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# Location, sex - and age - wise prevalence of brucellosis in camels in Bikaner region of India assessed through seropositivity by both Rose Bengal plate test and ELISA

**Neharika Saxena and Rajani Joshi**

**Abstract**
Blood samples from 177 camels (108 males and 69 females) from Bikaner and nearby villages Gadwala, Gadola and Naurangdesar were analyzed for Brucellosis by Rose Bengal Plate Test (RBPT). Fifteen camels [7 males and 8 females] were positive. Prevalence in camels by RBPT was 8.47%. Out of 166 camel sera analyzed by ELISA, four were positive. The prevalence through ELISA was 2.25%. Out of 15 RBPT positive sera, 4 (26.66%) were also positive by ELISA. Combined prevalence (both RBPT and ELISA) in camels was 1.61% in Bikaner and 6.12% in Naurangdesar, respectively. The study revealed that Brucellosis is prevalent in camels in Bikaner and adjoining villages in Rajasthan state of India and is of public health significance being a zoonotic disease transmissible to humans in contact with camels or consuming raw milk or handling meat.

**Keywords:** Camel brucellosis, brucellosis, *Brucella*, Bikaner, prevalence, Rajasthan

## 1. Introduction
According to the FAO Live Animals' Statistics (FAO, 2019) [13], the worldwide camel population is about 35 million heads. Among the large camelids (Dromedary and Bactrian), Dromedary camels compose about 95% of the population (Bornstein and Younan, 2013) [7]. As per the 20th Livestock Census of India, the total camel population in India was 2.5 Lakhs (0.25 million) in 2019. This included 0.08 million males and 0.17 million females (Vikaspedia, 2021) [39]. Rajasthan had the highest camel population across India, at about 213 thousand in 2019 (Statista, 2021) [38].

Camels have developed, through millennia, the ability to produce quality meat, milk, and fiber in some of the hottest and most hostile environments in the globe. Due to their unique physiology and in light of the current climate change impacts on ecosystems, camels are excellent candidate species for production (Hoffmann, 2010) [20]. Camels can not only contribute in boosting food security but also in job creation, poverty alleviation and economic diversification. They can browse and graze on a wide range of plants that are avoided by or are inaccessible to other livestock such as thorny bushes.

Beside their adaptation to harsh environments, camels are multipurpose animals used for milk and meat production, hair/felt, racing, transportation, and tourism. Camels also have a slow metabolism which results in comparatively less feed requirements compared to other ruminant livestock. As a result, they produce less methane on the basis of body mass index (Dittmann *et al*., 2014) [11]. Moreover, camels' milk and meat are highly nutritional and are comparable and sometimes deemed better than cattle beef and milk. For instance, camel meat contains less fat than lamb or beef (Kadim *et al*., 2008) [22] and its protein quality, assessed by the index of essential amino-acids in meat, is the highest among red meat (Raiymbek *et al*., 2015) [31]. Its milk contains between 3 and 10 times more vitamin C than cows' milk (Faye, 2011; Konuspayeva *et al*., 2009) [14, 24]. It also contains lower β-casein and no β-lactoglobulin resulting in its hypo-allergic property (Konuspayeva *et al*., 2009) [24]. During the last decade, demand for camel milk and meat products have increased both locally (in arid regions) and internationally with products varying from milk and its derivatives to beauty products to hump fat (Gossner *et al*., 2014) [14].

The Food and Agriculture Organization estimated the gross production value of camel milk to be US$ 342 million and of meat to be US$1000 million in 2009 (FAO, 2011) [12]. Camels are mostly reared by nomads in Africa and Asia for meat, fiber (hair and wool), milk and transport and its dung is used for fuel. About 5% of the total milk produced in sub-Saharan regions of Africa is contributed by camels.

Camel dairy products in arid and semi-arid areas have given the nomadic livestock herders a rich source of income along with a source of food (FAO, 2019) [13]. In the arid areas of Asia and Africa, there are many developing countries where camel holds a key position as a livestock for nomadic and rural populations (Gwida *et al.*, 2012) [18].

Camels are highly susceptible to Brucellosis caused by *Brucella melitensis* and *Brucella abortus*, both of which are pathogenic to man (Omer *et al.*, 2010) [29]. Camel Brucellosis can be readily transmitted from camels to humans by milk or its products (Dawood, 2008) [10]. Camel Brucellosis is a significant but neglected disease in India. Camel Brucellosis on the animal - human interface is an emerging problem from public health point of view. There have been few studies on prevalence of Brucellosis in camels and the risk of Brucellosis at the camel - human interface in Thar desert of India. Therefore, the present study was undertaken to determine the seroprevalence of Brucellosis in camels in desert areas of Bikaner and adjoining villages in the Rajasthan state of India.

## 2. Materials and Methods

The present study was carried out on camels to find out the prevalence of brucellosis employing serological methods in and around Bikaner region of Rajasthan state of India. RBPT and ELISA are the most widely used tests for the laboratory diagnosis of Brucellosis in cattle, camel and humans (Alton, 1990) [2]. To diagnose Brucellosis in suspected serum samples, approved serological tests - Rose Bengal Plate Test and Enzyme Linked Immunosorbent Assay were carried out.

