Outline: to invade macrophages, epithelial cells, dendritic

Outline: Brucellosisis the second most important zoonotic disease in the world. Different Brucella spp. and their differentbiovars have been reported all over the world in both animals and humans. InPakistan most of the work is done on animals.

Most prevalent biovar of B. abortus in animals, in Pakistan is biovar1and regarding humans, it is still unknown. The main focus of this study is toidentify, isolate and molecularly characterize the different biovars of Br. abortus, which are prevalent in humans of Pakistan.

This study will increasethe understanding regarding B. abortusin humans in Pakistan. IntroductionBrucellaeare Gram-negative, nonmotile, aerobic, non-spore forming, facultativeintracellular bacilli. They have the ability to invade macrophages, epithelialcells, dendritic cells, and placental trophoblasts (Asif et al., 2014).

It has the ability to escape from theimmune system and thus can live intracellularly (Al Dahouk et al., 2013). On the bases of difference in host specificity the genus is divided into 10nomo-species. Among six classical species, two major species are; B.

melitensis biovar 1–3; B. abortus bv 1– 9 (Din et al., 2013). In Pakistan, a serious threat for expecting womenand their unborn children is Brucellosis. The seroprevalence of Brucellosis inpregnant women with history of an abortion, having contacts to aborted women orcontact to animals that aborted were 14. 6 %, 15.

8 %, and 12. 5 % respectively (Ali et al., 2016). In humans, it causes undulant fever, and malesterility in humans and in animals, B. abortus causes abortion (Din et al., 2013). Primarily the diseaseis presented as fever (of unknown origin) with various clinical signs andsymptoms.

These include serious focal problems such as neurobrucellosis orBrucella endocarditis and spondylitis. Besides that failure of treatment by primaryantibiotic, relapses, and chronic disease occur frequently (Baba et al., 2001; Grillo et al., 2006). Brucellosisis primarily a contagious disease of domestic animals – goats, sheep, cattle, swine, camels, and dogs. People are infected through ingestion of fresh milk, cheese, and cream; through direct contact with infected animals (e.

g., amongshepherds, farmers, and veterinarians); and through inhalation of infectiousaerosols (e. g.

, by workers in abattoirs and microbiology laboratories). Brucellosis is a global zoonoticdisease associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also in humans. The disease is widely distributed throughout the developing world, considered to be serious problem in atleast 86 countries (Din et al., 2013).

Morethan 500, 000 human cases had been reported worldwide each year, but the numberof undetected cases is believed to be considerably higher (Geresu et al., 2016). This alarmingsituation can be attributed to the non-speci? c clinical picture of human Brucellosis, low awareness of the disease in non-endemic countries and shortcomings inlaboratory diagnosis.

The number of human cases is directly correlated with thenumber of infected animals within a de? ned region. Effective countermeasures toreduce the incidence of human brucellosis are therefore based on surveillanceand control of livestock and pasteurization or cooking of contaminated foodproducts. Once the disease has been transmitted from its animal reservoir to humans, only early diagnosis and adequateantibiotic therapy can prevent serious sequelae in patients (Al Dahouk et al., 2013). Pakistanis bordered by Afghanistan and Iran in the west, India in the east, and China inthe far north east.

In most of the neighboring countries of Pakistan, brucellosis outbreaks have been reported. According to the World HealthOrganization, Brucellosis is considered a neglected disease, particularly inPakistan (World Health Organization (WHO) 2012). In Pakistan, 23. 9 % of thepopulation lives below the poverty line. Cattle or buffaloes are mainly kept bythe poor rural farmers as subsistence farming (Ali et al., 2014). A study (Buchanan et al.

1974)revealed that brucellosis is an abattoir associated disease and slaughter houseworkers have the greatest risk of contracting the disease. Ina study, theprevalence of Brucellosis among humans, based on RBPT, was 14%. The prevalence rates of Brucellosis in cattleand buffalo at slaughterhouses were 10% and 9.

5%, respectively, with RBPT, and8. 33% and 7%, respectively, with ELISA (Hussainet al., 2008). Thebrucellosis is diagnosed on the basis of serological, bacteriological, allergicskin reaction and molecular methods. The most important confirmatory method of Brucella infection is

bacteriologicaldiagnosis since its specificity is much higher than that of other diagnosticmethods and it is used as a gold standard diagnostic method (Sathyanarayanan et al., 2011). Also currently Brucella specie is detected by molecular diagnostic methods invarious samples.

