Science, Engineering & Education, 11, (1), 2026, 103-108

Analysis of Statistical Data for Diagnosing Brucellosis in Blood Samples from Cows

Petyo Rizakov*, Lilyana Koleva

University of Chemical Technology and Metallurgy, 8 Kliment Ohridski Blvd., Sofia 1797, Bulgaria

Received 10 Semptember 2025, Accepted 05 January 2026
DOI: 10.59957/see.v11.i1.2026.14

ABSTRACT

Brucellosis is a chronic disease that often affects animals such as sheep, goats, cattle, and wild boars. Symptoms of the disease may include abortions, inflammation of the testes (orchitis), inflammation of the joints (arthritis), and infertility. People working with these animals are most at risk of contracting the disease. Early detection of brucellosis is achieved through the Rose-Bengal test. Proper sanitary and hygienic practices are also of great importance; they play a key role in preventing the spread of brucellosis.

Keywords: brucellosis, animals, abortions, blood samples, statistical analyses.

INTRODUCTION

Under natural conditions, brucellosis most commonly affects sheep, goats, cattle, pigs, and dogs. Other animal species are less frequently affected, with brucellosis occurring sporadically among them. The infection can be transmitted both vertically (from mother to offspring) and horizontally (between individuals). Infected mothers give birth to sick or infected, non-viable offspring. Transmission most often occurs through contaminated food, milk, or water [1, 2]. Brucellosis is an infectious disease that poses a threat to the lives of both animals and humans. The brucellosis microbe can take the form of a sphere or a rod.

EXPERIMENTAL

Analytical methods

The Rose Bengal Test (RBT) is a spot agglutination method that utilizes an antigen stained with Bengal rose dye and buffered to a low pH (typically 3.65 ± 0.05). The antigen for this test is prepared by suspending unnaturalised bacterial suspension of Brucella abortus S99 or S1119-3, which is centrifuged at 23 rpm for 10 min at 4°C. The resulting pellet is then resuspended in 0.5 % phenol at a ratio of 1 g in 22.5 mL. To each 35 mL of this suspension, 1 mL of 1 % Rose Bengal solution in distilled water is added, and the mixture is stirred for 2 h at room temperature. After stirring, the mixture is filtered

**Correspondence to: Petyo Rizakov, University of Chemical Technology and Metallurgy, 8 Kliment Ohridski, 1797 Sofia, Bulgaria, e-mail: petyo.rizakov@abv.bg*

through cotton cloth and centrifuged at 10 rpm until the coloured cells pellet. The pellet is then resuspended in diluent at a ratio of 1 g cells in 7 mL. The suspension should be intensely pink, and the supernatant should be free of colouring material, with a pH of 3.65 ± 0.05. Next, the suspension is filtered two more times through a pre-filter (Sartorius No. 13 430) and standardized to approximately 8 % packed cell volume (PCV). The final standardization is calibrated against serum using OIEISS and stored in the dark at 4°C. It is crucial that the antigen is never frozen.

The antigen for the Rose Bengal Test (RBT) should exhibit a clear positive reaction at a dilution of 1/45 but not at 1/55 dilution in the standard test procedure. It is recommended that new and old batches of antigens are compared using a panel of specific sera to ensure their reactivity.

The test is conducted using a pipette or micropipette to place 0.03 mL of the serum being tested onto a plate or glass slide. To the serum, 0.03 mL of antigen is added, and then the serum and antigen are mixed using a glass rod and spread over a 2 cm area. The plate is gently rocked for 4 min, and the result is read. Any positive reaction that appears after the 4 min mark is disregarded. Before starting work, two controls are always set up: one with positive brucellosis agglutinating serum and antigen, and another with saline solution and antigen. The positive brucellosis serum is used to check the antigen's activity, while the saline solution control verifies that the antigen is not self-agglutinating.

In Fig. 1, a shaker machine is shown for shaking a plate to read blood samples. The homogenization time for the samples is 3 min at 130 rpm.

In Fig. 2, a plate with applied blood samples and controls can be seen. In the positive control (P+), formations like clumps are observed, while in the negative control (P–), no such formations are observed.

Brucellosis is a disease that spreads among both animals and humans who care for them. The primary carriers of the disease are individuals working with animals (workers caring for animals, slaughterhouse employees, livestock farmers, veterinarians, hygienists, and zoo technicians) [1, 3]. Brucellosis transmission occurs through contaminated tools, work clothing, and equipment. It spreads via airborne droplets, inhalation of contaminated air, and contact with bodily fluids of infected animals. Another mode of transmission is through contaminated food or water [2].

