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A pilot study to explore the bacterial strain interference between two laboratory adapted strains of *Brucella abortus*

Varsha Paladan, Vinod Kumar Chaturvedi and Bablu Kumar

Abstract
A pilot approach to explore the strain interference between two strains of *Brucella abortus* were attempted. Two common laboratory adapted strains of *B. abortus*, strain19 and strain 99 which could be differentiated based on their metabolic requirements and antibiotic susceptibility were selected and their interactions were studied. Experiments were made by direct inoculation of the selected strains in Brucella broth and to understand any interference of one strain on other. The interference or interaction between the selected strains was evaluated by co-cultivation and pre-culture techniques under varying conditions. The study results suggest that there is no significant difference in viable count if both the strains were inoculated together in to broth or when the S99 was inoculated into a 3h growth of S19, except for a 1-log (log_{10}) enhancement. At the same time, the growth of S99 got inhibited when S19 was inoculated 3h after the S99 inoculation into the same broth. In conclusion, there is some *in vitro* interference of S19 on S99 strains, suggesting that they could be promising candidates for protection against brucellosis if supporting evidences unveiled. The clinical significance of the obtained results must be judged with caution as the interference studies were performed *in vitro* and only two bacteria were used. The present research approach can be considered as one among the pilot studies, further studies are warranted to evaluate the preventive effect of S19 against S99 and to suggest the possible therapeutic use if feasible.

Keywords: Brucella, Bacterial Strain Interference, Strain 19 and Strain 99

1. Introduction
Brucellosis is one of the most ignored zoonoses having significant public health and economic impact in developing countries like India. This highly infectious bacterial zoonosis remains one of the most important diseases affecting livestock worldwide. As control and eradication of brucellosis from domestic livestock is cornerstone in reducing the prevalence of the disease in humans, much effort has been implemented to control and eradicate the disease in livestock. Several countries including India are adopting national level brucellosis control programs to mitigate the economic losses and to control transmission to humans.

As brucellosis causes significant economic losses in the country, the disease should be controlled on a priority basis (Singh *et al*. 2015) [28]. The key factor in any brucellosis control programme is calf hood vaccination of female calves, and *Brucella abortus* strain 19 and RB51 are the two most commonly used vaccine strains in most control programs throughout the world (Manthei, 1968; Olsen and Stoffregen, 2005; Dorneles *et al*. 2015) [18, 24, 12]. However, the vaccination cannot be solely relayed to eradicate brucellosis. Most of the countries implement test and slaughter policies. In a country like India, where cattle slaughter is legally banned because of religious sentiments, it is high time to think on therapeutic measures of disease control. There is no recommended antibiotic treatment protocol to control the disease in infected animals. For these reasons, the conventional therapeutic approach needs to be reconsidered; the future directions should be sought to find alternate therapeutics.

Bacterial interference is one of the interesting areas to explore and studies are undergoing on the application of these bacterial interferences in to clinical platform to prevent the degree of infection. These interactions can be either within the different species of bacteria or among the different strains of same bacteria. The history of bacterial interference begins right from the Alexander Fleming's famous laboratory observation that *Penicillium chrysogenum* could inhibit the growth of *Staphylococcus aureus* (Fleming, 1929) [14]. Before the advent of chemotherapy and antibiotics, many physicians had explored the possibility of interference into a therapy. Many studies have proved the use of prior infection or colonization with an avirulent *Staphylococcus aureus* to afford significant protection against the subsequent

challenge with virulent strains (Shinefield *et al*. 1963; Boris *et al*. 1964; Drutz *et al*. 1966; McCabe, 1967; Darouiche and Hull, 2000; De Gregorio *et al*. 2014; France and Remold, 2016) [26, 3, 9, 20, 8, 11, 15]. However, there are no literatures available on bacterial strain interference among the different strains of *B.abortus.* At the same time, there are studies suggesting the safe use of a live S19 vaccine strain in pregnant animals (Plommet and Fensterbank, 1976; Beckett and Mac Diarmid, 1985; Cardena *et al*., 2009; Chand *et al*. 2015) [25, 2, 4, 5]. But there is no data available on how the vaccine strain interacts with the challenge strain and prevents disease. This needs to be clarified that whether there is any strain interference between the vaccine strain and the virulent field strains. With this in mind, we thought of an alternate therapeutic based on bacterial interference. For this, we tried to explore and evaluate new strategies to prevent the colonization of virulent strains and thus the degree of infection.

