed to 0. 4 %[7]. These 141 cattle were slaughered and sampled for pathological and bacteriological examinations. A disease-control veterinarina and some researchers and technicians in the diagnostic laboratory became infected with brucellosis during this period. Before this

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outbreak of bovine-origin human brucellosis, only one human clinical case that occurred in 1978 had been diagnosed in Taiwan (Cheh LL, personal communication). That patient was a graduate dtudent who acquired the infection in a laboratory of the Department of Veterinary Medicine, National Taiwan University. In the new outbreak, the infection was found not only in laboratory personnel, but also in a disease-control veterinarian and a few dairy-farm workers. As the brucellosis is impotrant to public health, the gathering of the epidemiologic information is imperative. Here we report an epidemiologic survey of the outbreak of the bovine-origin human brucellosis in 1979～1981 in Taiwan. The clinical signs, antibody response of the patients and characteristics of *B. abortus* isolates are described.

MATERIALS AND METHODS

Sera

Sixty-three serum samples were collected from researchers or technicians of the diagnostic laboratory, diseas-control veterinatians, farmers of workers of contaminated dairy farms during 1980～1981. Serum samples were also collected at various intervals from four patients (all reseachers or technicians of the diagnostic laboratory) for ten years (1981～1990).

Tube Agglutination Test

A strain 19 *B. abortus* commercial antigen (National Institute of Animal Health, Ibaraki, Japan) was used. The serum samples were twofold serially diluted in 0.5 % phenol saline starting at 1 : 5 dilution. Each dilution (0.5 mL) was thoroughly mixed with the same amount of antigen and incubated at 37 C for 20 ～24 h. The agglutination titer was determined by the end point of the tube showing 50 % agglutination. Agglutination titers 1 : 40 (equal to 100 IU) or greater were considered positive.

Complement-fixation (CF) Test

The serum samples were serially diluted with saline containing 0.01 % magnesium sulfate starting at 1 : 5. Each serum dilution (0.25 mL) was mixed with equal amount of 2 units of a commercial CF antigen (National Institute of Animal Health, Ibaraki, Japan) and 0.5 mL of 2 units of complement. After incubation at 4 C for 18～24 h, sheep red-blood cells (0.5 mL, 2 %) were added to each tube. The CF antibody titer was determined after incubation at 37 C for 30 min. The endpoint that had 50 % RBC hemolysis was regarded as the CF antibody titer, and titers at 1 : 5 or greater were considered positive.

Bacteria Isolation and Identification

Blood clots were separated from blood samples of four researchers or technicians of the diagnostic laboratory. The clots were first incubated in trypticase soy broth (Gibco Diagnostics, Wisconsin, USA) for seven days. Then one loop of cultivated broth was transferred onto brucella agar (Gibco) and trypticase soy agar (BBL Microbiology Systems, Maryland, USA) containing 10 % horse serum. The inoculated agars were incubated in an environment (CO_2 5 %) and examined for bacteria growth for a week. The Gram-stained negative small bacilli that agglutinated with *B. abortus* antiserum were further tested by the method of Nicoletti[4]. Eight strains of Brucella (Table 2 and 3) that were gifts from Dr. Y. Isayama at National Institute of Animal Health, Ibaraki, Japan, were included as reference steains.

Growth on Dyes

Dyes were added into trypticase soy agar containing 2 % inactivated horse serum and 1 % dextrose (SD − TSA). Two dyes, thionin, and basic fuchsin (The National Aniline Division, Allied Chemical and Dye Company) were added at concentrations 1 : 50,000 respectively. The ability of the Brucella strains to grow on SD − TSA in the presence of dyes was examined for four days after cultivation.

Agglutination by Monospecific Serum

Rabbits were intravenously inoculated with strain 544 *B. abortus* or strain 16 *B. melitensis* (5×10^9 in total). Immune sera were collected seven days after inoculation. Ten mL of immune sera were absorbed with Ig heat-inactivated, precipitated heterologous bacteria at 37 C for 2 h, and then at 4 C

overnight. The cross-absorbed sera that had agglutinating titer against homologous antigen 16 times as great as against heterologous antigen were used as monospecific anti-A antigen or anti-M antigen serum respectively. Anti-rough (Anti-R) antigen serum was prepared from QE 13 strain *B. canis* by a simliar method.