The use of protective clothing, including gloves and goggles, is mandatory, along with regular disinfection of tools and animal enclosures. Vaccination is particularly important for female calves aged between 4 and 6 months. Personnel must routinely disinfect animal enclosures and

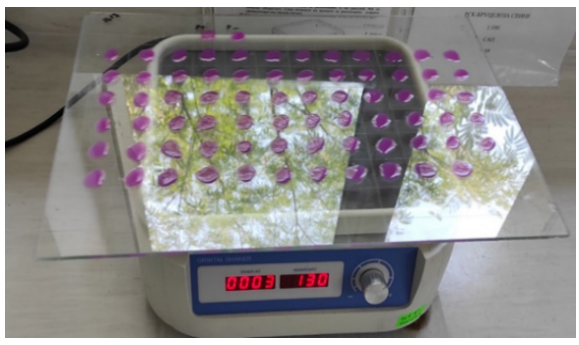

Fig. 1. Shaker machine, blood sample plate reader.

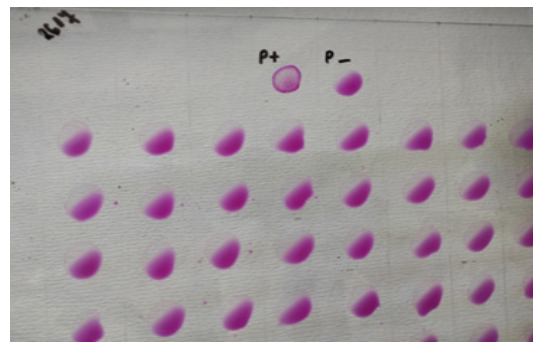

Fig. 2. Plate with applied blood samples and controls ((P+) positive and (P–) negative).

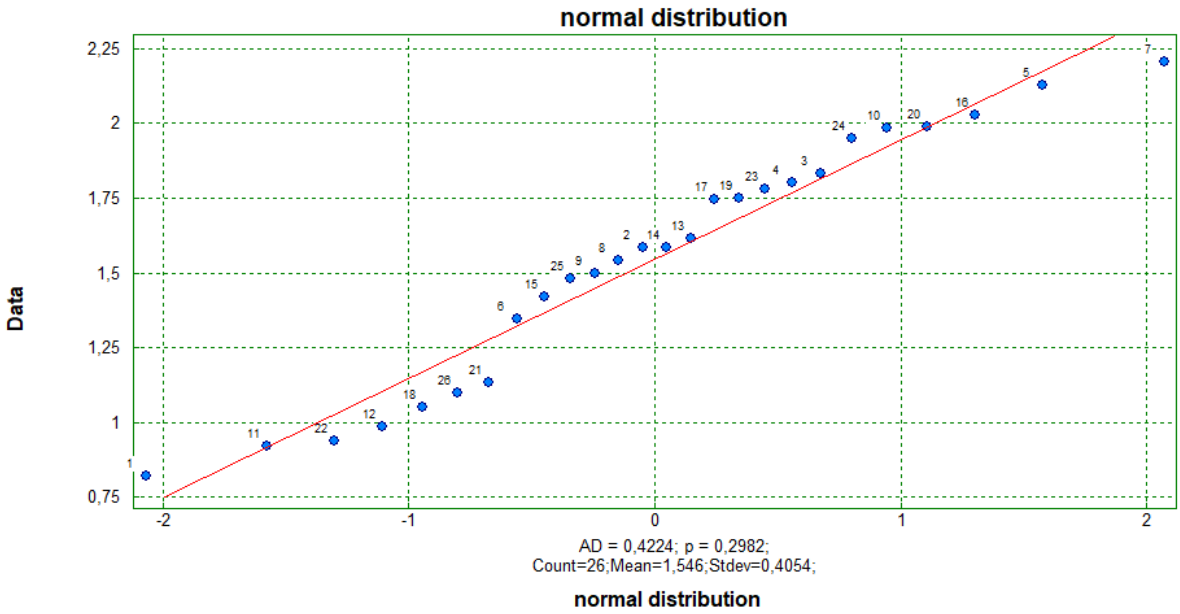

Fig. 3. Normality test of blood sample data.

strictly adhere to hygiene standards, as these are crucial for the health of both animals and humans [4, 5].