With the aim to investigate this interference and to exploit its future clinical usefulness, we decided to conduct a pilot study on the bacterial interference among two different strains of *B.abortus* and to further explore the usefulness of this alternative approach in clinical management of brucellosis in the hope for a favorable outcome. Experiments were designed accordingly to investigate whether there is any increase or decrease in the growth of one strain by the interference of another strain. A culture dependent pairwise interaction study between two different strains of *B.abortus* was attempted with two most commonly used laboratory adapted strains of *B.abortus*, Strain 19 and strain 99. Strain19 (S19), a live vaccine strain with a low pathogenicity in animals, while strain 99 (S99) is virulent and can cause disease symptoms in animals, could be differentiated each other based on their erythritol utilization and penicillin sensitivity were selected for the study. We have conducted experiments in different phases in view to assess whether the use of live S19 at a lower dose could reduce the virulent strain colonization and thus the degree of disease. In particular, we explored the presence or absence of any inhibitory activity of S19 against S99.

2. Materials and methods

2.1 *Brucella abortus* strains used

Two laboratory -adapted strains of *Brucella abortus*, strain 19 (S19) and strain 99 (S99) obtained from the Brucellosis Laboratory of the Biological Products Division, Indian Veterinary Research Institute, Bareilly, India were used in this study. Brucella strains were identified and were maintained as per the standard protocols (Alton *et al*., 1988) [1]. Further, strain differentiation was performed by penicillin sensitivity and growth on erythritol agar (Miranda *et al*., 2015) [22]. Erythritol agar was prepared by depriving the media with any other carbohydrate source and supplemented with erythritol sugar (5 mg/ ml).

2.2 *In-vitro* Evaluation of *Brucella abortus* Strain Interference

Morphologically and biochemically characterized strains maintained on Brucella agar (Himedia, India) were inoculated in to Brucella broth and were then incubated under microaerophilic conditions at 37^0C. The 18 h culture of both strains S19 and S99 were adjusted to equal turbidity using Braun's opacity tube no.8. Aliquots of these suspensions having equal turbidity were being used as inoculum throughout the experimental study.

The following experiments were conducted in order to evaluate the interference between the *Brucella abortus* strain 19 and strain 99 (Fig.1).

2.2.1 Experiment I (EI): Co-culture

In EI, as a preliminary investigation, we examined whether there is any interaction between the two selected strains of *Brucella abortus* in co-culture, which could interfere the growth pattern when plated for colony counting. For this, 100 µl each of S19 and S99 were inoculated in to 5 ml Brucella broth along with separate S19 and S99 controls. Incubation was carried out for three days at 37^0 C with shaking (180-220 rpm).

2.2.2 Experiment II (EII): Pre-culture

During EII, two sets of experiments were made by changing the time of inoculation of second strain into the co-culture. This was performed by inoculating S99 into 3h gown S19 culture in the Pre-culture 1 and, similarly in second tube by inoculating S19 into 3 h growth of S99 (Pre-culture 2). These tubes were also allowed to grow for 3 days under similar conditions as in EI.

Bacterial growth was determined at every 24 h (24h, 48h and 72 h growth) from both EI and EII cultures and controls by the standard plate dilution method. The viable counts of bacteria were made by plating the serially diluted growth on to Brucella agar plates followed by incubation at 37 °C for 3-4 days and the number of c.f.u /ml was determined.

2.2.3 Experiment III (EIII): Differentiation of stains

This phase was aimed to find which strain growth is being enhanced or altered during co-culture experiments (EI and EII) by differential plating for viable count estimation. For this, growths at every 24h interval were serially diluted and plated on to both Brucella plates and erythritol agar plates. The absence of growth on erythritol plates can differentiate S19 from S99. Each assay (EI, EII and EIII) was replicated thrice on separate days.

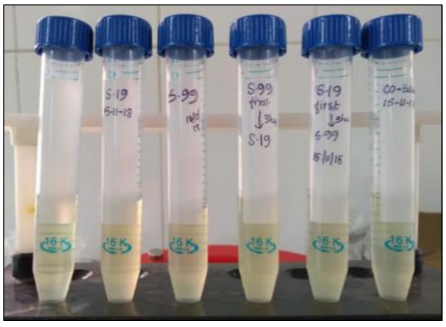

Fig 1: Different interference experiments conducted: EI, EII and EIII

3. Results

3.1 *Brucella abortus* strains used

Strain 99 could be differentiated from strain 19 based on its ability to grow on erythritol agar as it can utilize erythritol as a source of carbon, whereas strain 19 cannot. Further differentiation was made based on the sensitivity of S19 to penicillin.