The existence of different Brucellabiotypes among the Brucella spp. and their identification in human is important to confirm the infection and tracethe source of the infection. Sero-prevalencestudies showed that Brucellosis is regional in animals in Pakistan.

However, thebiovars of Brucella species, whichare endemic in Pakistan are still unknown. on the basis of species-specific PCRand biochemical tests, the evidence have been collected in previous studies thatbiovar 1 of B. abortus is present incattle and buffaloes (Ali et al., 2014). Hence, thereis no up to date knowledge of any Pakistani investigation of Brucella in humans. Available pieces ofinformation related to Brucellosis especially in human beings are scarce.

B. abortus biovar 1 is the cause of mostinfections all over the world primarily, but distribution of biotypes alsodepends on geographic differences. B. abortus biovars 1, 2, and 4 are found in cattle in Indiaand confirmed by the characterization of Brucellaabortus. (Kanani 2007) Aims& Objectives InPakistan most studies on Brucellosis were conducted on two platforms 1. organizedgovernment livestock farms and 2.

private livestock farms, and, to some extent, in humans. There is very little knowledge is gathered about the prevalence of B. abortus in humans in Pakistan. The current study aims to isolate andcharacterize the Brucella

abortus andalso to investigate the biovars of B. abortus (bv)1–6 and 9 in the human population.

· Sampling of Brucella positive humanblood from hospitals· Isolation of B. abortus by using culture media (Modified Farrell's Serumdextrose Agar)· Characterization of B. abortus through AMOS PCR· Identification of different species ofBrucella and its biovars through bio-chemical tests· Comparisonof molecular characteristics of the obtained isolates with other strains in theregion and outside.

Material& MethodsOutline: Brucella positive human blood samples will be collected fromhospitals of Rawalpindi and Islamabad. First, we will isolate Brucella spp. on selective media anddifferent biochemicals will confirm our organism. Secondly, DNA will beextracted using kit method from human blood sample. This DNA will be amplifiedin AMOS PCR and will confirm different species of Brucella. Finally, different set of tests proposed by Alton andJones (1967), will be performed for biovar typing of Br.

abortus. MethodologyFlow Chart 1. Sample CollectionAtotal of 300 Brucella positive bloodsample will be randomly collected from different hospitals of Rawalpindi andIslamabad. 2.

IsolationBacteriological analysis will be performed in asafety level-3 bio-containment facility. According tostandard procedures (Farrell and Robinson 1972; Alton et al. 1988) isolation of Brucella spp. will be done by inoculating the samples on modified Farrell's serumdextrose agar.

The components of modified Farrell’s serum dextrose agar are 5 %horse serum, 1 % dextrose. In the presence of 5–10 % carbon dioxide at 37 °C, we will inoculate the plates with samples and incubate it aerobicallyBiochemical testsCatalase, oxidase and urease tests will confirm the Brucella isolates after isolation onselective media. 3. CharacterizationDNA extraction fromblood samples Wewill use DNA purification kit (MBI Fermentas, Graiciunau 8, Vilmius 2028, andLithuania) to extract DNA from all the blood samples, as per the directions ofmanufacturer. Purified DNA pellet will be dissolved in 100 µl of doubledistilled deionized water and will store at -20o C.

DNA Amplification byPCRPositivesamples will be subjected to AMOS PCR for species identification. The extractedDNA samples will be amplified by PCR using specific set of primers for B. abortus. After that amplified DNA, PCR products will be examined by using Gel Electrophoresis.

Biovar Typing of B. abortusBrucella isolates, confirmed by results ofcatalase, oxidase, and urease tests, will be examined for biovar typingaccording to the standard methods described by Alton et al. Briefly, biovars willbe identified based on agglutination with A and M monospecific antisera, CO2 requirement for growth, H2S production, and growth on media containing20?? g/mL basic fuchsin and thionin dyes. Ganttchart Activity 1st Month 2nd Month 3rd Month 4th Month 5th Month 6th Month 7th Month 8th Month 9th Month 10th Month Human Blood Sampling Isolation via Selective media Bio-Chemical Tests for B. abortus Mol. Characterization by AMOS PCR & Biovar Typing ReferencesAL DAHOUK, S.

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