Serum samples were collected from camels from farms as well as individual owners in rural households from Gadola, Gadwala and Naurangdesar villages and Bikaner. The experimental work for the study was carried out at the Department of Veterinary Public Health and the Department of Microbiology and Biotechnology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan.

The investigation was approved and informed consent was obtained.

### 2.1 Camel serum samples analyzed

A total of 177 serum samples from camels were included in this study. Out of these, 108 were males and 69 were females. The samples were collected from Bikaner, Gadwala, Gadola and Naurangdesar villages. The age of camels ranged from 0.2 to 20 years (Table 1).

Blood was collected from 177 adult camels including those suspected for or having characteristic lesions of Brucellosis in and around Bikaner, Gadwala, Gadola, and Naurangdesar villages. Serum was separated from clotted blood and stored at -20$^0$C till use. Blood samples collected aseptically from the camels were kept in a slanting position for the separation of serum from the blood clot. After retraction of the clot, serum was separated by centrifugation at 1200 rpm for 15 minutes. The serum samples were preserved at -20˚C in serum collection vials for conducting further serological studies. Serum samples from camels were subjected to analysis with the common diagnostic techniques – Rose Bengal Plate Test (RBPT) and Enzyme Linked Immunosorbent Assay (ELISA).

### 2.2 Rose Bengal Plate Test (RBPT)

RBPT was conducted as per the method of Morgan *et al.* (1978) [26]. RBPT antigen obtained from Punjab Veterinary Vaccine Institute, Ludhiana, India was used for the test. Serum samples and RBPT antigen were brought to the room temperature and one drop (30 µl) of serum was placed on a clean, dry and non-greasy glass slide with the help of a micropipette. The antigen bottle was shaken well to ensure homogenous suspension and one drop of antigen was added. The test serum and antigen were mixed with the help of a clean sterilized toothpick and slide was gently rotated for four minutes. Negative control known brucellosis negative serum and positive control was known brucellosis positive serum. The result was noted after four minutes. RBPT antigen and normal saline solution were mixed thoroughly on a separate glass slide in order to detect auto agglutination. Definite clumping / agglutination were considered as positive reaction, whereas no clumping / agglutination were considered as negative reaction. Grading / degree of agglutination as per duration of time were as following (Alton *et al.*, 1975) [3]:

(i) 0-30 sec., quick = ++++
(ii) 30 sec.-2 min. = +++
(iii) 2-3 min. = ++
(iv) 3-4 min. = +
(v) Negative = -

### 2.3 Enzyme Linked Immunosorbent Assay (ELISA) on camel sera

AsurDx$^{TM}$ Brucella Antibodies Test Kit **(**Biostone Animal Health, Dallas, USA) was used for conducting ELISA on camel sera. The kit is designed for the detection of IgG antibodies specific to *Brucella abortus* and *Brucella melitensis* in the serum. The O.D. was read at 450 nm using Multiskan Go ELISA Reader (Thermo Scientific, USA).

**2.3.1 Principle**: The method is based on a colorimetric ELISA. Purified *Brucella* antigens have already been coated in the plate wells. During analysis, diluted serum sample is added. If antibodies to *B. abortus* or *B. melitensis* are present in the sample, they will bind to the coated antigen. The secondary antibody, tagged with a peroxidase enzyme, targets anti-*Brucella* antibodies, and the resulting color intensity, after the addition of substrate, is related to the amount of anti-*Brucella* antibodies in the sample.

**2.3.2 Sample preparation:** Serum samples were diluted to 20-fold dilution using lx Diluent Solution at room temperature and used immediately.

**2.3.3 Reagent preparation:** All frozen reagents were brought up to room temperature before use (1- 2 hours at 20 - 25°C); Solutions were prepared just prior to performing the ELISA. All reagents were mixed by inversion prior to use.

**2.3.4 Preparation of 1x Diluent Solution**

One volume of 10x Diluent Solution was mixed with 9 volumes of distilled water.

**A. Preparation of 1x Wash Solution**

One volume of 20x Wash Solution was mixed with 19 volumes of distilled water.

**ELISA Protocol:** All controls were run in duplicate.

1. The *Brucella* antigen-coated plate and all reagent components were brought to room *Brucella* temperature for an hour.
2. Assay Diluent (90µL) was added to each well of antigen coated plate.