The mean viable counts of three separate replicates (expressed in c.f.u /ml) are summarized in table 1 and graphically represented in Fig.2

Table 1: Mean viable counts of different experiments

	Mean viable count of different set of experiments expressed in cfu/ml				
	Pure S19	**Pure S99**	**Co-culture**	**Pre-culture 1**	**Pre-culture 2**
24 h growth	$3*10^8$	$1.5*10^8$	$2.5*10^8$	$3.5*10^8$	$1*10^4$
48 h growth	$7*10^8$	$5*10^8$	$3.6*10^9$	$3.3*10^9$	$6*10^8$
72 h growth	$9*10^8$	$7*10^8$	$4.6*10^9$	$3.4*10^9$	$6*10^8$

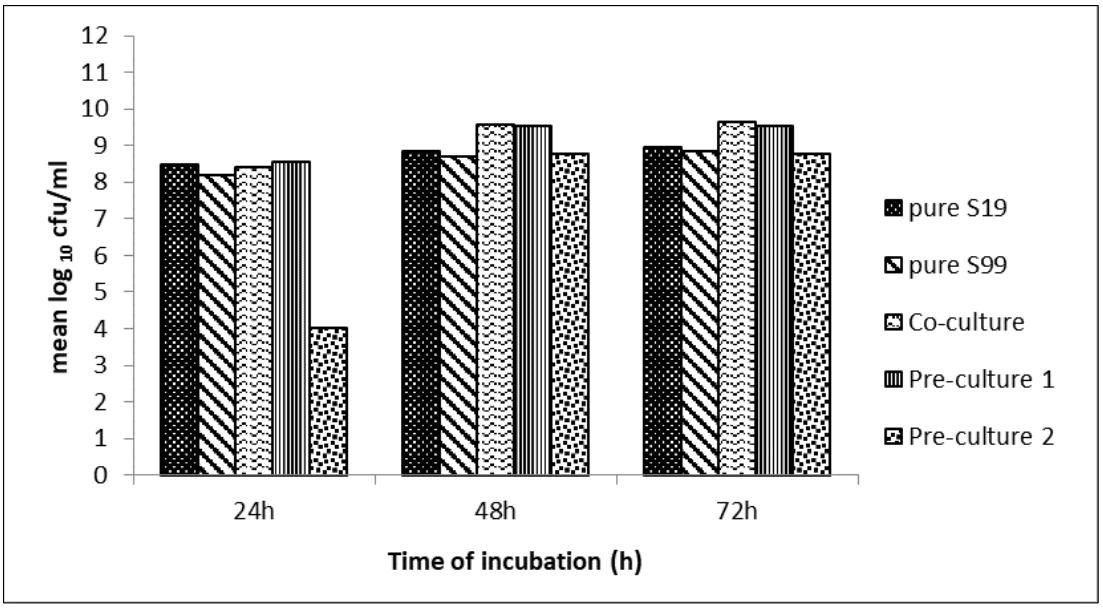

Fig 2: Mean Viable Count (log 10 cfu/ml) of different experiments at different time intervals

In EI, where 24 hour growth was plated for viable growth count, there was no significant difference among the growth pattern of co-culture and their respective individual controls. However, there was a 4-log reduction in the viable count of one of the pre-culture (preculture-2), where S19 was inoculated 3 hours after the S99 inoculation.

The 48h and 72h co-cultures and Preculture-1 have shown a 1-log enhancement in c.f. u/ml. At the same time, no significant difference could be observed in the other group in which, S19 was inoculated into 3h growth of S99 at any stage of growth (48h and 72h). All these observations were made by comparing the viable bacterial count with that of corresponding individual controls. During the third phase of study (EIII), the growth of strain 19 was differentiated from that of S99 based on its inability to grow on erythritol agar. The differential growth patterns of S19 and S99 are tabulated in table 2 and graphically represented in fig.3 and fig.4. This phase was aimed to find which strain growth was being enhanced or altered during co-culture experiments (EI and EII) by differential plating for viable count estimation. For this, erythritol agar was used to differentiate the growth of S19 and S99. It is evident from the data that there is no significant difference in the growth pattern of the two strains except for the pre-culture 2 group, where there was almost 2-log reduction in S99 growth compared to growths of S19 and its own control.

