Introduction

Brucellosis causes abortion, retention of placenta, and reduced productive and reproductive efficiency, consequently, the livestock industry suffers heavy economic losses as a result of this disease. It is the most widely spread zoonotic infection, transmissible from animals to humans (20). The incidence of brucellosis in Pakistan is on the increase, particularly in large dairy herds. While earlier studies indicated a low prevalence, i.e. 0.33% to 0.65% (18), a much higher prevalence, i.e. 21.05% to 26.1% has been recorded in recent studies (5, 16, 17). The incidence is higher in animals maintained on organised farms rather than on small holdings (2, 3, 4, 11). In spite of this increasing prevalence, no mandatory measures have yet been adopted to curtail the spread of the disease, as the country has no official policy of brucellosis control and/or eradication.

Because of the high prevalence of brucellosis, the socioeconomic conditions, the nature of the Veterinary Services (which have no legal powers or compensatory funds to distribute) and the system of livestock farming in the country, the most appropriate method for the control of this disease is the immunisation of susceptible animals. However, vaccination against brucellosis has not been carried out, because the efficacy of the available buffalo vaccines is not known and no locally prepared vaccine is available. It is generally accepted that immunisation against diseases caused by facultative...
intracellular microbes, such as brucellosis, is more effective with live vaccines (13, 19). The most popular live attenuated vaccine for brucellosis in large ruminants is *Brucella abortus* strain (S) 19. This study was designed to produce *B. abortus* S 19 vaccine locally and to determine its immune response in buffalo calves.

**Materials and methods**

**Preparation of strain 19 vaccine**

The vaccine was produced using *B. abortus* strain 19 imported from Germany. Bacteria were grown on tryptone soya agar supplemented with 0.1% yeast extract in roux flasks at 37°C for 48 to 72 hours. The growth was closely observed to ensure uniformity and prevent contamination. Roux flasks with uniform colonies were harvested in 8 ml to 12 ml sterile phosphate buffer saline (PBS) by gentle agitation. Suspension from each flask was harvested in separate containers and checked for purity by both Gram and modified Ziehl-Neelsen stains. The purity and absence of contamination was also checked by inoculating the suspension from each tube on tryptone soya agar plates. The colonies were checked for smoothness. The pure *B. abortus* S 19 suspensions from the containers were pooled and the number of the bacteria per ml of suspension was determined as described by Hulse et al. (10). The bacterial suspension was adjusted as per required number of bacteria.

The safety of the prepared vaccine was tested in guinea pigs as described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (the Manual) (15). Briefly, guinea pigs were given 5 × 10^8 viable organisms per animal intramuscularly. Unvaccinated control guinea pigs were given 2 ml PBS. Blood was collected from the guinea pigs through cardiac puncture on day zero and day 10 post-vaccination. The animals were observed for any obvious adverse effect or mortality. After ten days, the animals were killed and their spleens were collected. Each spleen was weighed and the number of bacteria per gram of spleen was determined as described by Alton et al. (7).

A potency test was carried out in guinea pigs as described in the Manual (15). Briefly, vaccinated and unvaccinated guinea pigs were challenged with virulent *B. abortus* biotype 1, isolated locally from a Jersey cow, as a single dose of 5,000 viable organisms injected intramuscularly eight weeks after vaccination. At six weeks post-challenge, all the animals were weighed and killed and the blood and spleens were collected. Sera were separated and stored at −20°C for future use. The spleens were examined for any gross lesions and weighed in aseptic conditions. The spleen to body ratio of the animals was determined and the spleno-somatic index was calculated. One way analysis of variance was applied on spleno-somatic indices of different animals and the results were compared at 0.01 level of significance. The least significant difference test was used to compare different means.

**Vaccination of buffalo calves**

The prepared *B. abortus* S 19 vaccine, containing 7 × 10^10 viable bacteria, was injected subcutaneously into the neck area of five 8 to 10.5 month old female buffalo calves. Blood samples were collected by jugular venipuncture on day 0, 7, 14, 21, 28, 35, 42, 49, 63, 77 and 91 post-vaccination. Sera were separated and stored at −20°C for future use.

