Successful therapeutic regimens for treating *Brucella melitensis* and *Brucella abortus* infections in cows


**Summary:** Three therapeutic regimens were evaluated in 121 cows naturally infected with *Brucella melitensis* or *Brucella abortus*, using a combination of long-acting oxytetracycline (LA-OTC), streptomycin (ST) and OTC-intramammary infusion (IMI). Cessation of shedding of *Brucella* in udder secretions and absence of *Brucella* in selected tissues were considered criteria for successful treatment.

Regimen A (tested on 35 cows) consisted of LA-OTC 25 mg/kg administered intramuscularly (i.m.) every 3 days for 42 days, ST 25 mg/kg i.m. daily for 8 days, and OTC-IMI 20 ml/teat daily for 4 days. Regimen B (tested on 53 cows) was similar to regimen A, except that ST was administered every 2 days for 16 days and OTC-IMI every 2 days for 8 days. Both regimens were equally effective in eliminating *Brucella* organisms from all cows involved in the tests and no relapses were recorded. However, regimen C, which was similar to regimen A, except that ST was administered every 3 days for 24 days and OTC-IMI every 3 days for 12 days, resulted in the elimination of *Brucella* organisms from only 30 (91%) of 33 cows.

Before commencement of the therapeutic regimens, *B. melitensis* biovar 1 or 2 had been repeatedly isolated from udder secretions of 103 cows and *B. abortus* biovar 1 from mammary secretions of 18 cows.


**INTRODUCTION**

Several chemotherapeutic agents have been employed in recent decades for the treatment of *Brucella abortus* infection in cows; however, none of these has been entirely successful. Numerous chemical agents, general antimicrobials (phenols or dyes), trace elements, minerals and mixtures of vitamins (A and E) have been tried unsuccessfully. Furthermore, attempts using antibiotics such as penicillin or sulfonamides failed to cause cessation of the shedding of *B. abortus* from the mammary secretions of infected cows, or caused only temporary cessation (7, 11, 37).

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The use of broad-spectrum antibiotics such as aureomycin, terramycin, tetracyclines and streptomycin (ST), singly or in combination, has resulted in the reduction of abortions in infected herds or individual cows (20, 21). However, the cost of therapy, the presence of antibiotic residues in milk and the failure to cure udder infections in many cases have led to the general conclusion that such treatment is unsuitable for the control of bovine brucellosis.

With the development of Oxytetracycline (OTC) and long-acting (LA)-OTC, the use of these agents alone or in combination with ST has succeeded in eliminating the symptoms of this disease and reducing the shedding of brucellae by infected cows. Consequently, such therapeutic regimens have been applied in infected herds to prevent abortions and reduce the spread of brucellosis. However, these regimens have failed to result in complete cure. Furthermore, serious local reactions were reported in cows, due to repeated intraperitoneal (i.p.) inoculations with OTC (12, 13, 24, 25, 29). In addition, the regimens previously tried involved small doses and few injections of the antibiotics used. In one of these studies, injections of LA-OTC (20 mg/kg intramuscularly [i.m.] every 3 days for 2 weeks) combined with ST (20 mg/kg i.m. daily for 1 week) resulted in cessation of shedding of *B. abortus* in 10 (71.4%) of 14 cows (24). In another study, inoculation of LA-OTC (20 mg/kg i.m. every 3 days for 2 weeks) alone was successful in curing 3 (21%) of 14 cows. However, when LA-OTC was combined with ST (20 mg/kg intravenously [i.v.] or i.m. daily for 1 week) 14 (67%) of 21 cows were successfully treated (25).