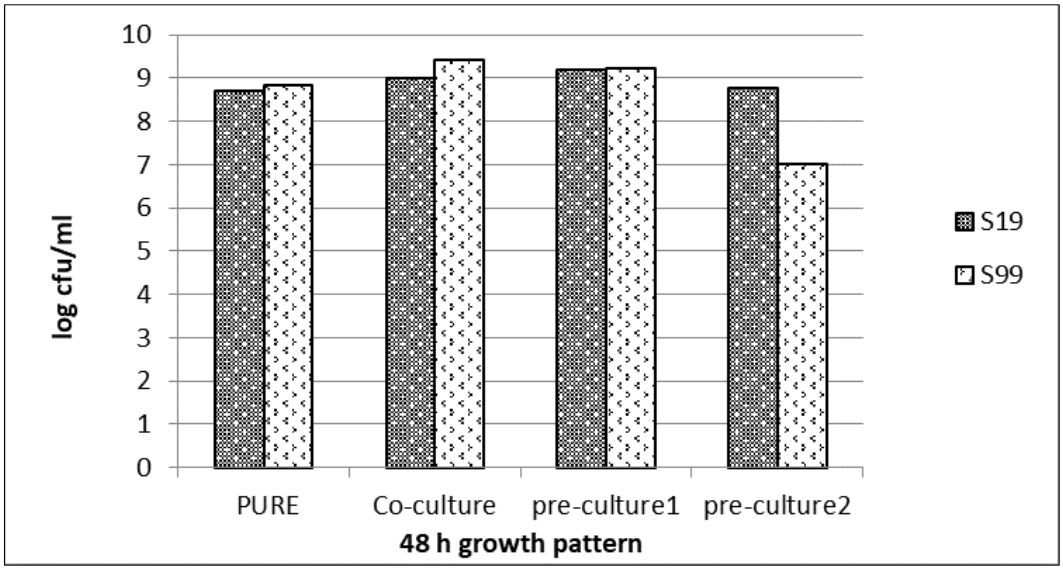

Fig 3: Differential growth of S19 and S99 at 48h of incubation in different experimental groups

Table 2: Differential growth of S19 and S99 in different experimental groups

	Mean viable count of different set of experiments expressed in cfu/ml							
	Pure Culture		Co-culture		Pre-culture 1		Pre-culture 2	
48 h growth	$5*10^8$	$7*10^8$	$1*10^9$	$2.6*10^9$	$1.6*10^9$	$1.7*10^9$	$6*10^8$	$1*10^7$
72 h growth	$7*10^8$	$9*10^8$	$2.6*10^9$	$2*10^9$	$1.7*10^9$	$1.7*10^9$	$6*10^8$	$1*10^7$

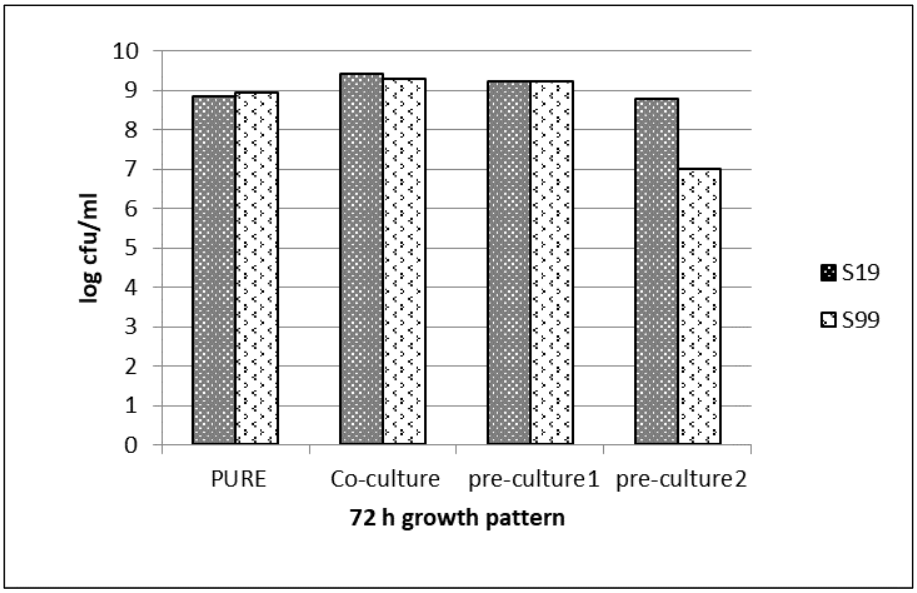

Fig 4: Differential growth of S19 and S99 at 72h of incubation in different experimental groups