3. *Brucella* Negative Control (10 µL/well) was aliquoted to two wells of the antigen coated plate.
4. *Brucella* Positive Control (10 µL/well) was aliquoted to two wells of the antigen coated plate.
5. Added 10µL of previously diluted serum sample (1:20) per well.
6. The solution in the wells was mixed by gently rocking the plate manually for 1 minute.
7. The plate was covered with foil and incubated for 45 minutes at room temperature avoiding direct sunlight and air vents during incubation.
8. The solution in the wells was discarded.
9. The plate was washed by adding 250 µL of 1x Wash Solution to each well of the plate. The wash solution was discarded and tapped dry on paper towels. This step was repeated four additional times for a total of five washes. The next step was performed immediately. The plate was not allowed to dry.
10. Added 100 µL of HRP-Conjugated Secondary Antibody to each well of the plate. The plate was covered with foil and incubated for 30 minutes at room temperature.
11. The plate was washed by adding 250 µL of 1x Wash Solution to each well of the plate. The wash solution was discarded and tapped dry on paper towels. This step was repeated two additional times for a total of three washes.
12. Added 100 µL of TMB Substrate to each well of the plate. The plate was covered with foil and incubated for 15 minutes at room temperature.
13. Added 100 µL of Stop Solution to each well. The plate was read as soon as possible on a plate reader at 450 nm wavelength.

**Reading the results**

a) The optical density (OD) of the wells was measured at 450 nm within 15 minutes after color development has been stopped.
b) The mean OD of the Positive Control and the mean OD of the Negative Control were calculated.
c) The percent positivity (PP) of all samples was calculated and expressed as percent positivity (PP) relative to the mean OD of the Positive Control as below:

$$PP = \frac{OD_{450} \text{ of test sample}}{\text{Mean } OD_{450} \text{ of Positive Control}} \times 100$$

**Interpretation of the result**

a) The PP for Negative Control must be < 40%
b) The mean OD of the Positive Control must be $\geq$ 0.5
The results of the specific test run not meeting any of these criteria were discarded.
c) serum samples with

PP < 40% (negative) indicated that *Brucella* antibodies were absent in the test sample.
PP $\geq$ 40% (positive) indicated that *Brucella* antibodies were present in the test sample.

**Statistical analysis of data**

The data was analyzed by utilizing MedCalc software online.

## 3. Results

There have been few studies on prevalence of Brucellosis in camels and the potential risk of Brucellosis on the camel - human interface. The present study was therefore undertaken on camels in and around Bikaner region of Rajasthan state of India to assess the extent of prevalence of Brucellosis in camels since the disease causes great economic losses and is of public health importance.

### 3.1 Camel samples analyzed

A total of 177 serum samples from camels were included in this study. Out of these, 108 were males and 69 were females. Out of 177 samples collected, 62, 36, 30 and 49 samples were from Bikaner, Gadwala, Gadola and Naurangdesar villages, respectively. The age of camels ranged from 0.2 to 20 years.

### 3.2 Analysis of camel sera by RBPT

Out of 177 camels, 15 were positive by RBPT (Table 2). The prevalence of Brucellosis in camels was found to be 8.47% by RBPT.

### 3.3 ELISA on camel sera

A total of 166 camel serum samples were subjected to ELISA. Out of 166 samples, four were positive by ELISA (Table 3). One was male and 3 were females. The overall seroprevalence elucidated through ELISA was 2.25%. Out of 15 RBPT positive sera, 4 (26.66%) were also positive by ELISA (Table 4).

**3.4 Prevalence by RBPT and ELISA taken together:** Since RBPT detects antibodies to particulate antigens whereas ELISA detects antibodies to soluble antigens, prevalence was calculated taking into account results of both RBPT and ELISA for confirmatory results.

**3.5 Location – wise prevalence in camels by RBPT and ELISA combined:** Prevalence in camels by RBPT and ELISA combined was found to be 1.61% in Bikaner and 6.12% in Naurangdesar, respectively (Table 5, Fig. 1).

**3.6 Sex - wise prevalence in camels by RBPT and ELISA combined:** Prevalence in camels by RBPT and ELISA combined was found to be 0.92% in males and 4.34% in females, respectively (Table 6, Fig. 2).

**3.7 Age - wise prevalence in camels by RBPT and ELISA combined:** Among the camels positive by RBPT and ELISA combined, 50% camels were between 5 – 8 years of age and 25% each were aged between 8 – 12 years, and those above 12 years of age, respectively (Table 7, Fig. 3). Prevalence was 3.22% in 5-8 yrs age, 1.85% in 8-12 years age and 2.22% in camels of more than 12 years age.

## 4. Discussion

Mathur and Bhargava (1979) [25] reported seroprevalence of Brucellosis in camels in Jorbeer village of Rajasthan to be 3.8%. Camels are usually kept mixed with ruminant species in Indian households and farms and cattle have been considered to be the potential source of infection for camels. Seroprevalence of camel brucellosis tends to follow two distinct trends with a low prevalence of below 5% in nomadic or extensively kept camels and a high prevalence of 8–15% in intensively or semi-intensively kept camels (Abbas and Agab, 2002) [1]. Various Biotypes trigger the infection like *B. abortus* and *B. melitensis*. Bitter (1986) [5] examined 948 camels from various herds in eastern Sudan and recorded 16.5 – 32.3 percent prevalence. Musa (1995) [27] studied 416

camels from seven herds of western Sudan owned by nomads, found a prevalence rate of 23.3 percent, and concluded that camels ranked second only to cattle in the rate of *Brucella* infection. The spread of brucellosis in camels depends on the species of *Brucella* that are prevalent in other animals in their habitat.