With regard to brucellosis in sheep, 9 sheep experimentally infected with *B. abortus* were injected daily for 6 days with 1 g chlortetracycline, then 20 days later with 1.4 g together with immune serum for 3 days. Only one sheep was found to be bacteriologically positive at slaughter, 46 days after infection (30). Furthermore, Radwan and colleagues (34) conducted a long-term treatment trial using a variety of doses of OTC, alone or in combination with ST, on 118 sheep naturally infected with *B. melitensis*. In this trial, groups of infected Najdi sheep were injected with 250, 500 or 1,000 mg OTC daily for 6 weeks, by the i.p. route. In the respective groups, 52%, 69% and 100% of the sheep were found to be *Brucella*-free at the end of the trial. In addition, treatment with OTC (250 mg i.p. daily for 6 weeks) combined with ST (1 g i.m. daily for 3 weeks) showed a synergistic effect, increasing the percentage of *Brucella*-free sheep to 82%. Moreover, when 8 infected Najdi sheep were inoculated with 1,000 mg LA-OTC i.p. every 3 days for 6 weeks, 6 sheep were *Brucella*-free at necropsy (34). In this trial, the majority of the treated sheep developed subcutaneous sterile abscesses in the flank at the site of repeated i.p. inoculation with OTC. Recently, Radwan and colleagues (35) evaluated six different long-term treatment regimens on 480 sheep and goats naturally infected with *B. melitensis*, using high doses of LA-OTC combined with ST. One of these regimens (LA-OTC 25 mg/kg i.m. every 2 days for 4 weeks combined with ST 20 mg/kg i.m. every 2 days for 2 weeks) proved to be the most practical, safe, effective and least expensive means of achieving complete elimination of *B. melitensis* in the 80 sheep and goats treated (35).

In view of the encouraging results in cows (24, 25) and the successful results in sheep and goats (35), the present study was undertaken to evaluate the efficacy of three long-term therapeutic regimens using a combination of LA-OTC, ST and OTC intramammary infusion (IMI) in eliminating *B. melitensis* or *B. abortus* from naturally-infected cows. The aim of the study was to obtain a therapeutic regimen which is effective, practical, without side-effects and relatively inexpensive, in order to make treatment of *Brucella*-infected cows with superior genes a viable alternative to slaughter.
MATERIALS AND METHODS

Animals

A total of 121 cows were selected, which gave positive results for brucellosis after serological and bacteriological tests. These animals were selected to provide a more conclusive evaluation than that obtained in previous studies. The cows originated from three large commercial dairy herds raised in the central and eastern provinces of Saudi Arabia. The animals belonged to two breeds (108 Holstein x Friesian and 13 Jersey). The cows were aged 3-10 years and weighed between 450 kg and 750 kg. The three herds were composed of highly valuable breeding cows originally imported from the United States of America (USA) and Europe. Herd records indicated that none of the cows had been vaccinated during calfhood.

In two herds, *B. melitensis* was isolated from udder secretions of 103 cows (biovar 1 from 97 cows and biovar 2 from 6 cows). These two herds originally tested seronegative on arrival in Saudi Arabia, approximately four years prior to this study. The cows contracted brucellosis from sheep and goats infected with *B. melitensis* (biovars 1 and 2) which had been introduced to the two farms 8-12 months before bovine brucellosis occurred in the two cattle herds (unpublished findings).

In the third herd, *B. abortus* biovar 1 was isolated from the mammary secretions of 18 cows. This herd contained few seropositive cows on arrival in Saudi Arabia, eight years prior to the present study.

Shortly before initiation of the present treatment regimens, 89 cows were found not to be pregnant (having already aborted or calved 4-8 months before treatment) and 32 cows were in various stages of pregnancy. Milking was ceased in lactating cows. The 121 cows were divided into three treatment groups (A, B and C), without regard to age, number of previous pregnancies, date of recent pregnancy and number of previous abortions due to *Brucella*. The reason for the division into three treatment schedules was to choose the most practical and effective regimen, particularly because LA-OTC is a long-acting antibiotic, in contrast to ST and OTC-IMI which are not. Since the number of available cows infected with *B. abortus* was small, these were placed in group B together with 35 cows with *B. melitensis*. All cows in the study were isolated from other animals. Non-treated cows were not included as controls, as several previous studies have indicated that *Brucella* infections persist in cattle and spontaneous recovery is uncommon (10, 22, 28).