4. Discussion

If one kind of bacteria can interfere the growth of another kind of bacteria inhabiting in same host either by inhibiting or promoting their colonization, the phenomena can be referred to as bacterial interference (Johansson *et al*. 1994; Stubbendieck *et al*. 2016) [16, 29]. Mechanisms of bacterial interference are quite complex and remains not well understood till date. Brucellosis is one among the most common zoonotic diseases having significant public health impact and which is often ignored. Since there are no approved human vaccines for this zoonosis, prevention and control of animal brucellosis is gaining more importance. There is no standardized or approved antibiotic therapy to be followed in animals. So, the disease is mainly controlled through calf-hood vaccination and culling of infected animals (de Baguds *et al*. 1991; Corbel, 2006) [7]. As we cannot rely only on vaccination and culling to prevent or control the disease in low income countries, further studies are needed for the development of novel therapeutic strategies and prevention of brucellosis.

Here, we were having keen interest in the interactions or interference among the different strains of *B.abortus* when they simultaneously inhabit a single host. There are no supporting data as such depicting this inter-relationship. However, there undergone many researches and clinical trials on the interference among different strains of other bacteria and the possible use of a less virulent strain to prevent the colonization of a more virulent strains of the same bacteria (Shinefield *et al*. 1963; Boris *et al*. 1964; Darouiche and Hull, 2000; De Gregorio *et al*. 2014; France and Remold, 2015) [26, 3, 8, 11, 15]. However, there are no reference literatures available for the strain interaction among *Brucella abortus* strains and their probable clinical use in therapeutic management of the disease.

In order to test this hypothesis, two aerobically adapted laboratory strains of *B.abortus*, S19 and S99 were selected and the strains could be easily differentiated form each other by their differential growth on media containing penicillin or on erythritol agar. Varying levels of erythritol agars were used to differentiate different strains of *B.abortus* (Miranda *et al*. 2015) [22]. Colonies of strain 99 which can utilize erythritol sugar could grow on erythritol agar, while S19 cannot utilize erythritol sugar as a source of carbon and thus cannot growth on erythritol agar plates (Whatmore *et al*. 2007) [30]. This facilitated the differentiation of S19 and S99 strains. Strain 19 is relatively less pathogenic in animals and is used as a live vaccine candidate. *Brucella abortus* S19 vaccination is commonly followed as part of brucellosis control programs in most countries including India (Manthei, 1968; de Baguds *et al*. 1991; Olsen and Stoffregen, 2005) [18, 24], while S99 is pathogenic. As S19 can interfere the serodiagnosis or may induce abortion in pregnant animals, S19 vaccination is not usually advised in adult animals (Meyer and Nelson, 1969; Jones and Hooper, 1976; Olsen and Stoffregen, 2005; Dorneles *et al*. 2015; Simpson *et al*. 2018) [21, 17, 24, 12, 27]. However, there are studies, suggesting the safety of strain 19 vaccination at a reduced dose (1x 10^6- 5 × 10^9 c.f.u) through conjunctival route in adult animals (Plommet and Plommet, 1976; Fensterbank and Plommet, 1979; Chand *et al*. 2013) [25, 13, 6]. There are even literatures stating the use of reduced dose of S19 vaccine in both pregnant animals and infected herds (Plommet and Fensterbank, 1976; Beckett and Mac Diarmid, 1985; Cardena *et al*. 2009; Chand *et al*. 2015) [25, 2, 4, 5]. Findings of Chand *et al*. (2015) [5] suggested that a reduced dose of S19 vaccine by conjunctival route did not cause any adverse effects like abortion in pregnant animals and there were no persistent vaccinal antibody titers, whereas subcutaneous vaccination can cause these two. However, there are no clear experimental demonstrations of the interference of live S19 with other disease causing field

strains of *B.abortus*. If a pregnant animal was already injected with a *B.abortus* strain and simultaneously given a dose of S19, the results are unpredictable. Therefore, the aim of the study was to access the strain interaction between the two different *B.abortus* strains as a preliminary approach in this field and to further explore its future use in the therapeutic management. With this in mind, we have designed the present study. For *in-vitro* strain interaction analysis, further experiments by direct inoculation of the selected strains were made in Brucella broth. The interference or interaction between the selected strains was evaluated by co-cultivation and pre-culture techniques of varying incubations. The study results suggest that there was no significant difference in viable count when both the strains were inoculated together in to broth except for a 1-log enhancement. Even when the S99 was inoculated into a 3h growth of S19, there was no considerable enhancement in growth than this 1-log increase.

