A total of 282 blood samples from patients having clinical symptoms and signs of brucellosis was collected from Allied and District Headquarter Hospitals, Faisalabad. Slide agglutination test for initial screening and standard tube agglutination test for further confirmation of Brucella species were used. Two major antigens, Br. abortus and Br. melitensis were used for each test. Frequency of the disease with Br. abortus was 97.7% and with Br. melitensis was 90.3%. Seroprevalence of brucellosis in patients having family history (30%), fever (95%), body aches (90%), lack of energy (91%), joint pain (85%), back pain (60%), chills (44%), headache (67%), loss of appetite (48%), weight loss (58%), abdominal pain (13%), sleep disturbance (26%), constipation (36%), signs of ill-looking (46%), pallor (48%), lymphadenopathy (23%), joint swelling (45%) and spinal tenderness (10%) was assessed. Minor differences of prevalence between married (51%) and unmarried patients (49%) was found. The age group of 21-30 years showed the highest seroprevalence (46%). Patient's hygienic conditions, consumption of raw milk, occupation and contact with animals were the main risk factors.

Key words: antigens, brucellosis, seroprevalence

INTRODUCTION

Brucellosis is a zoonotic bacterial disease. The causative organism, Brucella are non-encapsulated, non-motile, non-sporing, gram negative and intracellular aerobic bacilli. Br. melitensis causes disease in sheep, goats and camels; Br. abortus in cattle and buffaloes (Bang, 1897); Br. suis in swine (Mohler, 1914); Br. ovis is specific for sheep (Buddle and Boyes, 1953); Br. neotomae for rats (Stoenner and Lackman, 1957); Br. canis for dogs (Carmichael et al., 1966).

Human brucellosis may be caused by one of the four species: Br. melitensis, Br. abortus, Br. suis or Br. canis. No human infection due to Br. ovis or Br. neotomae has been reported (Meyer, 1974). The disease is transmitted to man from infected animal reservoirs through several routes: including ingestion of raw or unpasteurized milk and milk products derived from the infected animals (Betas et al., 1986), animal owners, butchers, veterinarians, individuals who come in contact with the infected animals, dairy farm workers and abattoir workers usually get exposed to the pathogen by contact with the animals uterine contents and discharges contaminated floors, utensils, dust, etc. Other potential sources of human infection include direct or indirect inoculation, inhalation (Parker and Collier, 1990), through conjunctiva (Vanrooney, 1981; Williams, 1982), by blood transfusion, by bone marrow transplantation (Naparastek et al., 1982), by transplacental transmission, by sharing needles among drug addicts (Romero et al., 1984) and via milk to breast fed infants of infected mothers. The purpose of this study was to investigate the clinical epidemiological aspects of human brucellosis.

MATERIALS AND METHODS

A total of 282 serum samples from patients having clinical symptoms and signs of brucellosis was collected from Allied and District Headquarter Hospitals, Faisalabad. Each of the sera was analysed first by slide agglutination test (SAT) and only the Brucella positive or doubtful sera were further analysed by standard tube agglutination test (STAT). Immuno-fibrile antigens containing Br. abortus and Br. melitensis made of immunostics, Inc. USA were used in the test. For SAT one drop of serum was placed on a clean glass slide. A drop of the antigen was added and thoroughly mixed. The mixture on slide was examined for evidence of agglutination. The results were recorded 2-3 minutes after mixing the test antigens. Known positive and negative sera were used as controls (Brown, 1974). For STAT serial two-fold dilutions of the test serum starting from 1:10 up to 1:640 (volume 0.5 ml) were prepared in phenol saline (0.85 % NaCl solution containing 0.5 % phenol). The antigens were diluted and an equal amount was added to each tube. Contents of the
Fig. 1. Seroprevalence of brucellosis in 144 clinical cases based on family history and clinical symptoms.

Fig. 2. Seroprevalence of brucellosis in 144 clinical cases based on clinical signs.

Fig. 3. Age-wise seroprevalence of brucellosis in 144 clinical cases.
tubes were mixed thoroughly and incubated at 37°C for 48 hours. The degree of agglutination was determined by the degree of clearing without shaking the tubes. Known negative and positive sera were used as control (Stemshaorn et al., 1985). Complete agglutination and sedimentation with 100% clear supernatant was marked as four plus (+++), similarly 75%, 50%, 25% were marked as three, two and one plus, respectively. No agglutination and no clearing was considered as negative. The highest serum dilution showing 50% clearing (++/-+) was considered as titre of that serum. A titre of 1:80 or higher was considered as positive for brucellosis (Alton and Jones, 1967).

RESULTS
On the basis of clinical symptoms and signs, blood of the patients was collected for seroconversion studies (Fig. 1 and 2). Of 282 suspected patients, 144 were having antibodies against Br. abortus and Br. melitensis. The higher prevalence was recorded with Br. abortus (97.7%) as compared to Br. melitensis (90.3%). The age group of 21-30 years showed the highest (46%) seroprevalence (Fig. 3). Serological prevalence of disease was higher in males (59.7%) than in females (40.3%). Similarly, married persons showed higher prevalence of disease (50.7%) than unmarried (49.3%). The prevalence of brucellosis in rural patients was 66% while in urban it was 34%. Similarly, animal handlers showed higher prevalence of disease (78.5%) as compared to other workers (21.5%). Other risk factors found to be involved were contact with animals (91.7%) and consumption of raw milk (63.2%).

DISCUSSION
The standard serological procedures for the diagnosis of brucellosis since the inception of its serology are SAT and STAT (Contini et al., 1973 and Rahman et al., 1990). The validity and reliability of these tests has further been confirmed by Brown (1974), Kulshreshtha et al. (1978) and Stemshaorn et al. (1985). The use of STAT in order to confirm human brucellosis has been extensively evaluated, yielding the highest degree of reproducibility and accuracy (Buchanan et al., 1974). Higher prevalence of disease recorded with Br. abortus as compared to Br. melitensis might be due to the fact that majority of patients has frequent contact with cattle and buffalo and less with sheep and goat. This finding is in contrast to the finding of Madkour (1989) that Br. melitensis is the most common cause of human brucellosis. Higher serological prevalence of disease among 21-30 years might be due to the fact that this age group is most active and directly involved in handling the livestock or their products. These findings are in accordance with Russo et al. (1984) who also reported a higher incidence in the age group '20–29' years. Married persons showed higher serological prevalence of disease which might be due to sexual intercourse. This mode of transmission is supported by Goossens et al. (1983) and by Stantic et al. (1983). According to them in the absence of all other possible routes of transmission, the pathogens may spread through sexual contact. However, this route of transmission has not been proven (Ruben et al., 1991).

Higher serological prevalence of the disease on the basis of consumption of raw milk was supported by Guercio et al. (1985) and Matheos (1990) who reported similar results. From this finding, the present view that raw milk taken directly from the animal is safe from all bacterial transmissions needs reconsideration, for Brucella as the pathogen are secreted along with milk and may cause infection. The above findings indicate that the prevalence of the disease in human beings seems somewhat correlated with the prevalence of disease in animals. Similar observations were also reported by Masoumi et al. (1992).

REFERENCES
Akhtar, Mahboob & Latif


