Identification of the *Brucella melitensis* Vaccine Strain Rev.1 in Animals and Humans in Israel by PCR Analysis of the *Pst*I Site Polymorphism of Its *omp2* Gene

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Adverse effects of strain persistence and secretion in milk have been encountered with the *Brucella melitensis* vaccine strain Rev.1. Field isolates obtained from vaccinated animals and from a human resembled the vaccine strain Rev.1 by conventional bacteriological tests. The lack of a specific molecular marker that could specifically characterize the commercial vaccine strain prevented confirmation of the homology of the Rev.1-like field isolates to the vaccine strain. The composition of the *omp2* locus from two gene copies with differences in their *Pst*I restriction endonuclease sites was used to establish an epidemiologic fingerprint for the *omp2* gene in the Rev.1 vaccine strain. Primers designed to amplify DNA sequences that overlap the *Pst*I site revealed a single 282-bp DNA band common to all *Brucella* spp. Agarose gel electrophoresis of the *Pst*I digests of the PCR products from strains 16M and the vaccine strain Rev.1 revealed a distinctive profile that included three bands: one band for the intact 282-bp fragment amplified from *omp2a* and two bands resulting from the digestion of the amplified *omp2b* gene fragment, 238- and 44-bp DNA fragments, respectively. Amplified fragments of 37 Rev.1-like isolates, including 2 human isolates, also exhibited this pattern. In contrast, DNA digests of all other Israeli field isolates, including atypical *B. melitensis* biotype 1 and representatives of the biotype 2 and 3 isolates, produced two bands of 238 and 44 bp, respectively, corresponding with the digestion of both *omp2a* and *omp2b* genes. This method facilitates identification of the Rev.1 vaccine strain in both animals and humans in Israel.

Brucella melitensis causes a worldwide zoonosis. It is one of the major causes of abortions in sheep and goats, and the organism is secreted in the milk of infected animals. People contract the disease by direct contact with contaminated fetal membranes or, more commonly, as a result of the consumption of contaminated unpasteurized milk and cheese products. The organisms are small, gram-negative coccobacilli that grow in the host as nonobligatory intracellular pathogens of the reticuloendothelial system. Derivatives of tetracycline are often used to treat human infection, while a slaughter policy is recommended for livestock in order to eradicate the disease (11).

In the late 1950s, Elberg developed a live attenuated vaccine, strain Rev.1 (12, 13). It was shown that although the vaccine prevented abortion, it did not provide protection against infection. Bosseray demonstrated that different lots of Rev.1 vaccines showed variable immunogenicity in mice according to their level of virulence (5, 6). This study emphasized the instability of the biological properties of the vaccine strain, stressing the need for stringent control of vaccine production (7).

The problems in the laboratory were reflected by similar results in the field. In South Africa, selection of a few smooth colonies as seed stock led to production of a virulent vaccine strain which infected sheep and caused human disease (22, 27, 28).

Throughout the last decade Israel maintained a conservative vaccination policy in which only young female livestock between the ages of 2 to 6 months were vaccinated, using a full dose by the subcutaneous route. Nevertheless, retrospective data demonstrated that the Rev.1 vaccine led to the adverse effects of strain persistence in the vaccinated animals and was occasionally spread horizontally (4, 30). Moreover, in two cases it was shown that the vaccine strain caused human infection, demonstrating the zoonotic hazards of its virulence. The fact that vaccination did not always protect the animals in the field and the several cases of secretion of the field strain in milk had proven the inefficacy of the whole vaccination program.

International agencies, in their assistance to developing countries, suggested that national control programs should depend on a whole-flock vaccination scheme as a cost-effective method until the prevalence of the disease was reduced. Only then should test and slaughter be implemented to eradicate the disease (10). There was opposition to this proposal (4) due to the adverse effects encountered in the field and the public concerns of possible risks to the human population following secretion of the vaccine strain in milk. The absence of a specific molecular marker that could be associated with the identity of the commercial vaccine strain prevented those opposing the

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TABLE 1. *Brucella* strains used in this study