One month after treatment, all non-pregnant cows were artificially inseminated. Three months later, a total of 12 pregnant cows (4 from each treatment group) were slaughtered, and selected tissues were collected for bacteriological examination. In addition, immediately after birth, a total of 3 calves (1 from a cow in each treatment group) were sacrificed, and selected tissue samples were cultured.

Therapeutic agents

The pharmaceutical products used in this study were as follows:

- LA-OTC injectable solution (from France) containing 200 mg OTC base per millilitre
- ST sulphate (from Giza, Egypt) supplied in vials, each containing 1g or 4 g, dissolved (just before use) in 3 ml or 12 ml sterile distilled water, respectively
OTC-IMI (from Athens, Greece) in 10 ml syringes, each containing 200 mg OTC hydrochloride, 100 mg neomycin sulphate, 100 mg oleandomycin phosphate, 5 mg Prednisolone and 10 ml special excipient.

**Therapeutic regimens**

The treatment regimens of the three groups of infected cows are shown in Table I. Both ST and OTC-IMI were administered with LA-OTC at the commencement of therapy. The LA-OTC and ST were injected i.m. in the cervical, thigh and shoulder muscles, at a dosage of 20-30 ml per site. The date of previous abortions in relation to the initiation of treatment was not recorded on the farms involved.

**Serological testing**

All animals were subjected to three successive serological examinations at monthly intervals. At each examination, animals were initially screened for the presence of *Brucella* agglutinins by the rose bengal test (3). The rose bengal test antigen was obtained from the Central Veterinary Laboratory, New Haw, Weybridge, United Kingdom. Animals testing positive to the rose bengal test were retested by the standard US plate agglutination procedure for the determination of *Brucella* antibody titres (3). The standard *Brucella* plate antigen (made of *B. abortus* strain 1119-3) was obtained from the United States Department of Agriculture in Ames, Iowa, USA. Agglutination at 1:100 or greater was considered positive.

Following the three serological examinations, milk samples from all seropositive cows were subjected to bacteriological examination for identification of *Brucella* shedders. Furthermore, sera from the selected 121 serologically- and bacteriologically-positive cows were serologically tested again immediately before initiation of the present treatment regimens and at monthly intervals after completion of treatment (for 4 months in 12 sacrificed cows and for 16 months in the remaining 109 cows). In addition, sera from all calves born from treated cows were examined immediately after birth.

**Bacteriological examination**

The medium for *Brucella* culture was prepared as described previously (35). Immediately before initiation of the therapeutic regimens, cultures of udder secretions were made from the selected 121 positive cows to confirm shedding of *Brucella* organisms. Separate 30 ml samples of udder secretions from each quarter were also cultured every week during treatment, every month after completion of treatment until calving and again every 2 weeks after calving for 6 months. In addition, from the 12 pregnant sacrificed cows (4 cows from each treatment group which were slaughtered at 4 months post-treatment) the following tissue specimens were aseptically collected for bacteriological examination: supramammary, prescapular, iliac, precrural, mediastinal, mesenteric, popliteal and head lymph nodes, udder secretion, and sections of udder, uterus, ovary, brain, liver and spleen. In addition, samples of stomach contents, liver, lung and spleen of foetuses from the sacrificed animals were cultured. Similar selected tissue specimens were cultured from 3 sacrificed newborn calves (1 from a cow in each treatment group).

Aliquots from each udder secretion sample were spread with sterile cotton swabs on 2-4 freshly prepared plates of culture medium. Each tissue specimen was individually homogenised in a tissue grinder and aliquots were spread with sterile cotton swabs on several (4-8) freshly prepared plates of culture medium. The plates were incubated at 37°C for 7 days in the presence and absence of 10% CO₂ atmosphere. The isolated
TABLE I

*Efficacy of three treatment regimens in eliminating Brucella melitensis or B. abortus from naturally-infected cows*