Khadjeh *et al*. (1999) [23] studied camel brucellosis in Boushehr province of Iran during the year 1997. A total of 258 serum samples were collected and serologically examined. Out of these samples, 5 cases (1.93%) showed laboratory evidence of *Brucella* infection. In bacteriological examination, the lymph nodes of all serologically positive camels were cultured. *Brucella melitensis* biotype 1 was isolated from two cultures.

Dawood (2008) [10] carried out a study of the prevalence of camel brucellosis in the south province of Jordan during the years 2006 and 2007. Six hundred forty camel sera from 44 herds were randomly collected and analyzed. Rose Bengal plate test was used to screen all serum samples. The positive samples were subjected to confirmation by complement fixation test. The true prevalence of *Brucella* seropositive was 15.8%. *Brucella melitensis* biotype 3 was isolated from 2 aborted fetuses and from 2 milk samples. 64.8% of the positive camels were adults more than 4 years old and the remaining 35.2% were young ranging from 6 months to 4 years old.

Abbas and Agab (2002) [1] studied the seroprevalence of brucellosis in camels. They speculated that it follows two distinct patterns: low (2-5%) prevalence in nomadic or extensively kept camels and high (8-15%) prevalence in camels kept intensively or semi-intensively.

Hadush *et al*. (2013) [19] conducted a cross-sectional study in three selected districts of Afar region of Ethiopia to determine the seroprevalence of camel brucellosis. A total of 1152 camels from 168 camel herds were included in the study. All serum samples were consequently tested and confirmed serologically using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Risk factors analysis was also conducted using multivariable and univariate logistic regression analysis. As a result, 58 (5.0%) were RBPT reactors in which 47 (4.1%, 95% CI: 2.9 to 5.3%) were confirmed to be positive using CFT and at least one reactor camel was found in 37 (22.0%) of the total herds sampled. The statistical analysis indicated that herd size and contact with other ruminants were the major risk factors for the presence and transmission of the disease between animals. In addition, pluriparous (4.7%), abortive (5.7%), pregnant (6.6%) and lactating (4.1%) camels were found with higher seropositivity which contributed in transmission of the disease to calves, other ruminants as well as to humans, but this was not a statistically significant association ($P > 0.05$).

In our present study, the overall prevalence in camels through ELISA was 2.25%. In a study conducted on 78 camels by Shome *et al.* (2013) [36] during 2008-2012 in Rajasthan state of India, a prevalence of 4.9% and 8.9% by ELISA and RBPT respectively was reported.

In our present study, out of 15 RBPT positive sera, 4 (26.66%) were also positive by ELISA. Combined prevalence (both RBPT and ELISA) in camels was 1.61% in Bikaner and 6.12% in Naurangdesar, respectively. Sex-wise combined prevalence was 0.92% in males and 4.34% in females. Among the double positive camels, 50% camels were aged 5 – 8 years and 25% each were aged 8 – 12 years, and above 12 years of age, respectively.

Chauhan *et al.* (2017) [8] conducted a study to determine the *Brucella* specific antibodies in camel from three camel rearing districts of Gujarat using RBPT, i-ELISA and MRT. On screening of 352 serum samples, 41(11.64%) and 16 (4.54%) samples found positive by RBPT and i-ELISA, respectively. 8 (15.38%) samples of milk were found positive from total 52 samples by MRT. It was also revealed from the study that the female camels have high seroprevalence of brucellosis than males. Hosein *et al.* (2016) [21] carried out a serological study using 1126 blood samples from Dromedary camels. The modified Rose Bengal Plate Test (mRBPT) and competitive ELISA (cELISA) were used as screening and confirmatory tests, respectively. The overall sero-prevalence of *Brucella* antibodies was 4.17% and 3.73% as detected by the mRBPT and c-ELISA respectively.

Rose Bengal test is fast, but in its chronic form this test has many false-negative results (Roushan *et al*., 2005) [32]. A high proportion of animals in infected areas give results negative in RBT, but positive in CFT (Blasco *et al*., 1994) [6]. Different diagnostic tests have been validated for diagnosing Brucellosis in camels and humans, but only the Rose Bengal test (RBT) and the complement fixation test (CFT) are approved for diagnosis of camel and human Brucellosis in the European Union (EU) legislation on intra-community trade (Council Directive 91/68/EEC). However, there is evidence that both tests are less sensitive and specific for the diagnosis of Brucellosis in camels and humans than in cattle (Blasco *et al*., 1994; Garin-Bastuji *et al*., 1998) [6, 15].

