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Brucellosis causing economic losses in small ruminants of Pakistan

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Abstract

Brucellosis is an economically important remerging bacterial zoonosis that not only affects health and productivity of animals but it is also of public health concern. Brucellosis is prevalent in Pakistan, economic losses due to brucellosis have never been estimated for small ruminant population of the country. This study was conducted for assessing economic impact of brucellosis in small ruminants. A herd comprising of 250 sheep with clinical signs of late term abortions was selected. Brucellosis positive status of herd was confirmed through Rose Bengal plate test (RBPT) and indirect enzyme linked immunosorbent assay (I-ELISA). Bacterial isolation and Polymerase chain reaction (PCR) was used to identify species of brucella. The component for calculating economic loss were loss due to reduction in meat yield by abortion, still birth, repeat breeding, veterinary expenses and increased cost of management due to rearing of nonproductive animals and weak lambs. A total of 52% of animals were found to be infected with brucellosis in sheep herd. The isolates obtained from aborted fetuses were characterized as *B. melitensis* through culture and molecular technique. The annual economic loss was calculated as Rs. 2745/ sheep. Based on results it was concluded that Brucellosis causes significant economic losses in small ruminants of Pakistan.

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Introduction

Brucellosis is highly infectious bacterial disease of animals and human. It affects many species of animals including sheep and goats (Dijkhuizen *et al.,* 1995; Bernues *et al.,* 1997). It is the most important disease in many countries throughout the world due to its economic significance (Nicoletti, 1980; Silva *et al.*, 2000; McDermott and Arimi, 2002). Mortality rate in Brucella infected animals is very low but economic losses are mainly due to abortion, still birth, repeat breeding, infertility, decrease milk yield (Walker, 1999: Rodositis *et al.*, 2003), lowerd commercialization of dairy products and international trade (Benkirane. 2006).

Brucella species belong to alpha-2 subdivision of the Proteobacteria (Yanagi and Yamasato, 1993), brucellae are gram-negative, non-motile coccobacilli. Currently genus *Brucella* contains ten species. Brucella species affecting terrestrial animals include *B. abortus* (infects cattle), *B. melitensis* (goats), *B. suis* (pigs), *B. canis* (canines), *B. ovis* (sheep), *B. neotomae(rodents), B. microti* and *B. inopinata* (Verger *et al.,* 1987: Scholz *et al.,* 2008). The *Brucellae* isolated from marine animals are *B. ceti* from cetaceans (whales, porpoises and dolphins) and *B. pinnipedialis* from pinnipeds (seals, sea lions and walruses) (Foster *et al.,* 2007).

Although small ruminants are carrier for both *B. ovis* and *B. melitensis* but predominant etiologic agent of brucellosis in small ruminants is *B. melitensis*. Brucellosis is major cause of stillbirths, late term abortion, low milk production and decreased fertility in small ruminants (Lilenbaum *et al.,* 2007). South-east Asia has brucellosis prevalence of 2.9% and 164 brucellosis outbreaks in small ruminant of this region has been reported in the year 2010 to World Animal Health Organization (ILRI, 2012).

Economic losess due to brucellosis are associated with prevalence (McDermott *et al.*, 2013). Yearly economic losses because of brucellosis vary from one country to another. Many countries do not report their losses but few countries like Argentina, has reported an annual loss of US$ 60,000,000 based on 5% prevalence (Samartino, 2002) similarly official reports from Nigeria has estimated losses as US$ 575,605 per year at prevalence of 7% - 12% (Ajogi *et al.*, 1998).

In the U.S.A. the cost of abortion and reduced milk production in 1952 alone were put at US$ 400 million (Acha and Szyfres, 2003). Khartoum State, the capital State of one of eighteen states of the Sudan experienced total economic loss of US$ 6, 587, 400 per annum due to bovine brucellosis (based on reduced milk production only) (Angara *et al.*, 2016). Brucellosis is one of the neglected disease among diseases of animals in Pakistan. Studies conducted in various parts of Pakistan has reported a brucellosis prevalence of 3.3-7% in small ruminants (Iqbal *et al.*, 2013; Ameen-ur-Rashid *et al.*, 2017). Previously losses due to brucellosis have never been estimated in small or large ruminants of Pakistan.