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of treated cows</th>
<th>Therapeutic agents</th>
<th>Route</th>
<th>Dosage</th>
<th>Periodicity (days)</th>
<th>No. of inoculations</th>
<th>Success/treated Tissues (a)</th>
<th>Udder secretions (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>LA-OTC&lt;sup&gt;(e)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>3</td>
<td>14</td>
<td>4/4</td>
<td>31/31 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST&lt;sup&gt;(f)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>1</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&amp; OTC-IMI&lt;sup&gt;(g)&lt;/sup&gt;</td>
<td>IMI</td>
<td>20 ml/teat</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>35&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>LA-OTC&lt;sup&gt;(e)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>3</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST&lt;sup&gt;(f)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>2</td>
<td>8</td>
<td></td>
<td>49/49 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&amp; OTC-IMI&lt;sup&gt;(g)&lt;/sup&gt;</td>
<td>IMI</td>
<td>20 ml/teat</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>33&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>LA-OTC&lt;sup&gt;(e)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>3</td>
<td>14</td>
<td></td>
<td>27/29 (91%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ST&lt;sup&gt;(f)&lt;/sup&gt;</td>
<td>i.m.</td>
<td>25 mg/kg</td>
<td>3</td>
<td>8</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>&amp; OTC-IMI&lt;sup&gt;(g)&lt;/sup&gt;</td>
<td>IMI</td>
<td>20 ml/teat</td>
<td>3</td>
<td>4</td>
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</table>

i.m.: intramuscular (injection)
IMI: intramammary infusion

a) number of sacrificed cows from which selected tissues were cultured for *Brucella*
b) number of cows from which mammary secretions were repeatedly cultured for *Brucella*
c) cows naturally infected with *B. melitensis*
d) cows naturally infected with *B. abortus*
e) long-acting oxytetracycline injectable solution (from France) 200 mg/ml
f) streptomycin sulphate (from Egypt)
g) oxytetracycline intramammary infusion (from Greece)
Brucella cultures were identified morphologically, microscopically, biochemically and serologically (3). The biotyping of the identified isolates was performed at the Central Veterinary Laboratory, New Haw, Weybridge, United Kingdom.

When Brucella organisms could no longer be recovered from udder secretions nor from any of the selected tissue specimens collected at necropsy, the respective cow was considered to be cured (successful treatment). Continued or resumed shedding of Brucella organisms in udder secretions or the isolation of Brucella organisms from any of the tissue specimens obtained at necropsy were considered as conclusive evidence of treatment failure.

In this study, aborted foetuses from the three cattle herds involved were cultured for Brucella isolation, in addition to routine bacteriological examination for listeriosis, salmonellosis, mycoplasmosis and mycotic abortion.

RESULTS

Breeding performance

A comparison was made between breeding performance in the three herds prior to identification of the infected cows and that in the treated animals in the breeding season following treatment. Before treatment, the cows had been living in an infected environment for periods of between 1 and 8 years. The abortion rate in the three herds ranged between 5% and 15% and the infertility rate was approximately 15%. In addition, there were unusually high rates of metritis, retained placenta and mastitis, with milk production reduced by 10-15%. However, in the breeding season following treatment, the cows were living in an uninfected environment. Of the cows treated with regimens A, B and C, 94% became pregnant and experienced normal calving. No abortions were observed among the treated cows. On the whole, the treated cows expelled placenta from the womb without requiring any special manual manipulation, or the use of chemotherapeutic measures. Furthermore, the 32 cows which were in various stages of pregnancy at the time of initiation of the therapeutic regimens A, B and C experienced normal calving within 4 months following completion of the treatment. Moreover, there were no cases of metritis or mastitis in the treated cows, and milk production was within the normal range for uninfected animals of the same breed.

Side-effects of the therapeutic agents

In 8 cows, there was very light swelling and signs of painful sensations at the site of repeated LA-OTC injections, particularly in the thigh muscles. These slight local reactions disappeared within 36 hours. Meat inspection of the twelve cows which were slaughtered (4 months post-treatment) revealed complete absence of any detectable abnormalities in the muscles at the site of intramuscular inoculations of LA-OTC and ST. Furthermore, no systemic reactions were observed in any of the treated cows during the treatment period.