The classical Rose Bengal Plate Test (RBPT) is often used as a rapid screening test for the diagnosis of brucellosis (Oomen and Waghela, 1974; Ruiz-Mesa *et al*., 2005) [30, 34]. The sensitivity of RBPT is high but the specificity can be disappointingly low (Barroso *et al*., 2002) [4]. As a result, the positive predictive value of the test is low and a positive test result thus requires confirmation by a more specific test (Smits and Kadri, 2005) [37]. The RBPT could sometimes give a false positive result because of S19 vaccination or of false positive serological reactions (OlE, 2009). In endemic areas, there is a low level of antibody titer in normal population, making it difficult to set up a threshold to balance the sensitivity and specificity, which causes some false positive as well as false negative results. In endemic areas, there is background positivity in normal population, which may lead to false positive results. The positivity of agglutination test decreases as the disease prolongs. Even in acute stage with positive bacterial culture, patients can have false negative results. Another potential problem for agglutination test is the presence of cross reactivity with other bacteria, such as *Yersinia enterocolitica*, *Salmonella urbana* group N, *Vibrio cholera*, *Escherichia coli* O:157 and *Francisella tularensis*, causing false positivity. Gram negative bacteria such as *Campylobacter fetus* and *Bordetella bronchiseptica* may cross react with smooth *Brucella* spp. (Corbel, 1985) [9].

## 5. Tables and figures

**Table 1:** Sex and age-wise distribution of camels included in the study

| S. No. | Location | Numbers | | Total | Age range (years) |
|---|---|---|---|---|---|
| | | Males | Females | | |
| 1 | Bikaner | 41 | 21 | 62 | 0.2 - 18 |
| 2 | Gadwala | 32 | 4 | 36 | 5 - 17 |
| 3 | Gadola | 5 | 25 | 30 | 3 - 20 |
| 4 | Naurangdesar | 35 | 14 | 49 | 3 - 10 |

**Table 2:** Camel serum samples positive for Brucellosis by RBPT

| S. No. | Camel number | Age (yrs) | Sex | RBPT | Location |
|---|---|---|---|---|---|
| 1 | CB1 | 10 | Male | + | Bikaner |
| 2 | CB6 | 10 | Male | + | Bikaner |
| 3 | CB10 | 8 | Female | + | Bikaner |
| 4 | CB12 | 8 | Male | + | Bikaner |
| 5 | CB16 | 9 | Female | + | Bikaner |
| 6 | CB59 | 18 | Male | + | Bikaner |
| 7 | CB60 | 18 | Female | + | Bikaner |
| 8 | CB61 | 18 | Female | + | Bikaner |
| 9 | CW14 | 17 | Female | + | Gadwala |
| 10 | CW19 | 13 | Male | + | Gadwala |
| 11 | CW27 | 13 | Male | + | Gadwala |
| 12 | CO9 | 3 | Female | + | Gadola |
| 13 | CN3 | 9 | Female | + | Naurangdesar |
| 14 | CN5 | 7 | Female | + | Naurangdesar |
| 15 | CN6 | 8 | Male | + | Naurangdesar |

**Table 3:** Camel serum samples positive for Brucellosis by ELISA

| S. No. | Camel number | Age (yrs) | Sex | ELISA | Location |
|---|---|---|---|---|---|
| 1 | CB61 | 18 | Female | + | Bikaner |
| 2 | CN3 | 9 | Female | + | Naurangdesar |
| 3 | CN5 | 7 | Female | + | Naurangdesar |
| 4 | CN6 | 8 | Male | + | Naurangdesar |

**Table 4:** Camel sera positive for Brucellosis by RBPT and / or ELISA

| S. N. | Camel number | Age (yrs) | Sex | RBPT | ELISA | Location |
|---|---|---|---|---|---|---|
| 1 | CB1 | 10 | Male | + | - | Bikaner |
| 2 | CB6 | 10 | Male | + | - | Bikaner |
| 3 | CB10 | 8 | Female | + | - | Bikaner |
| 4 | CB12 | 8 | Male | + | - | Bikaner |
| 5 | CB16 | 9 | Female | + | - | Bikaner |
| 6 | CB59 | 18 | Male | + | - | Bikaner |
| 7 | CB60 | 18 | Female | + | - | Bikaner |
| 8 | CB61 | 18 | Female | + | + | Bikaner |
| 9 | CW14 | 17 | Female | + | - | Gadwala |
| 10 | CW19 | 13 | Male | + | - | Gadwala |
| 11 | CW27 | 13 | Male | + | - | Gadwala |
| 12 | CO9 | 3 | Female | + | - | Gadola |
| 13 | CN3 | 9 | Female | + | + | Naurangdesar |
| 14 | CN5 | 7 | Female | + | + | Naurangdesar |
| 15 | CN6 | 8 | Male | + | + | Naurangdesar |

**Table 5:** Location – wise prevalence of Brucellosis in camels by both RBPT and ELISA

| Location | Count (percentage) | | | | Prevalence |
|---|---|---|---|---|---|
| | Total Examined | RBPT +ve | ELISA +ve | Both +ve | |
| Bikaner | 62 | 6 (40.0%) | 1 (25.0%) | 1 (25.0%) | 1.61% |
| Gadwala | 36 | 3 (20.0%) | 0 (0.0%) | 0 (0.0%) | 0.0% |
| Gadola | 30 | 1 (6.7%) | 0 (0.0%) | 0 (0.0%) | 0.0% |
| Naurangdesar | 49 | 5 (33.3%) | 3 (75.0%) | 3 (75.0%) | 6.12% |
| Total | 177 | 15 | 4 | 4 | 2.25% |