Bacteriophage Typing

Two bacteriophages were kindly supplied by Dr. Y. Isayama at National Institute of Animal Health, Ibaraki, Japan. Bacteriophage Tbilisi (Tb) was cultivated on 544 strain *B. abortus,* and bacteriophage Weybridge (Wb) was cultivated on strain 1330 *B. suis.* The test was conducted by inoculating Brucella strains on SD − TSA, and then one routine test dilution (RTD) of bacteriophage was added on agar and incubated at 10 % CO_2 and 37 C. Bacteriophage lysogeny was determined by lysis of bacteria 48 h after incubation.

Pathogenicity to Mice

Two isolates (LU stain and LEE strain) were intraperitoneally inoculated into five 5-week-old mice at a dose 10^{10}, 10^{1} and 10^{0} respectively. Only those mice from which bacteria were reisolated from the carcass were included in the calculation of LD_{50}. One month after inoculation, all surviving mice were sacrificed; the liver, spleen, cardiac blood, and lymph nodes were cultured for bacteria reisolation; the ID_{50} was determined and recorded.

RESULTS

Epidemiology Study

Sixty-three human serum samples were collected between 1981 and 1982. Information about the occupation, history of contact with contaminated cattle or materials, and results of the serological tests appesrs in Table 1. Thirty-eight of 63 serum samples were collected from persons who had direct contact with infected cattle or contaminated materials, and of them 42.1 % (16/38) had the antibody against *B. abortus*. All other 25 serum samples from persons who had no direct contact with the infected cattle or contaminated materials were *B. abortus* antibody negative. The ages of these 16 *B. abortus* antibody-positive patients were in the range 30 ~ 60 years. Among them 14 were male and 2 were female. All had common clinical signs of fever, cold at extremities, lassitude, anorexia. Besides, one patient claimed poor libido, another person felt pains in muscles and joints, and two other parients had orchitis.

Bacteriological Study

Blood samples of four patients were collected for bacterial isolation at 7~10 days after onset of the symptoms. Pure organisms were isolated from two patients and nsmed "LU" and "LEE" strains. The isolates were identified as *B. abortus* biotype 1 (LEE strain) and biotype 2 (LU strain) according to reaults of biochemical and serological tests (Table 2), their ability to grow on dye, the pattern of agglutination with anti − A, − M, or − R monospecific antiserum, and the sensitivity to Tb and Wd strain bacteriophages (Table 3).

Table 1. Serological survey on human brucellosis in Taiwan in 1981-2

Occupation of the persons under survey	History of link with infected cattle or contaminated materials	
	Yes	No
Veterinarians and technicians	1 / 10[a]	0 / 19
Personnel in the laboratory	9 / 16	0 / 0
Workers on dairy farms	6 / 12	0 / 0
Wives of patient	0 / 0	0 / 3
Others	0 / 0	0 / 3
Total	16 / 38	0 / 25
%	42.1	0

[a] Number of persons with positive test / Number of persons tested

Table 2. Biochemical properties of LU and LEE strain isolates

Bacteria (strain)	Urease	Nitrate	Catalase	Oxidass	MR	VP	DHL
LU	+	+	+	+	−	−	−
LEE	+	+	+	+	−	−	−
B. abortus (544)	+	+	+	+	−	−	−
B. abortus (83/8/59)	+	+	+	+	−	−	−
B. melitensis (16M)	+	+	+	+	−	−	−
B. suis (1330)	+	+	+	+	−	−	−
B. neotomae (5K33)	+	+	+	−	−	−	−
B. ovis (63/290)	−	−	+	−	−	−	−
B. canis (RM6/66)	+	+	+	+	−	−	−
B. canis (QE13)	+	+	+	+	−	−	−

[a] Strain 544 is a biotype 1 *B. abortus*, strain 86/8/59 is a biotype 2 *B. abortus*.

Table 3. Characteristics of LU and LEE strain isolates

Strain[a]	CO_2[b]	Hs	Growth on dyes		Agglutination with antiserum			Lysis by phage	
			Thionin	Fuchsin	A	M	R	Tb	Wb
LU	+	+	−	−	+	−	−	+	+
LEE	+	+	−	+	+	−	−	+	+
B. abortus (544)	+	+	−	−	+	−	−	+	+
B. abortus (83/8/59)	+	+	−	−	+	−	−	+	+
B. melitensis (16M)	−	−	+	+	−	+	−	−	−
B. suis (1330)	−	+	+	−	+	−	−	−	+
B. neotomae (5K33)	−	+	+/−	−	+	−	−	+/−	+
B. ovis (63/290)	+	−	+	+	−	−	+	−	−
B. canis (RM6/66)	−	−	+	−	−	−	+	−	−
B. canis (QE13)	−	−	+	−	−	−	+	−	−