Cost of therapeutic agents and animals

The weight of the treated cows ranged between 450 kg and 750 kg, and the average cost of the therapeutic agents alone used (regimen A, B or C) in treating a cow weighing 600 kg was US$186 (SR700).
The treated cows were imported a few years previously as pregnant heifers, at a cost of US$3,600 each, including the cost of air freight to Saudi Arabia.

Serological findings

Among the treated animals, there was a decrease of only one dilution in the US plate agglutination test (from 400 to 200) from the beginning of treatment until 4 months post-treatment in 4 of 12 slaughtered cows. The remaining 8 slaughtered cows did not show any decrease in titre over the same period.

The Brucella agglutinin titres were also compared with results obtained pre-treatment and 16 months post-treatment in the remaining 109 treated cows. There was a decrease of two, three and four dilutions in 42%, 43% and 10% of the treated cows respectively. In addition, only 5% of the 109 cows became seronegative 16 months post-treatment, while all calves born from treated cows were seronegative for brucellosis.

Bacteriological findings

In the present study, no Listeria, Salmonella, Mycoplasma or pathogenic fungi were isolated from the aborted foetuses examined in the three herds; only B. melitensis or B. abortus were isolated. The efficacy of the three treatment regimens applied in eliminating B. melitensis or B. abortus from 121 naturally-infected cows is shown in Table I.

In regimen A, the selected tissue specimens from 4 sacrificed cows (at 4 months post-treatment) and all repeated udder secretion samples from the remaining 31 cows (for 16 months post-treatment) were all found to be free from Brucella organisms.

When 53 cows (of which 35 were naturally infected with B. melitensis and 18 infected with B. abortus) were treated with regimen B, all selected tissue samples from 4 slaughtered cows and the repeated udder secretion samples were found to be Brucella-free.

However, when 33 cows (naturally infected with B. melitensis) were treated with regimen C, B. melitensis biovar 2 was isolated (1-5 colonies per plate) from mammary secretions, udder tissue and supramammary lymph nodes of only 1 of 4 sacrificed cows (at 4 months post-treatment). In addition, B. melitensis biovar 1 was isolated from the repeated udder secretion samples from only 2 of 29 cows. The average number of Brucella colonies recovered per plate from the udder secretion samples of these 2 cows prior to treatment was approximately 30 times higher than the number recovered following treatment, which ranged between 2 and 8 colonies per plate. However, when these 2 Brucella-shedding cows were re-treated with regimen B, they ceased shedding Brucella.

All selected tissue specimens from the 3 newborn calves (1 calf from a cow in each treatment group) were found to be free from Brucella organisms.

In all successfully treated cows in the present study (118 cows treated with regimen A, B or C), shedding of Brucella organisms in mammary secretions had ceased when these secretions were cultured (1 week after initiation of the antibiotherapy) and did not recommence. However, in the 3 cows of regimen C where treatment failed, the amount of shedding declined after the initiation of treatment and remained low until 4 months post-treatment in 1 slaughtered cow, or after calving in the other 2 cows (these animals were then re-treated with regimen B).
DISCUSSION

In order to increase milk production, commercial dairy production was introduced to Saudi Arabia through the importation of valuable and highly productive exotic cattle at a very high cost. These cattle were originally imported as pregnant heifers from the USA and some European countries, namely: the Netherlands, Germany and France. On arrival in Saudi Arabia, the cattle were raised in large numbers under intensive management systems and sometimes together with small ruminants.

Brucellosis has been reported and confirmed in both livestock and humans in Saudi Arabia. *B. melitensis* biovars 1, 2 and 3 were responsible for all infections in sheep, goats, camels and dairy cattle, except cows in one dairy herd which had *B. abortus*. Consequently, *B. melitensis* accounted for 92% of human cases and *B. abortus* for 8%, while *B. suis* was not found in animals or humans (1, 2, 4, 5, 6, 16, 18, 19, 23, 31, 32, 33, 34, 35, 39).