Fig. 3 presents the normal probability plot of the observed blood sample data. The distribution of the blood sample measurements can be regarded as a random variable with a normal distribution. From the figure, the following values can be noted: AD = 0.4224, the Anderson-Darling statistic; p = 0.2982; and the number of observations N = 26. The mean value of all data is $\bar{x}$ = 1.546, and the standard deviation is S = 0.4054. Based on the Anderson-Darling normality test, since p = 0.2982 is greater than the threshold value p = 0.05 (0.2982 > 0.05), it can be concluded that the distribution is normal.

Twenty-six blood samples were analysed. Fig. 3 presents a histogram of the optical density (OD) of these samples. The figure also displays the Lower Natural Tolerance Limit (LNTL) and the Upper Natural Tolerance Limit (UNTL), which indicate the range of a normally distributed variable according to the three-sigma rule.

The histogram presented in Fig. 4 for the analysis of the blood samples shows that a small proportion of the animals have values below the lower specification limit (LSL) of 1 OD. This indicates that these animals are infected with Brucella bacteria. Most of the analysed samples show values above LSL = 1 OD, which indicates that these animals are not infected with Brucella bacteria.

Fig. 5 presents the individual values from the blood sample analysis chart. It is evident that the process is under control, as all observations fall within the control limits: the lower control limit (LCL) is 0.3155 OD, and the upper control limit (UCL) is 2.7769 OD. The chart also indicates the lower specification limit (LSL) of the test, which is 1 OD. This implies that any animals with values below this threshold are infected with Brucella bacteria.

It can be seen from the control chart of individual values in Fig. 4 that four samples are below the LSL line of 1 OD, indicating that four of the tested animals have tested positive and should be separated for initiation of vaccination or antibiotic therapy. Additionally, three other animals are near the LSL = 1 OD threshold, making them suspicious, and it is advisable for

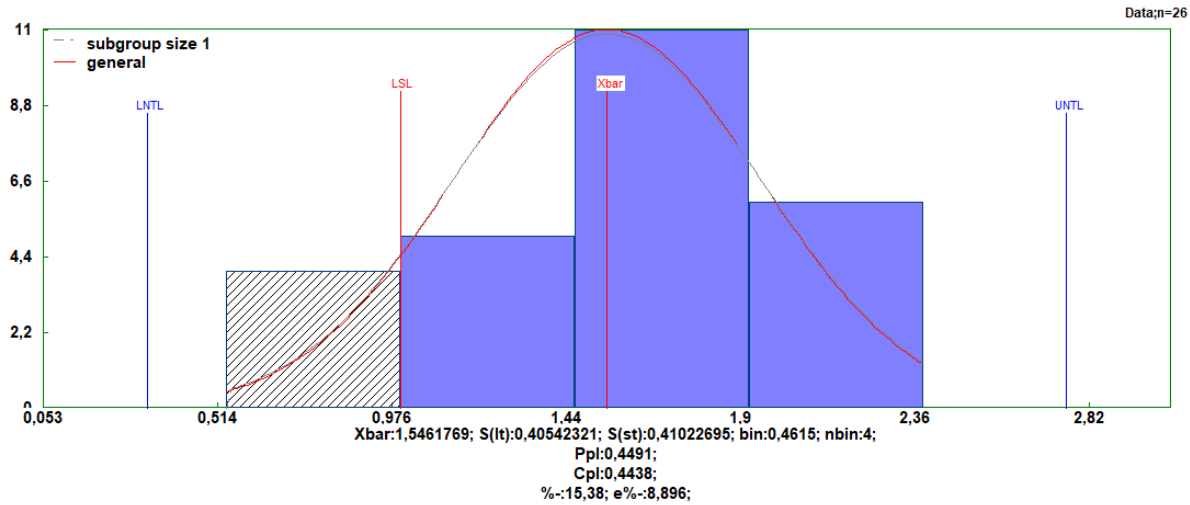

Fig. 4. Histogram of the optical density (OD) of blood samples.