Therefore present study was conducted with objective to estimate economic losses in small ruminant population of Rawalpindi, Pakistan.

Materials and methods

A herd comprising of 250 sheep with clinical signs of late term abortions suggestive of brucellosis was selected for assessing economic impact of disease in small ruminants. Serum samples (144) were collected from all adult animals of herd. Fetuses aborted within 24 hour were also collected for isolation of brucella. Brucellosis positive status of herd was confirmed through Rose bengal plate test (RBPT) and indirect enzyme linked immunosorbent assay (I-ELISA). Bacterial isolation and Polymerase chain reaction (PCR) was used to identify *Brucellae*.

Blood sample collection

Each animal was bled by jugular vein puncture and 5ml of blood was collected aseptically in a vacutainer using sterile needles and vacutainers were labeled. The collected blood was to allowed clot and then packed carefully to avoid cross contamination. Individual samples were placed in a zipper bag with enough absorbent material to prevent any risk of leakage. Blood samples were transported to microbiology laboratory of Animal Health program, Animal Sciences Institute (ASI), National Agricultural Research Center (NARC), Islamabad in cold box with ice packs. Sera were harvested by centrifugation (Sigma, Germany) at 1500×g for 10 minutes.

Rose bengal plate test

Initial screening of the sera was carried out by Rose Bengal Plate test (RBPT) (MacMillan, 1990: John *et al*., 2010). A total of 25µl of serum sample was mixed with same quantity of RPBT antigen (IDEXX, USA) to produce a circular zone of about 2cm in diameter. The plate was rotated gently for 4 minutes at room temperature, any visible clumping within 4 minutes was indicative of a positive result. Any test showing agglutination beyond this time was considered negative. Positive and negative controls for RBPT were tested for reference.

Indirect enzyme linked immunosorbent assay

Serum samples initially tested by RBPT were further evaluated through indirect enzyme linked immunosorbent assay using I-ELISA kit (IDEXX,USA), following method described in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2009). Optical densities values of samples and control were measured at 450nm. Sample percentage (s/p) values were calculated to find out level of antibodies in serum. Samples with s/p % ≤ 110% were considered negative for presence of *Brucella* antibodies while Sample with s/p % > 110 and <120 were considered as suspected. Samples with s/p % ≥120 were taken as positive for *Brucella* antibodies, as per manufacturer's instructions.

Isolation of Brucella

Stomach contents of aborted fetuses were streakrd on sterilized agar media according to the techniques defined by Alton *et al.* (1988). Commercially available *Brucella* specific tryptone soya agar (Oxoid, England) was used. The media was supplemented with 5% v/v inactivated fetal bovine serum (GIBCO, Invitrogen, USA). The duplicate sets of plates were incubated at 37°C under both aerobic and anaerobic conditions under 10% CO_2 tension (Carbon dioxide jar). The *Brucella* suspected colonies were picked up with sterilized aluminum wire and transferred to tryptone soya agar plates and incubated again to get pure culture.

Identification and characterization of Brucella melitensis

For identification of *Brucella,* staining techniques (modified Ziehl-Neelsen & Gram' s staining), biochemical tests (oxidase test, catalase test, nitrate reduction, urease test, indole test, H_2S production, CO_2 Utilization) and agglutination (monospecific sera A, M, A+M and acriflavin) were applied. PCR following Koichi *et al* (2007) was also used to identify species of *Brucella.*