In the present study, two of the cattle herds contracted *B. melitensis* infection from sheep and goats raised on the same farms. Transmission of this infection to cattle produced an infection similar to that produced by *B. abortus*. Such infection was directly responsible for the high rate of abortions, infertility, metritis, retained placenta and mastitis with reduced milk production, leading to serious consequences for cattle performance and cattle handlers. However, in *B. abortus* infection, it is recognised that the economic consequences of infection are encountered principally during initial infection and become less prominent in further pregnancies.

In a campaign against brucellosis, it is impossible to apply only one method which would suit every country. The "test and slaughter" method combined with calfhood vaccination no doubt contributes to considerable reduction in the number of infected herds; however, for economic reasons this method cannot be applied in all cases. An eradication campaign through slaughter and compensation is not within the economic scope of developing countries, especially where it is difficult to change agricultural customs or social habits. On the other hand, mass vaccination of infected herds protects only uninfected animals without altering the course of infection. The infected vaccinated animals continue to present a serious public health risk. In addition, one worker assessed the average cost of an infected cow at approximately US$3,200, not just for abortion or stillbirth, but from the subsequent problems or irregular breeding, loss of milk production, and the reduced human productivity resulting from a spread of infection (38). Moreover, with the development and widespread use of techniques such as artificial insemination and embryo transfer, cattle with superior genetic potential have become increasingly valuable. Slaughter of infected animals with superior genetic potential has serious economic and genetic consequences (24). Livestock producers in Saudi Arabia and in many other developing countries cannot afford the traditional "test and slaughter" approach used in developed countries. An effective, practical and safe antibiotherapy would be of enormous benefit to producers in these countries as an alternative to slaughter of infected animals of high value. Although this has been the goal of several workers, none has been fully successful.

The following explanations were given for the previous treatment failures and difficulties:

a) Brucella organisms are known to survive within phagocytic cells of the reticuloendothelial system, particularly in lymph nodes, liver, spleen, bone marrow, the
mammary glands, reproductive organs and tissues with a weak blood supply, and are therefore protected from antibodies, complement and antibiotics. The ability of Brucella organisms to survive intracellularly can result in long-term chronic infection. In addition, relapses after antimicrobial treatment were also explained by the intracellular location of brucellae (9, 24, 25, 26).

b) Incorrect choice of antibiotics (7, 11, 37).

c) Insufficient doses of effective antibiotics (12, 13, 24, 25, 26, 29).

d) Insufficient duration of treatment (8, 12, 13, 24, 25, 26, 29).

e) Use of the intraperitoneal route for administration of antibiotics created serious reactions in some cases (17, 34).

f) Use of only one antibiotic (20, 21, 24).

g) High cost of antibiotherapy (24, 25, 34).

h) Failure to cure udder infections in many cases (24, 25, 26).

i) Presence of antibiotic residues in milk and meat of treated animals, and the possible harmful effect on consumers (27).

The present study aimed to overcome the above problems and involved work under particularly difficult conditions with regard to the level of infection among the cows used, which were all serologically and (particularly) bacteriologically positive. Consequently, the following points were taken into account when attempting to obtain more successful results. Since successful therapy in human beings appears to depend on the permeability of cell walls to drugs (24), the OTC base was used because it is capable of penetrating intracellularly and inhibits bacterial protein synthesis at the level of the ribosomes. The LA-OTC variant was selected to save time and effort and to provide long-lasting OTC concentration in blood plasma, as one injection gave an effective concentration of 0.6 µg/ml for three days (26). ST was also used, because it is known to inhibit protein synthesis of Gram-negative bacteria. Furthermore, ST acts synergistically with OTC to inhibit growth of *B. abortus* within bovine cells cultured in vitro. OTC at 0.5-1.0 µg/ml, permitted growth or was bacteriostatic for brucellae within tissue cells. However, OTC proved to be effectively bactericidal when combined with 10 µg/ml of ST (15, 25, 34, 36). In addition, a combination of LA-OTC and ST proved very effective in curing sheep and goats naturally infected with *B. melitensis* (35). However, early studies (20, 24, 25) on the treatment of bovine brucellosis indicated poor success rates when LA-OTC or ST was used alone. For these reasons, LA-OTC combined with ST was used for the treatment of cows naturally infected with *B. melitensis* or *B. abortus*.