**Table 6:** Sex – wise prevalence of Brucellosis in camels by RBPT and ELISA combined

| Sex | Count (percentage) | | | | Prevalence |
|---|---|---|---|---|---|
| | Total Examined | RBPT +ve | ELISA +ve | Both +ve | |
| Male | 108 | 5 (33.3%) | 1 (25.0%) | 1 (25.0%) | 0.92% |
| Female | 69 | 10 (66.7%) | 3 (75.0%) | 3 (75.0%) | 4.34% |
| Total | 177 | 15 | 4 | 4 | 2.25% |

**Table 7:** Age–wise prevalence of Brucellosis in camels by RBPT & ELISA combined

| Age | Count (percentage) | | Prevalence |
|---|---|---|---|
| | Total examined | Both (RBPT & ELISA) Positive | |
| < 5 Years | 16 | 0 (0.0%) | 0.0% |
| 5-8 Years | 62 | 2 (50.0%) | 3.22% |
| 8-12 Years | 54 | 1 (25.0%) | 1.85% |
| > 12 Years | 45 | 1 (25.0%) | 2.22% |
| Total | 177 | 4 | 2.25% |

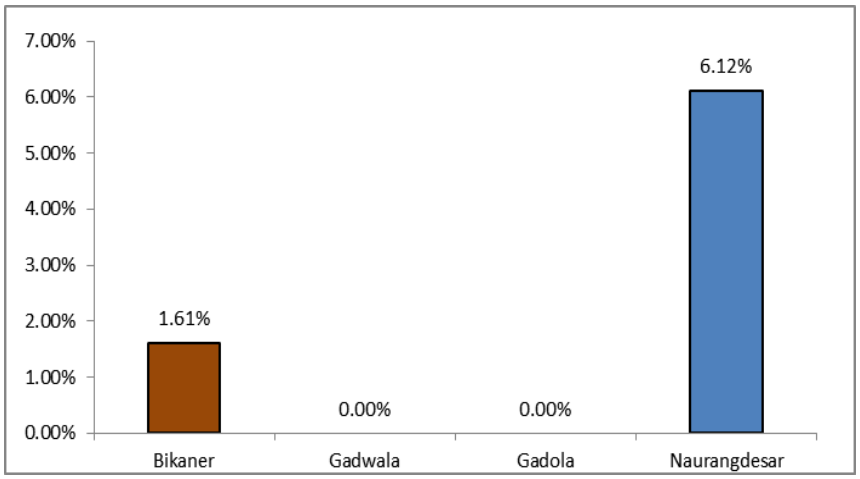


**Fig 1:** Location–wise prevalence of Brucellosis in camels by RBPT and ELISA combined

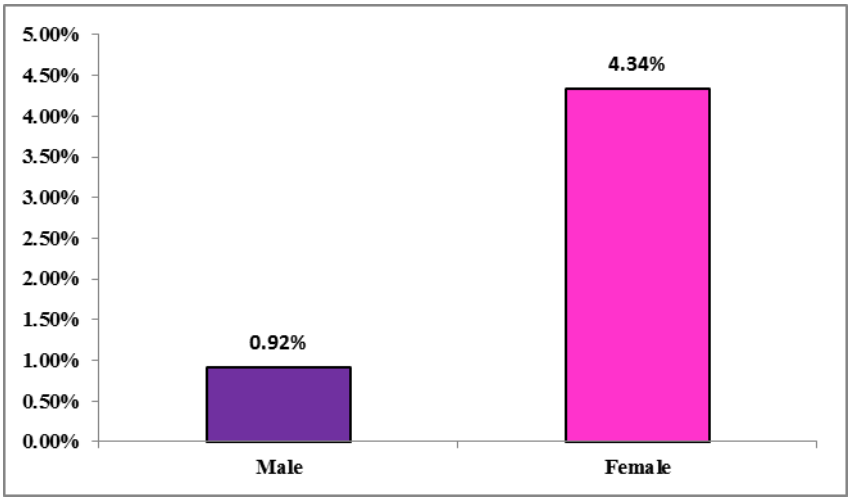


**Fig 2:** Sex – wise prevalence of Brucellosis in camels by RBPT and ELISA combined

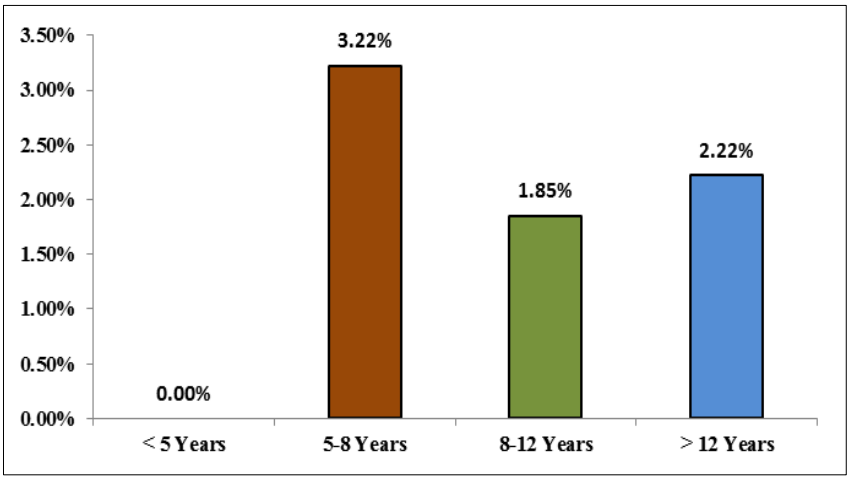


**Fig 3:** Age - wise prevalence of Brucellosis in camels by RBPT & ELISA combined

Sanaei *et al* (2012) [35] opined that one single serodiagnostic test may not be reliable and to get a more accurate diagnosis, a combination of RBPT and ELISA may be recommended. It is believed that serological tests used for *B. abortus* infection in cattle are also adequate for diagnosis of *B. melitensis* infection in camels and humans. However, the serological findings and conclusions drawn should be considered carefully, because the classical serology tests CFT, RBT and SAT for the *Brucella* spp have been licensed for use only in livestock (Godfroid *et al*., 2002) [16].

## 6. Conclusion

The present study was undertaken to assess the extent of prevalence of Brucellosis in camels in and around Bikaner region of Rajasthan state of India. The overall prevalence of Brucellosis in camels was 8.47% by RBPT. The overall prevalence in camels through ELISA was 2.25%. Out of 15 RBPT positive sera, 4 (26.66%) were also positive by ELISA. Combined prevalence (both RBPT and ELISA) in camels was 1.61% in Bikaner and 6.12% in Naurangdesar, respectively. The findings of our study suggest that Brucellosis is prevalent

in camels and is a problem of serious public health concern in and around Bikaner district of Rajasthan state of India.