Economic loss estimation

Serological and questionnaire data collected from 144 animal ≥ one year (140 female & 4 male) was used for calculating economic losses due to brucellosis. The total economic losses due to *B. melitensis* infection were estimated following Singh and Prasad (2008). The component for calculating economic loss were loss due to reduction in meat yield by abortion, still birth, repeat breeding, veterinary expenses and increased cost of management due to rearing of nonproductive animals and weak lambs. Average milk production per sheep per year in Pakistan is reported as 64litre (Hassan *et al*., 2014). Cost of meat was taken as Rs.639/Kg based on market rate. Abortion rate in herd was calculated by technique of Chaudhary *et al*., 2013. The weight of lambs and kids after 12 months is reported to be around 30-33kg (Ahmad *et al*., 2001) yielding 12-14kg of meat (Khan *et al.,* 2014). Treatment cost, increased cost of management due to repeat breeding and rearing of weak kids from infected animals were difficult to compute where records and estimates on cost of feeding, rearing and treatment were missing. In the absence of any correct data in this respect, these costs were taken as 20% of the cost of animal as described by Singh and Prasad (2008). Economic loss was calculated by using following equation.

Economic loss = C1+C2+OC+M1+M2+W

Cost of Live Weight Loss due to Increased Abortions

$C1= [(12/K_I) – \{12/ (K_I + 9.5 A)\}] (I – D) P_I N_K B_W P_W$

Cost of Live Weight Loss due to Increased Inter-kidding Period

$C2= [(12/K_I) – \{12/ (K_I + W)\}] (I – D) P_I N_K B_W P_W$

Cost of Milk Loss due to Increased Inter-kidding Period

$M1= [(12/K_I) – \{12/ (K_I + W)\}] (I – D) P_I YP_M$

Cost of Milk Loss due to Increased Abortions

$M2= [(12/KI) – \{12/ (KI + 9.5 A)\}] (I – D) P_I YP_M$

Cost of decreased wool yield

$W = (I-D) P_H Y_L Y_W M_W$

Treatment Cost and Increased Cost of Management

OC= (I –D) ×Z

I = Number of infected animals

D = Number of animals died

P_l = Proportion of animals in milk (60 ewes out of 140) = 0.43

A = Increased abortion rate = 0.34 (calculated as described by Chaudhary *et al.*, 2013)

K_I = Inter kidding period = 10 months

N_K = Average number of kids per kidding = 1.5

W = Delay in next conception = 3 months

9.5= the average time for abortion was 3.5 months from conception, and a delay of six months in the next conception, the interkidding interval gets increased by 9.5 months

B_W = Average birth weight of a kid = 2.5kg

P_W = Price of meat per kg = Rs. 636/-(Market price)

P_M (Price of milk) = Rs. 50/lit from field estimates

W = Delay in next conception = 3 months

Y = 64litre (Annual average milk yield per sheep in milk: Hassan *et al.*, 2014)

Z = 1533/- Per infected animal surviving (20% of cost of animal: cost of animal was calculated by multiplying meat yield with market price of meat/Kg: 12 x 639 = 7668)

P_H = Proportion of animals in shearing age = 0.6 (144 out of 250)

Y_L= Proportion of wool yield lost= 0.20 (Singh *et al.*, 2014)

Yw= Average annual wool yield= 1.2kg (Munir *et al.*, 2010)

Mw=Price of wool per Kg= 16/- (Munir *et al.*, 2010)

Results

In small ruminant herd 52% of animals were found to be infected with brucellosis (Table 1). All ewes having history of abortion, still birth and repeat breeding were found sero positive through RBPT and I-ELISA. Culture, biochemical and molecular techniques confirmed *Brucella melitensis* as etiological agent causing late term abortion in sheep herd (Table 2). The annual economic losses were calculated as Rs. 2745/sheep (Table 3).

Table 1. Seropositive status of sheep herd.

Adult Animals	Number	Seropositive
Female	140	74(53%)
Male	4	01(25%)
Total	144	75(52%)

Fig.s in parenthesis shows percentage

Table 2. Identification of Isolates by cultural, biochemical, serological, staining and molecular technique.