Furthermore, in infected cows, despite the use of a combination of LA-OTC and ST, several authors failed to cure udder infections in many cases (24, 25, 26). For this reason, before initiation of the present treatment regimens, milking was stopped in lactating cows and udders were completely evacuated before each injection, in order to decrease the number of *Brucella* organisms in udder tissues and secretions. Consequently, OTC-IMI was used locally in combination with systemic (i.m.) inoculation of LA-OTC and ST. This combination proved to be highly effective in eliminating *Brucella* organisms from all infected cows in regimens A and B. However, OTC-IMI and ST, when administered every 3 days in regimen C, were less effective in eliminating *Brucella* organisms in infected cows. This may be due to the fact that both ST and OTC-IMI are
not long-acting antibiotics. Also, it seems unlikely that OTC-IMI alone would be effective for the treatment of lactating cows due to the continuous flushing of OTC by milking, and the protection of \textit{Brucella} organisms in the presence of milk.

Previous studies in cows and sheep (17, 34) concluded that i.p. inoculation of OTC was not safe, due to the development of several serious local reactions at the site of injection. In the present study, i.m. inoculation of LA-OTC did not result in harmful systemic effects or any obvious lasting local reactions at the site of inoculation.

The doses and duration of application of selected antibiotics in the present study indicate that the long-term therapy and the drug combinations used yielded better results than several other regimens (8, 12, 13, 24, 25, 26, 29).

In humans, although OTC has been considered the most effective antibiotic for treatment of brucellosis, a small number of relapses do occur. Relapses have also been reported in cows (14, 24, 25). In the present study, relapses occurred in only 3 of 33 cows treated with regimen C. However, relapses did not occur in any of the 88 cows treated with regimen A or B (provided that the treatment protocol is strictly observed). In the present trial, the majority (approximately 90%) of the cows treated were monitored bacteriologically for 16 months post-treatment in order to detect possible relapses. Even in relapsed cows (3 animals in regimen C), only a small number of \textit{Brucella} colonies were recovered from large tissue samples.

In the present study, all treated cows were dried before initiation of treatment; consequently, there was no risk of harmful effects on human beings from antibiotic residues in the milk from these cows during and after treatment. However, the residues of antibiotics in milk and meat of treated animals should be investigated to determine accurately when milk and/or meat can be safely consumed following completion of the treatment regimens.

The present results of serological examinations were of some use in evaluating the effectiveness of the treatment regimens. However, the results were not conclusive, as animals were only monitored for 16 months post-treatment, and there was no non-treated control group for comparison. The results obtained were in agreement with findings on successful treatment of brucellosis in sheep and goats when monitored for 8 months post-treatment (35). Other investigators, however, reported that serological findings were not useful in evaluating the effectiveness of treatment regimens (24, 34).

In Saudi Arabia, the treated cows were originally imported as pregnant heifers at an average cost of US$3,600, including the cost of air freight. The cost of antibiotics used was calculated according to sale prices in the Saudi market at the time of the experiment (US$1 = SR3.75). The average cost of the antibiotics used in regimen A, B or C for treating a cow of an average weight of 600 kg was US$186 (SR700). This did not include the cost of equipment or interventions, because all serological and bacteriological examinations were performed free of charge at Government institutions. However, in other situations, the cost of supplies and intervention should be considered. The cost of successful treatment of a cow represents approximately 5% of the original price of the animal, which is considered economically feasible in Saudi Arabia.