The antibody titres in the serum samples of the guinea pigs and vaccinated buffalo calves were determined by serum agglutination test (SAT) and 2-mercaptoethanol test (2-MET) following Morgan et al. (12) and Anderson et al. (8), respectively. The antigen was purchased from the Veterinary Research Institute, Lahore. The positive and negative control sera were procured from the National Veterinary Services Laboratories, Ames, Iowa, United States of America. The highest dilution with 50% agglutination was recorded as end point. The antibody titres were converted to international units per ml (IU/ml) following Morgan et al. (12) and the geometric mean titres of each group were calculated.

**Results**

**Preparation of *Brucella abortus* strain 19 vaccine**

The pooled harvested suspension from the roux flasks contained 3.73 × 10^12 colony-forming units (CFU). The suspension was pure and had smooth colonies. The suspension was diluted to 7 × 10^8 per ml, i.e. the standard dose for calf vaccination. Guinea pigs inoculated with the test vaccine containing 5 × 10^9 CFU showed no obvious adverse effect or mortality. The antibody titres of vaccinated guinea pigs ten days after vaccination ranged from 82 IU/ml to 656.5 IU/ml, with a geometric mean titre of 248.6 IU/ml. No anti-brucella antibodies were detected in the sera of the control group. The spleens of the vaccinated guinea pigs showed no gross lesions or enlargement. No bacteria were isolated from the spleens of the vaccinated guinea pigs.

The geometric mean antibody titres of different groups of guinea pigs vaccinated with *B. abortus* S 19 vaccine are shown in Table 1. The vaccine induced a good immune response in guinea pigs. The mean antibody titres of all vaccinated guinea pigs on day 10 post-vaccination was 328 IU/ml. Unvaccinated, unchallenged guinea pigs were negative for anti-brucella antibodies throughout the study. The vaccine provided protection in guinea pigs against experimental challenge.

The spleens of guinea pigs from the vaccinated challenged group showed no gross lesions or enlargement, whereas, those of the unvaccinated challenged group had a nodular appearance and were enlarged. Spleno-somatic indices of the
vaccinated challenged and unvaccinated challenged groups of guinea pigs were 0.14 and 0.30, respectively. There was a highly significant (P < 0.01) difference in the mean spleno-somatic indices of the vaccinated and unvaccinated challenged guinea pigs. Furthermore, *B. abortus* were isolated from the spleens of all the unvaccinated challenged guinea pigs. The mean number of bacteria isolated from unvaccinated challenged guinea pigs was 29,205 CFU per gram of spleen. Brucellae were not isolated from the spleens of vaccinated unchallenged, vaccinated challenged or unvaccinated unchallenged guinea pigs.

### Immune response in buffalo calves

All five buffalo calves were negative for anti-brucella antibodies before vaccination. The geometric mean titres of all the vaccinated animals, as measured by SAT and 2-MET, are shown in Figure 1. Significant SAT titres were seen on day 7 following vaccination with *B. abortus* S 19. The highest SAT titre was observed on day 14 post-vaccination, with a geometric mean titre of 216.4 IU/ml. The titre started declining after day 14 post-vaccination. The rate of decrease was slow between day 14 and day 49 post-vaccination. However, a rapid decrease in titres was seen between day 49 and day 91 post-vaccination. A negligible SAT titre of 3.3 IU/ml was observed on day 91 post-vaccination. The SAT titres became negative in one animal on day 63 post-vaccination, on day 77 post-vaccination in another animal and on day 91 post-vaccination in a third animal. However, two animals had low SAT titres on day 91 following vaccination.

The immunoglobulin G antibody titre, as measured by treating the serum samples with (2-MET), was also highest on day 14 post-vaccination, with a geometric mean titre of 124.3 IU/ml. Then a gradual decrease in 2-MET titre was observed until day 42. A sharp decrease in the titre was observed after day 42 and the titre became zero on day 91 post-vaccination. 2-MET titres became zero on day 49, 63 and 77 following vaccination in three animals. Two animals became negative for 2-MET titres on day 91 post-vaccination.

### Discussion

*Brucella abortus* strain 19 was produced and used in liquid form. The vaccine, prepared as described in the *Manual* (15), produced a good immune response in guinea pigs. The vaccine also protected the guinea pigs against challenge with a locally isolated *B. abortus* strain. This strain was virulent for unvaccinated guinea pigs, as evidenced by an increase in the size and weight of their spleens, and isolation of brucellae from their spleens six weeks post-challenge.