Test	Isolate
Agglutination with Acriflavin	-
Catalase	+
Oxidase	+
Urease	+
H_2S	-
Basic Fuchsin	+
Thionin	+
Streptomycin	-
CO_2	-
A	+
M	+
A+M	+
Nitrate Reduction	+
Colony Morphology	Smooth
Modified Ziehl-Neelsen Staining	Acid Fast
Gram's Staining	Gram negative
Phenotypic Appearance	Cocco Bacilli
OMP31 (Species specific segment)	Amplified

Table 3. Economic losses in small ruminants.

Sr. No.	Components of economic loss	Number of animals contributing loss	Total Economic loss (Pak rupees)	Economic losses per sheep (Pak rupees)
1	Loss due to reduction in meat yield by abortion and still birth	60*	17928	299
2	Loss due to reduction in meat yield by increased lambing interval	74**	21349	289
3	Loss due to reduction in milk yield by abortion and still birth	60	23942	426
4	Loss due to reduction in milk yield by increased lambing interval	74	28511	385
5	Veterinary and extra management expenses	74	113486	1344
6	Cost of decreased wool yield	74	170	2.29
Total				2745

* aborting ewes;**aborting plus repeat breeder

Discussion

In current study, sera sample were tested through RBPT and I-ELISA that is similar to Diaz *et al.* (2011) who suggested combination of two serological tests for confirmation of brucellosis. The reason of confirming our RBPT results through I-ELISA was to increase authenticity of brucellosis sero-positivity. Culture and isolation techniques were used for identification of *B. meltensis* in current study that is in complete agreement with a study conducted in Iran where isolates obtained from aborted sheep fetuses were identified by using standard microbiological methods (Behroozikhah *et al.*, 2012). In current study fetal stomach contents were used for isolation of *B. meltensis* as fetal stomach is considered preferred site for isolation of brucellae (Sahin *et al.*, 2008). In the present study annual losses due to brucellosis in small ruminant were calculated as Rs.2745/sheep. Other studies have reported economic losses due to brucellosis in different countries (Anon, 1995; Brisibe *et al.*, 1996; Sam-Bittner, 2004; Salih *et al.*, 2010; Sulima and Venkataraman, 2010; Singh *et al.*, 2014; Bamaiyi *et al.*, 2015). For example, a study conducted in India indicated annual economic loss of Rs.1180/- per sheep and Rs.2121.82/goat on an average (Sulima and Venkataraman, 2010). Another study estimated a loss of US $ 0.7 per sheep and US $ 0.5 per goat for *Brucella* affected Indian livestock populations (Singh *et al.*, 2014). A study conducted in Malaysia calculated losses in goats due to *Brucella* infection and reported an average loss of RM 503.91 per goat, parameter used for estimating economic loss were number of animals culled, farm value, herd size, weight loss of goats, cost per unit of animals and farm economic impact (Bamaiyi *et al.*, 2015). A little variation in amount of economic loss per sheep in current study may be due to difference in management expenses, veterinary charges, Pakistani currency value or miscellaneous contributing factors.

Brucella infection is surely obstructing economic growth of livestock industry in Pakistan as it directly affects the farmer. Such losses not only affect the livestock sector of Pakistan but also have huge impact on the national economy as agriculture sector accounts for 19.82 percent of Gross Domestic Product (GDP) in Pakistan and livestock contributes for 58.55% of agriculture sector (Economic survey of Pakistan, 2015-16). Current study has only estimated losses due to reduced milk production, abortion, still birth, repeat breeding, veterinary expenses, wool yield lost due to culling of animal, increased cost of management due to rearing of nonproductive animals and weak lambs. Losses due to culling of *Brucella* infected adult animal, losses based on the feeding expenses, the hours spent by the farmer in taking care of sheep, amount spent on other farm utilities and miscellaneous inputs at the farm were not estimated due to lack of information/data. If these costs would had been included that might have doubled currently estimated economic losses.

Conclusion

Brucellosis has a significant economic impact on the livestock production in Pakistan. The losses suffered by farmers due brucellosis can be minimize by developing a strategy for control of brucellosis.

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