In conclusion, when treatment protocols were strictly observed, regimens A and B proved to be effective, practical, without side-effects and relatively inexpensive in causing cessation of the symptoms of brucellosis and eliminating \textit{Brucella} organisms from infected cows. Consequently, at the present time, this treatment would probably be limited to valuable breeding animals. However, further research may yield a regimen
which could be used for the treatment of all infected animals in a herd and for prophylaxis of those animals recently exposed to Brucella infection. Furthermore, it is concluded that the failure of a small number of cows to respond to regimen C cannot be accounted for by the development of resistance by Brucella. The treatment of seropositive animals should be performed together with vaccination of all seronegative animals in the same herd to achieve effective control and minimise public health risks.

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Le protocole A (appliqué à 35 vaches) consistait en une administration intramusculaire (i.m.) de 25 mg/kg de LA-OTC tous les trois jours pendant 42 jours, de 25 mg/kg de ST (i.m.) quotidiennement pendant huit jours et en une OTC-IMI quotidienne de 20 ml par trayon pendant quatre jours. Le protocole B (appliqué à 53 vaches) était similaire au précédent, sauf pour la ST qui était administrée un jour sur deux pendant 16 jours et pour l’OTC-IMI, pratiquée un jour sur deux pendant huit jours. Les deux modes de traitement se sont révélés aussi efficaces l’un que l’autre pour l’élimination totale des brucelles des vaches objet de l’expérimentation et aucune récidive n’a été constatée. Quant au protocole C, similaire au protocole A, hormis pour la ST qui était administrée tous les trois jours pendant 24 jours et l’OTC-IMI, pratiquée également tous les trois jours pendant 12 jours, il n’a permis l’élimination des brucelles que sur 30 (91 %) des 33 vaches traitées.

Avant l’application de ces trois protocoles thérapeutiques, les biotypes 1 ou 2 de B. melitensis ont été régulièrement isolés dans les sécrétions mammaires de 103 vaches et le biotype 1 de B. abortus dans celles de 18 vaches.
**PROTOCOLOS TERAPÉUTICOS EFICACES PARA EL TRATAMIENTO DE LA INFECCIÓN POR BRUCELLA MELITENSI S Y BRUCELLA ABORTUS EN VACAS.**


**Resumen:** Se evaluaron tres protocolos terapéuticos en 121 vacas infectadas por vía natural por Brucella melitensis o Brucella abortus, a partir de una combinación de oxitetraciclina de acción prolongada (long-acting oxytetracycline: LA-OTC), estreptomicina (ST) e infusión intramamaria de OTC (OTC-intramammary infusion: OTC-IMI). Para considerar el tratamiento exitoso se tuvieron en cuenta dos criterios: el cese de la excreción de brucelas en las secreciones mamarias y la ausencia de brucelas en tejidos específicos.

El protocolo A, que fue experimentado en 35 vacas, consistía en la administración intramuscular (i.m.) de 25 mg/kg de LA-OTC cada tres días durante 42 días, de 25 mg/kg de ST (i.m.) diarios durante ocho días y en una OTC-IMI diaria de 20 ml por pezón durante 4 días. El protocolo B, que se experimentó en 53 vacas, era similar al anterior excepto en cuanto a la ST, que se administró cada dos días durante 16 días, y a la OTC-IMI, que se administró cada dos días durante ocho días. Uno y otro tratamiento se mostraron equivalentemente eficaces para la eliminación total de las brucelas en las vacas tratadas y no se constató ninguna recidiva. Por último, el protocolo C, similar al protocolo A salvo en que la ST se administraba cada tres días durante 24 días y la OTC-IMI también cada tres días durante 12 días, sólo permitió eliminación de brucelas en 30 de las 33 vacas tratadas, es decir, un 91%.

Antes de aplicar estos tres protocolos terapéuticos, los biotipos 1 o 2 de B. melitensis fueron regularmente aislados en las secreciones mamarias de 103 vacas y el biotipo 1 de B. abortus en las secreciones mamarias de 18 vacas.

**PALABRAS CLAVE:** Antibióticos – Bovinos – Brucella abortus – Brucella melitensis – Oxitetraciclina – Estreptomicina – Terapéutica.

**REFERENCES**