**7. Acknowledgement**
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**8. References**

1. Abbas B, Agab H. A review of camel brucellosis. Preventive Veterinary Medicine. 2002;55:47-56.
2. Alton GG. *Brucella melitensis*, Animal Brucellosis. In: Nielsen K and Duncan JR. Edn 1, CRC Press Inc., Boca Raton, Florida, 1990, 383-409.
3. Alton GG, Jones LM, Pietz DE. Laboratory techniques in brucellosis. World Health Organization. Monograph series. World Health Organization. 1975;55(1):1-163.
4. Barroso GP, Pelayo RC, Extremera G. Study of 1595 brucellosis cases in the Almeria province (1972–1998) based on epidemiological data from disease reporting. Review de Clinics de Espana. 2002;202(1):577-82.
5. Bitter H. Disease resistance in Dromedaries with particular reference to *Trypanosoma evansi* infection. Inaugural Desertation. Tierarticliche Hochschedule, Hanover, Germany. 1986;55(1):47-48.
6. Blasco JM, Marin C, Jimenez de Bagues M, Barberan M, Hernandez A, Molina L, *et al*. Evaluation of allergic and serological tests for diagnosing *Brucella melitensis* infection in sheep. Journal of Clinical Microbiology. 1994;32:1835-1840.
7. Bornstein S, Younan M. Significant veterinary research on the dromedary camels of Kenya: past and present. Journal of Camelid Science. 2013;6(1):1-48.
8. Chauhan HC, Patel KB, Patel SI, Patel BK, Chandel BS, Bhagat AG, *et al*. Serological survey of brucellosis in camel of Gujarat. International Journal of Current Microbiology and Applied Sciences. 2017;6(4):1815-1821.
9. Corbel MJ. Recent advances in the study of *Brucella* antigens and their serological cross reactions. Veterinary Bulletin. 1985;55(12):927-942.
10. Dawood HA. Brucellosis in camels (*Camelus dromedarius*) in the South Province of Jordan. Journal of Agricultural and Biological Sciences. 2008;3(3):623-626.
11. Dittmann MT, Runge U, Lang RA, Moser D, Galeffi C, Kreuzer M, *et al*. Methane Emission by camelids. PLoS One. 2014;9(4):e9436.
12. FAO. http://www.faostat.fao.org. 11 May, 2011.
13. FAO. Dairy production and products: Dairy animals. https://www.fao.org/dairy-production-products/production/dairy-animals/en/.July 24, 2019.
14. Faye B. Combating desertification: the added value of the camel farming. Ann. Arid Zone. 2011;50(3-4):1-10.
15. Garin-Bastuji B, Blasco JM, Grayon M, Verger JM. *Brucella melitensis* infection in sheep: present and future. Veterinary Research. 1998;29(3/4):255-274.
16. Godfroid J, Saegerman C, Wellemans V, Walravens K, Letesson JJ, Tibor A, *et al*. How to substantiate eradication of bovine brucellosis when aspecific serological reactions occur in the course of brucellosis testing. Veterinary Microbiology. 2002;90(1-4):461-477.
17. Gossner C, Danielson N, Gervelmeyer A, Berthe F, Faye B, Kaasik AK, *et al*. Human–dromedary camel interactions and the risk of acquiring zoonotic middle east respiratory syndrome coronavirus infection. Zoonoses Public Health. 2016;63(1):1-9. doi:10.1111/zph.12171.
18. Gwida M, El-Gohary A, Melzer F, Khan I, Rösler U, Neubauer H. Brucellosis in camels. Research in Veterinary Science. 2012;92(3):351-355.
19. Hadush A, Pal M, Kassa T, Zeru F. Sero-epidemiology of camel brucellosis in the Afar region of Northeast Ethiopia. Academic Journal. 2013;5(9):269-275. https://doi.org/10.5897/JVMAH13.0235.
20. Hoffmann I. Climate change and the characterization, breeding and conservation of animal genetic resources. Animal Genetics. 2010;41(1):32-46.