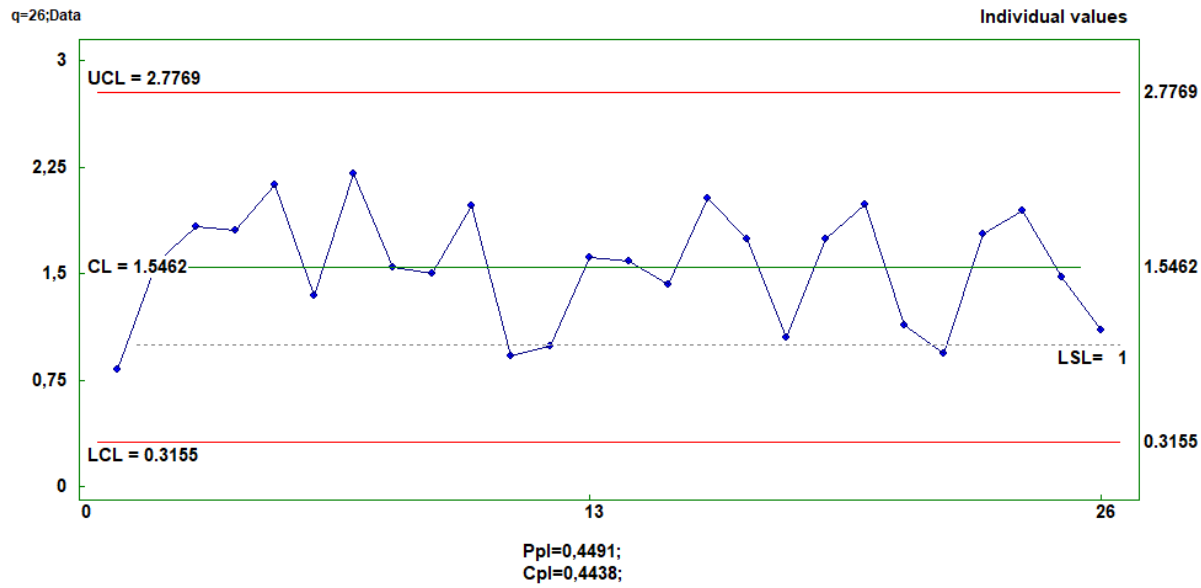

Fig. 5. Individual values, blood sample analysis chart.

them to be isolated from the rest of the animals as well. The blood test should be repeated to confirm the results and to rule out any possible equipment errors.

Analysis of variance

The initial set of 26 measurements was re-measuring two additional times using the same equipment to account for measurement variability and to enable the application of ANOVA. The results obtained from the Analysis of Variance are presented in Table 1.

From results in Table 1 can be seen that $S_x^2 < S_\varepsilon^2$ i.e., 0.00002 < 0.16442. In this case, it is concluded that the factor "measurement" is not significant.

In Fig. 6, a graph of the main effects of the measurement factor is presented. The black dots represent the mean values of the observed data groups, while the red dashed line represents the overall mean value of all measurements.

Fig. 7 presents the confidence interval plot

Table 1. Results of the analysis of variance (ANOVA).

Source of variance	Sums of squares	Degrees of freedom	Estimate of variance	Variance ratio
Factor	0.00004	2	0.00002	0.00012
Residual error	12.33128	75	0.16442	-
Total sum	12.33132	77	-	-

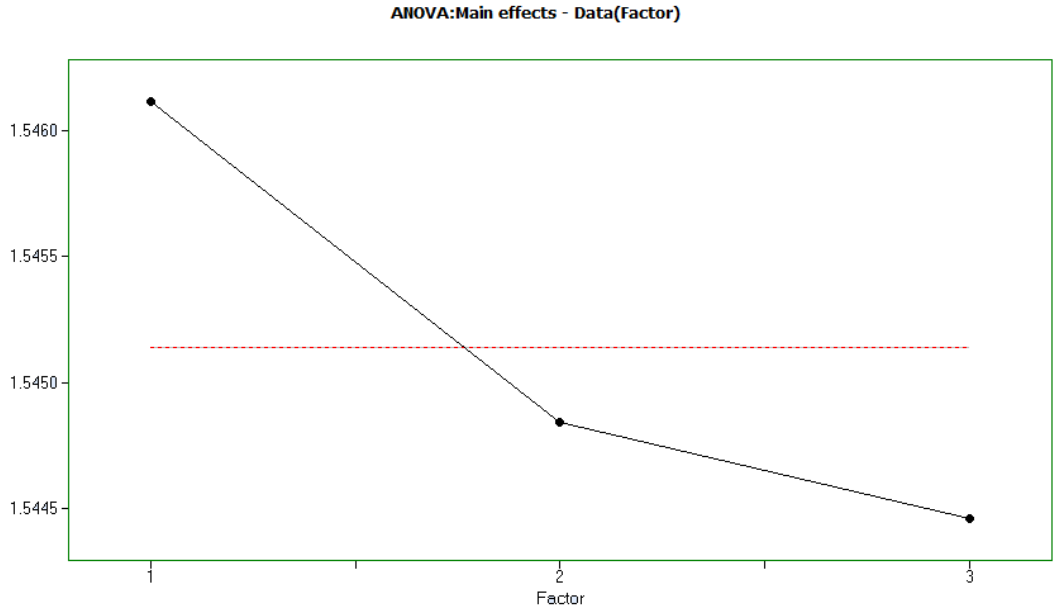

Fig. 6. Main effects of "measurement".