At the same time, the growth of strain 99 got inhibited when S19 was inoculated 3h after the S99 inoculation into the same broth. It is clear from the data that there is some interference or interaction between the two strains which may be due to some unknown factors produced during the early growth phase of one strain which may selectively inhibit the growth of second strain. In contrast, this can also be due to increased utilization of some growth promoting substance by S19 and thus preventing its availability to S99. There can be development of an unfavorable growth environment as a result of initial colonization and this may result from production of inhibitors or other unfavorable factors like pH or redox potential, accumulation of some toxic metabolic products or antimicrobial substance which may result in bacterial antagonism. These are only possible reasons, which need to be clarified by further studies. Growth and occupancy of any community is defined by flexible metabolic strategies according to the external environment. When different bacteria are co-infected, they can communicate each other by means of some diffusible signals and these signals can even cause activation of some silent secondary metabolite biosynthesis genes (Netzkeret *et al*. 2015). This can be reflected in their specific metabolic adaptations. Mao *et al*. (2015) [19] stated that "Bacteria undergoing constant growth will always outcompete neighbors whose growth rate is dependent upon the external environment". The resources in the environment may fluctuate on short timescales and therefore no bacteria have a constant growth rate under different environment. Bacterial cells will exploit their environment to produce more progeny and cells encountering suboptimal environments, relative to their metabolic adaptations, will be outcompeted.

5. Conclusion and Future Perspective

In conclusion, there is some *in vitro* interference of S19 on S 99 strains, suggesting that they could be promising candidates for protection against brucellosis if supporting evidences unveiled. The clinical significance of the obtained results must be judged with caution as the interference studies were performed *in vitro* and only two bacteria were used. Moreover, as the mechanism of bacterial interference in host is not well understood, it is difficult to predict the possible use of these results obtained in the management of infection in animals. That warrants thorough study on the interference among the strains at molecular level. The present research approach can be considered as one among the pilot studies, further molecular studies and animal trials in this area should be conducted to evaluate the preventive effect of S19 against S99 and to suggest the possible therapeutic use if feasible. And also, various interactions among the other strains of *B.abortus* are also recommended. This is the first time, to our knowledge that the strain interference between two different strains of *B.abortus* has been studied. Therefore, there is lack of substantial evidence to support our queries and their findings. However, we tried our maximum to find some clues into the strain interference among *B.abortus* strains. Future researches on different *B. abortus* strain interference and their *in-vivo* interactions should be done in detail for evaluating the clinical significance of these interactions.

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7. References

1. Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory. Institut National de la recherche Agronomique (INRA); c1988.
2. Beckett FW, MacDiarmid SC. The effect of reduced-dose Brucella abortus strain 19 vaccination in accredited dairy herds. British Veterinary Journal. 1985;141(5):507-514.
3. Boris M, Sellers TF, Eichenwald HF, Ribble JC, Shinefield HR. Bacterial interference: Protection of adults against nasal Staphylococcus aureus infection after colonization with a heterologous S aureus strain. American Journal of diseases of children. 1964;108(3):252-261.
4. Cardena AP, Herrer DM, Zamora JL, Pina FB, Sanchez BM, Ruiz EJ, *et al*. Evaluation of vaccination with Brucella abortus S19 vaccine in cattle naturally infected with brucellosis in productive systems found in the Mexican tropic. International Journal of Dairy Science. 2009;4(4):142-151.
5. Chand P, Chhabra R, Nagra J. Vaccination of adult animals with a reduced dose of Brucella abortus S19 vaccine to control brucellosis on dairy farms in endemic areas of India. Tropical animal health and production. 2015;47(1):29-35.
6. Chand P, Chhabra R. Herd and individual animal prevalence of bovine brucellosis with associated risk factors on dairy farms in Haryana and Punjab in India. Tropical animal health and production. 2013;45(6):1313-1319.
7. Corbel MJ. Brucellosis in humans and animals. World Health Organization; c2006.
8. Darouiche RO, Hull RA. Bacterial interference for prevention of urinary tract infection: An overview. The Journal of Spinal Cord Medicine. 2000;23(2):136-141.
9. Drutz DJ, Van Way MH, Schaffner W, Koenig MG. Bacterial interference in the therapy of recurrent staphylococcal infections: multiple abscesses due to the implantation of the 502A strain of Staphylococcus. New England Journal of Medicine. 1966;275(21):1161-1165.
10. de Baugés MJ, Marín CM, Blasco JM. Effect of antibiotic therapy and strain-19 vaccination on the spread of *Brucella melitensis* within an infected dairy herd. Preventive veterinary medicine. 1991;11(1):17-24.