[a] Strain 544 is a biotype 1 B. abortus, strain 86/8/59 is a biotype 2 B. abortus
[b] CO_2 requirement for growth

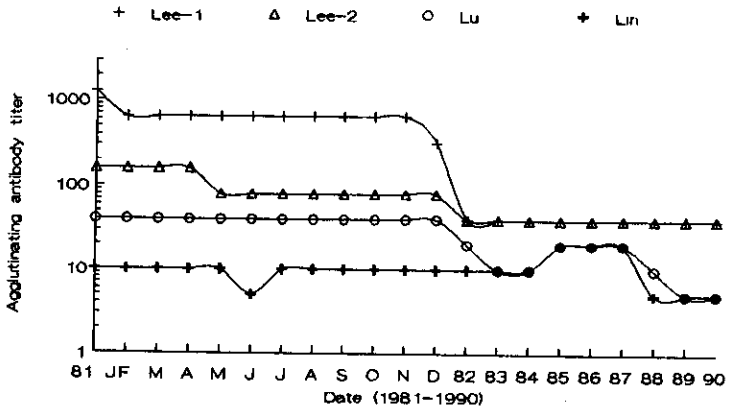

Fig 1 *Brucella abortus* agglutinating antibody titers of four patients (LEE−1, LEE−2, LU, LIN) within ten years after infection

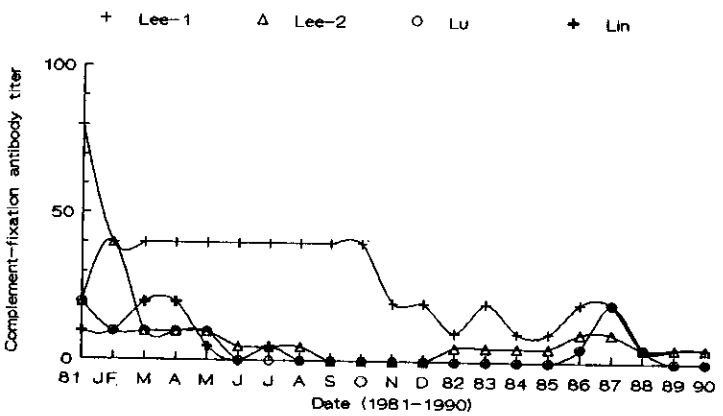

Fig 2 *Brucella abortus* complement-fixation antibody titers of four patients (LEE−1, LEE−2, LU, Lin) within ten years after infection

Pathogenicity of the LU and LEE Strain *B. abortus* Isolates

The LD_{50} and ID_{50} of the LU strain to mice were $10^{8.1}$ and $10^{0.9}$ respectively. The LD_{50} and ID_{50} of the LEE strain to mice were $10^{8.9}$ and $10^{0.1}$ respectively.

Longitudinal Study of Antibody Status of Four Patients for Ten Years

The antibody status of four patients was followed for ten years (1981 − 1990). The serum samples were collected monthly during 1981, and then annually until 1990. The agglutinating and complement-fixation antibody titers against *B. abortus* were determined and are summarized in Fig 1 and 2. Of four patients two maintained 1 : 40 agglutination antibody titers and 1 : 5 complement-fixation antibody titers ten years after infection. Fluctuations of antibody titers were noted during the observation period. Brucellosis is recognized as an important zoonosis worldwide. Veterinarians and people engaged in animal husbandry are at particularly high risk. According to an investigation conducted in England, of 309 veterinarians, 186 (63 %) had *B. abortus* antibody[2]. Among them, 85 veterinarians had clinical signs (27 %). In anther survey carried out in New Zealand, 90 % of veterinatians surveyed had antibosy titers[5]. In Taiwan, Liu et al.[3] confirmed the existence of *B. abortus* in Taiwan. Since that time, the policy of detection and slaughter was conducted in cattle population in Taiwan and no serologically positive dairy cattle were detected since 1989[7]. No epidemiological sruvey on human brucellosis has ever been conducted in Taiwan. Only one clinical case who acquired infection in the laboratory was reported (Cheh personal communication). This work was the first survey of human brucellosis in Taiwan.

According to our findings, all 16 persons infected with *B. abortus* had the history of direct contact with infected cattle or contaminated materials, whereas all others, including wives of three patients and colleagues of an infected veterinarian, who had not direct contact with infected cows or contaminated materials, all tested negative to the antibody. These results show that the chance to contract brucellosis from other human beings is minute.