The value of vaccination in controlling brucellosis in cattle is well known and various aspects of the use of *B. abortus* S 19 vaccine in cattle have been studied (6, 9, 14). However, the efficacy of vaccinating buffaloes against brucellosis had not been reported. The present study shows that effective *B. abortus* S 19 vaccine can produce good humoral immune response in buffalo calves. We reported in a previous study (1) that a lyophilised imported commercial strain 19 vaccine produced a good immune response in buffalo calves and heifers; the present study demonstrates that serological titres remain
detectable for somewhat longer periods when freshly prepared strain 19 vaccine, as opposed to a lyophilised vaccine, is used. Although strain 19 produced a good humoral immune response in buffalo calves, its efficacy in a challenge-protection study remains to be seen. Studies on the efficacy of B. abortus S 19 vaccine in buffaloes of different age groups are being planned.

La réponse immunitaire de cobayes et de bufflons à un vaccin local préparé avec la souche 19 de Brucella abortus

S.M. Jamal, M. Afzal & S. Ahmed

Résumé
Les auteurs rapportent la préparation d’un vaccin contre Brucella abortus à partir de la souche 19 importée d’Allemagne. Ce vaccin a induit une bonne réponse immunitaire chez les cobayes, comme l’atteste le titre d’anticorps sériques de 328 unités internationales (UI)/ml, 10 jours après la vaccination. Les cobayes vaccinés ont par ailleurs été exposés expérimentalement à une souche virulente de B. abortus locale (5 000 unités formant colonies). Ce vaccin, qui contient $7 \times 10^{10}$ micro-organismes vivants, a induit une réponse immunitaire significative chez de jeunes bufflonnes âgées de 8 à 10,5 mois. Des titres d’anticorps sériques significatifs ont été relevés lors du test d’agglutination, 7 jours après la vaccination. Ils ont atteint un pic 14 jours après la vaccination avant de diminuer graduellement. Cette baisse, relativement lente entre les 14e et 49e jours après la vaccination, s’est accélérée entre les 49e et 91e jours suivant la vaccination. Les titres d’anticorps observés lors des tests d’agglutination effectués 91 jours après la vaccination présentaient des valeurs négligeables. Les titres de l’immunoglobuline G agglutinée dans du sérum traité au 2-mercaptoéthanol ont suivi la même tendance : le sérum des cinq jeunes bufflonnes vaccinées ne contenait plus aucune trace de cet anticorps spécifique 91 jours après la vaccination.

Mots-clés
Buffle – Brucella abortus – Titre – Vaccination.

Respuesta inmunitaria de cobayas y terneras de búfalo a la vacuna de fabricación local con la cepa 19 de Brucella abortus

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Resumen
Los autores reportan la preparación de una vacuna contra Brucella abortus utilizando una cepa 19 importada de Alemania. Aplicada a cobayas, esa vacuna indujo una buena respuesta inmunitaria, que se tradujo en un título serológico de 328 unidades internacionales (UI)/ml a los 10 días de la administración. Los cobayos vacunados resistieron asimismo a la infección experimental con 5 000 unidades formadoras de colonias de una cepa virulenta local de B. abortus. La mencionada vacuna, que contenía $7 \times 10^{10}$ organismos viables, indujo una respuesta inmunitaria significativa en terneras de búfalo de entre 8 y 10,5 meses de edad, que a los siete días de la vacunación presentaban títulos significativos de anticuerpos en pruebas de aglutinación. El valor de los títulos alcanzó su nivel
máximo a los 14 días de la vacunación, y a partir de ese momento empezó a menguar. Entre el día 14 y el 49 el descenso fue lento, mientras que del día 49 al 91 los títulos de anticuerpos cayeron con rapidez. A los 91 días de haberse administrado la vacuna, los títulos de aglutinación en suero eran de un valor insignificante. Los títulos de inmunoglobulina G específica, calculados por aglutinación en suero tratado con 2-mercaptoetanol, siguieron una evolución similar, hasta llegar a cero al cabo de 91 días en las cinco terneras vacunadas.

**Palabras clave**
Brucella abortus – Búfalo – Título – Vacunación.

**References**