11. De Gregorio PR, Tomás MSJ, Terraf MCL, Nader-Macías MEF. *In vitro* and *in vivo* effects of beneficial vaginal lactobacilli on pathogens responsible for urogenital tract infections. Journal of medical microbiology. 2014;63(5):685-696.
12. Dorneles EM, Sriranganathan N, Lage AP. Recent advances in Brucella abortus vaccines. Veterinary research. 2015;46(1):76.
13. Fensterbank R, Plommet M. Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. IV. Comparison between two methods of vaccination. In Annales De Recherches Vétérinaires. 1979;10(1):131-139.
14. Fleming A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. British journal of experimental pathology. 1929;10(3):226.
15. France MT, Remold SK. Interference competition among household strains of Pseudomonas. Microbial ecology. 2016;72(4):821-830.
16. Johansson A, Bergenholtz A, Holm SE. Bacterial interference *in vitro*: comparison between a quantitative kinetic and a cocultivation blood agar test method. APMIS. 1994;102(7-12):810-816.
17. Jones FM, Hooper JA. Brucella abortus strain 19 calfhood vaccination: A review. Southwestern veterinarian; v1976.
18. Manthei CA. Application of research to bovine brucellosis control and eradication programs. Journal of dairy science. 1968;51(7):1115-1120.
19. Mao J, Blanchard AE, Lu T. Slow and steady wins the race: a bacterial exploitative competition strategy in fluctuating environments. ACS synthetic biology. 2015;4(3):240-248.
20. McCabe WR. Bacterial interference induced in embryonated eggs by staphylococci. The Journal of clinical investigation. 1967;46(3):453-462.
21. Meyer ME, Nelson CJ. Persistence of Brucella abortus strain 19 infection in immunized cattle. Us Animal Health Assoc. 1969.
22. Miranda KL, Dorneles E, Poester FP, Martins Filho PS, Pauletti RB, Lage AP. Different resistance patterns of reference and field strains of Brucella abortus. Brazilian Journal of Microbiology. 2015;46:265-269.
23. Netzker T, Fischer J, Weber J, Mattern DJ, König CC, Valiante V, *et al*. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Frontiers in microbiology. 2015;6:299.
24. Olsen SC, Stoffregen WS. Essential role of vaccines in brucellosis control and eradication programs for livestock. Expert review of vaccines. 2005;4(6):915-928.
25. Plommet M, Fensterbank R, Souriau A. Vaccination against bovine brucellosis with a low dose of strain 19 administered by the conjunctival route. III.-Serological response and immunity in the pregnant cow. In Annales de Recherches Veterinaires. 1976;7(1):9-23.
26. Shinefield HR, Ribble JC, Boris M, Eichenwald HF. Bacterial interference: its effect on nursery-acquired infection with Staphylococcus aureus. I. Preliminary observations on artificial colonzation of newborns. American Journal of Diseases of Children. 1963;105(6):683-688.
27. Simpson GJ, Marcotty T, Rouille E, Chilundo A, Letteson JJ, Godfroid J. Immunological response to Brucella abortus strain 19 vaccination of cattle in a communal area in South Africa. Journal of the South African Veterinary Association. 2018;89(1):1-7.
28. Singh BB, Dhand NK, Gill JPS. Economic losses occurring due to brucellosis in Indian livestock populations. Preventive veterinary medicine. 2015;119(3-4):211-215.
29. Stubbendieck RM, Vargas-Bautista C, Straight PD. Bacterial communities: interactions to scale. Frontiers in microbiology. 2016;7:1234.
30. Whatmore AM, Perrett LL, MacMillan AP. Characterisation of the genetic diversity of Brucella by multilocus sequencing. BMC microbiology. 2007;7(1):1-15.