21. Hosein HI, Rouby S, Menshawy A, Ghazy N. Seroprevalence of camel brucellosis and molecular characterization of *Brucella melitensis* recovered from dromedary camels in Egypt. Research Journal for Veterinary Practitioners. 2016;4(1):17-24.
22. Kadim IT, Mahgoub O, Purchas RW. A review of the growth, and of the carcass and meat quality characteristics of the one-humped camel (*Camelus dromedarius*). Meat Science. 2008;80(3):555-569.
23. Khadjeh G, Zowghi E, Zarif-Fard M. Incidence of brucellosis in one-humped camels of Boushehr, Iran. Archives of Razi Institute. 1999;50(1):83-86.
24. Konuspayeva G, Faye B, Loiseau G. The composition of camel milk: a meta-analysis of the literature data. Journal of Food Composition and analysis. 2009;22(2):95-101.
25. Mathur KN, Bhargava SC. Sero-prevalence of Q fever and brucellosis in camels of Jorbeer and Bikaner, Rajasthan State. Indian Journal of Medical Research. 1979;70(11):391-393.
26. Morgan WJB, Mackinnon DT, Gill KPW, Gower SGM, Norris PIW Brucellosis diagnosis: Standard Laboratory Techniques: Weybridge, England. 1978;1:159-163.
27. Musa MT. Brucellosis in Darfur States: The magnitude of the problem and methods of diagnosis and control of the disease. PhD Thesis, University of Khartoum, Sudan, 1995, 83-96.
28. OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Edn 8th, World Organization for Animal Health, Paris, France, 2018, 1. ISBN 978-92-95108-18-9
29. Omer MM, Musa MT, Bakhiet MR, Perrett L. Brucellosis in camels, cattle, and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Reviews in Science and Technology. 2010;299(3):663-669.
30. Oomen LJ, Waghela, S. The Rose Bengal Plate Test in human brucellosis. Tropical and geographical medicine. 1974;26 (3):300-302.
31. Raiymbek G, Kadim I, Konuspayeva G, Mahgoub O, Serikbayeva A, Faye B Discriminant amino-acid components of Bactrian (*Camelus bactrianus*) and Dromedary (*Camelus dromedarius*) meat. Journal of Food Composition and analysis. 2015;41(1):194-200.
32. Roushan MR, Amin MJ, Abdoel TH, Smits HL. Application of a user-friendly *Brucella*-specific IgM and IgG antibody assay for the rapid confirmation of Rose Bengal-positive patients in a hospital in Iran. Transactions of the Royal Society for Tropical Medicine and Hygiene. 2005;99(10):744-750.
33. Ruiz-Mesa JD, Gonzalez JS, Reguera JM, Martin L,

Palmero SL, Colmenero JD. Rose Bengal Test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clin. Microbiol. Inf,. 11(3), 221-225.
34. Ruiz-Mesa JD, Gonzalez JS, Reguera JM, Martin L, Palmero SL, Colmenero JD. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2005;11(3):221-5.
35. Sanaei DA, Karimi A, Javad V, Shiva F, Fallah F, Alaei MR, *et al*. ELISA cut-off point for the diagnosis of human Brucellosis: A comparison with Serum Agglutination Test Iranian Journal of Medical Sciences**.** 2012;37(1):9-14.
36. Shome R, Gupta VK, Bhardwaj B, Shome B, Mohandoss N, Rahman H. A report of seroprevalence of camel brucellosis in India. Journal of Camel Practice and Research. 2013;20(2):183-186.
37. Smits HL, Kadri SM. Brucellosis in India: a deceptive infectious disease. The Indian journal of medical research. 2005;122(5):375-384.
38. Statista. https://www.statista.com/statistics/1078163/camel-inventory-by-state-india/. 25 July, 2021.
39. Vikaspedia. https://vikaspedia.in/agriculture/agri-directory/reports-and-policy-briefs/20th-livestock-census. 25 July, 2021.