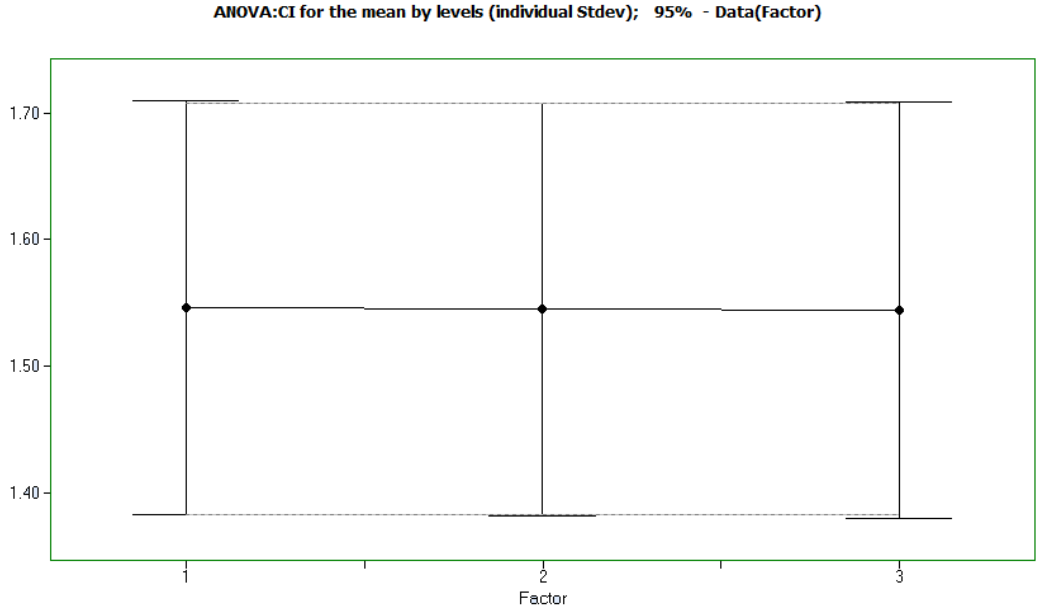

Fig. 7. Confidence intervals of the mean values for each level of the factor "measurement".

of the mean values for each level of the factor "measurement", calculated using the standard deviation corresponding to each factor level. The almost complete overlap of the confidence intervals indicates that the differences between the groups are not statistically significant.

CONCLUSIONS

The analysis of blood samples plays a crucial role in monitoring and implementing these control measures. Animals infected with brucellosis are isolated and culled. People in contact with these animals must adhere to hygiene standards and wear gloves, goggles, and masks. Foods of unregulated origin should not be consumed.

Human infection most commonly occurs through the consumption of contaminated or infected food and beverages.

From the conducted analyses, including histograms and control charts, it has been established that there are animals infected with brucellosis, as well as animals suspected of having the disease. Recommendations have been made to isolate the suspected animals from the herd and subject them to retesting.

ANOVA has shown that the "measurement" factor is insignificant and does not have a statistically significant effect on the dependent variable. The measured values obtained through different measurement methods are random and not a result of systematic influence by the "measurement" factor.

Acknowledgements

This study is funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0002, "BiOrgaMCT".

REFERENCES

1. I. Enev, Kr. Kostov, D. Ivanova, P. Badarova, Infectious Diseases in Animals, Publisher: MATCOM, 2005.
2. P.P. Penev, Milk Technology and Dairy Products, Second Edition, Hristo G. Danov State Publishing House, Plovdiv, 1962.
3. P. Ivanov, Iv. Ivanov, M. Deyanov, I. Tsachev, M. Lyutskanov, G. Mikhailov, A. Vachkov, V. Petrov, Diagnosis and Control of Infectious Diseases in Animals, Stara Zagora, 2001.
4. Brucellosis in Cattle, https://bg.farmafans.ru/kravi/25281-bruceloza-pri-kravite.html.
5. N. Dimov, Guide to Milk Control, ZEMIZDAT, State Agricultural Literature, Sofia, 1964.