In this sruvey only two of 14 infected persons were female, but this conditions implies no distinct in susceptibility between genders, because, most veterinarians and dairy-farm workers in Taiwan are male. A similar reason may apply about the survey by Hardy et al.[1] that of people infected with brucellosis in Iowa USA, two thirds were men. The ages of infected persons in our results were in the range 30～60 years whereas the age group reported by Hardy et al[1] was 20 ～ 45 years. This condition apparently reflects the age of persons engaged in the work.

The clinical signs of our patients were generally in accordance with those described by Spink and Thompson[6]. However, orchitis in two patients and poor libido claimed by one patient were not described by them. Whether these symptoms were psychologic effects[2], coincidental, or really linked to the disease is uncertain.

By a longitudinal study of antibody status of four patients, two patients maintained a positive antibody titer ten years after infection. One patient (LEE − 1) took medicine (tetracycline) for five days only; the other patient (LEE − 2) only took medicine for one week only. This condition may explain the high and enduring antibody titers of these two patients, as other two patients who took medicine for 3～4 weeks had lower and briefer antibody titers. There were fluctuations of antibody titers of all four patients during the observation period. No related clinical signs were repoted during this period. The intracellular nature of brucella make them somewhat inaccessible to chemotherapeutic agent. Although no bacteria were reisolated, it is suspected the fluctuation of the antibody titers may be due to recurring disease. Thus it is important to have patients to complete the full treatment course.

REFERENCES

1. Hardy AV, Jordan CF, Borts IH, Hardy GC. Undulant fever with special reference to a study of Brucella infection. U S Nat Inst Hlth Wash Bull 158 : 81. 1930

2. Kerr WR, Coghlan JD, Payne DJH, Robertson L. Chronic burcellosis in the practicing veterinary surgeon. Vet Rec 79 : 602, 1966
3. Liu JY, Lu YS, Yang SC, Liu YS. The isolation of *Brucella abortus* from dairy cows. Taiwan Prov Res Inst Anim Hlth Exp Rep 1 : 86, 1963
4. Nicoletti P. Brucella. In : Carter GR, Cole JR, Jr. eds. Diagnostic Procddures in Veterinatry Bateriology and Mycology. 5th ed. New York, Academic Press, Inc. 95－105, 1990
5. Robinson RA, Metcalfe RA. Zoonotic infections in veterinarians. New Zeal Vet Jour 24 : 201, 1976
6. Spink WW, Thompson H. Human brucellosis caused by brucella abortus, strain 19. JAMA 153 : 1162165, 1953
7. Wu YS, Chiu SY. The eradication of brucellisis of dairy cattle in Taiwan. Proceedings of Symposium on Animal Disease Prevention and Control in Asia L－1, 1991

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在臺灣發生之人布氏桿菌症

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摘要 在 1979～1981 年間，台灣有 14 個牧場之乳牛感染布氏桿菌（*Brucella abortus*），同時有 16 個人因直接與感染牛隻或污染病材接觸而感染，其中 9 人爲實驗室工作人員，6 人爲感染牧場員工，1 人爲現場獸醫師。爲調查台灣之人布氏桿菌症之發生情形，於 1981～1982 年間共收集 63 個人的血清標本，其中 38 個人曾與感染牛隻或病材直接接觸，結果發現其中 16 人（42 %）爲 *B. abortus* 抗體陽性，另外 25 個未曾與感染牛隻或病材直接接觸之人的血清則全爲 *B. abortus*抗體陰性。本病患者臨床症狀有發熱、疲倦、厭食、性慾降低、睪丸炎、肌肉及關節疼痛等。由 2 個病人之血液中分離到 2 株細菌，經由生物學、生化學、血清學、及噬菌體分類結果鑑定分別屬於 *B. abortus* 的生物型 1 及 2。自 1981 年起長期追蹤調查 4 個布氏桿菌症病人之 *B. abortus* 抗體消長情形，結果發現其中 2 人在 10 年後 (1990) 仍有 1：4 倍的凝集抗體力價及 1：5 倍的補體結合抗體力價。[**呂榮修、李永林、林地發、蔡向榮。在臺灣發生之人布氏桿菌症。中華獸醫誌 20 (1)：1－8, 1994, * 聯絡人 TEL (02) 621-2111, FAX (02) 622-5345*]

關鍵詞：人布氏桿菌症，凝集抗體，補體結合抗體

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