Strain no.	Species	Biovar	Strain designation	Strain type, host, or source
1	*B. melitensis*	1	16M	Prototype strain
2	*B. melitensis*	1	117790	Human
3	*B. melitensis*	1	118762	Human
4	*B. melitensis*	1 (atypical)	9413	Sheep
5	*B. melitensis*	1 (atypical)	6012	Human
6	*B. melitensis*	1 (atypical)	124386	Human
7	*B. melitensis*	1	Rev.1 (vaccine) (ocular lot)	Commercial source
8	*B. melitensis*	1	Rev.1 (vaccine) (subcutaneous lot)	Commercial source
9	*B. melitensis*	1 (Rev.1-like)	5000	Human
10	*B. melitensis*	1 (Rev.1-like)	204216	Human
11	*B. melitensis*	1 (Rev.1-like)	134172	Sheep milk
12	*B. melitensis*	1 (Rev.1-like)	116375	Sheep milk
13	*B. melitensis*	1 (Rev.1-like)	124596	Sheep milk
14	*B. melitensis*	1 (Rev.1-like)	225875	Goat's retropharyngeal gland
15	*B. melitensis*	1 (Rev.1-like)	226390	Sheep bulk milk tank
16	*B. melitensis*	1 (Rev.1-like)	226997	Sheep milk
17	*B. melitensis*	1 (Rev.1-like)	223713	Goat's membranes from aborted fetus
18	*B. melitensis*	2	118808	Human
19	*B. melitensis*	2	160621	Human
20	*B. melitensis*	2 (atypical)	124906	Human
21	*B. melitensis*	3	Ether	Prototype strain
22	*B. melitensis*	3	119917	Human
23	*B. melitensis*	3	119919	Human
24	*B. melitensis*	Rough	B115	Reference strain
25	*B. melitensis*	Rough	119056	Human
26	*B. abortus*	1	544	Prototype strain
27	*B. abortus*	1	2038	Reference strain
28	*B. abortus*	1	S19 (vaccine)	
29	*B. abortus*	3	Tulya	Prototype strain
30	*B. suis*	1	S2 (vaccine)	

vaccination program from linking Rev.1-like field isolates to the vaccine strain. By the same token, those in favor of the program could ignore the risks posed by the Rev.1 vaccine, using the same rationale.

Data presented below provide evidence supporting the existence of a *Pst*I site polymorphism in the *Brucella omp*2 gene. The *Pst*I digestion pattern of PCR-amplified fragments from the Israeli isolates was different from that of the prototype strain 16M. Curiously, the *Pst*I digestion profile of the *omp*2 amplified fragments from the vaccine strain Rev.1 resembled that of strain 16M, allowing the differentiation of Rev.1 isolates from *B. melitensis* field strains in Israel. This achievement could specifically address the potential misdiagnosis of the atypical *B. melitensis* biovar 1 strains as Rev.1 isolates due to similarities in their phenotypic susceptibility to penicillin.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study (Table 1) were from the collection maintained in the Israeli reference laboratory. *Brucella* field strains were isolated by conventional methods (1) from milk samples, aborted fetuses, and placentas. Human isolates were obtained from local medical laboratories. Strain 16M was obtained from J.-M. Verger, Institut National de la Recherche Agronomique, Nouzilly, France. Strain biotyping was performed by standard methods (1). *Escherichia coli* strain NCTC 9001 was used as a control (for non-*Brucella* DNA). *Rhizobium meliloti* strains 158M and 161M were obtained from D. Kishinevsky, and *Agrobacterium radiobacter* strains At5/r and At96216 were obtained from D. Zutra, The Volcani Center, Beit Dagan, Israel. Bacterial

strains other than *Brucella* were obtained from the Department of Bacteriology, The Kimron Veterinary Institute, Beit Dagan, Israel, or from the Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Bacterial DNA. To prepare chromosomal DNA, bacterial cells were harvested in saline and incubated for 20 min at 4°C with lysozyme (4 mg/ml). Sodium dodecyl sulfate (0.5% [wt/vol]) and proteinase K (200 mg/ml) were then added, and incubation was continued at 37°C for 1 h. The cell lysate was extracted once with phenol-chloroform-isoamyl alcohol (1:1:49) and once with chloroform-isoamyl alcohol (1:24). The purified DNA was alcohol precipitated, resuspended in TE (50 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and stored at 4°C.

PCR and oligonucleotide primers. The *Brucella omp*2 gene was used as target DNA. The forward 5′ primer (p1 [TGGAGGTCAGAAATGAAC]) and reverse 3′ primer (p2 [GAGTGCGAAACGAGCGC]) of an *omp*2 gene segment were obtained from National Biosciences, Inc., Plymouth, Minn.

PCR amplification was performed by the method of Mullis and Faloona (26). A typical reaction mixture contained 50 mM KCl, 1.5 mM MgCl$_2$, 0.1% (wt/vol) Triton X-100, 0.2 mg of bovine serum albumin (fraction IV; Sigma) per ml, and 10 mM Tris-HCl (pH 8.5). Each reaction mixture was supplemented with 100 mM each of the four deoxyribonucleotides, 100 ng of sample DNA, and each oligonucleotide primer. For slide PCR, sample DNA was replaced with brucellae that were laid on a glass slide, air dried, and fixed by being heated. A sample of the dried cells was then collected with a needle, the needle was dipped in 10 μl of double-distilled water, and 2 μl from this solution was put in the PCR mixture. Otherwise, sample DNA (2 μl from a bacterial cell suspension in double-distilled water boiled at 100°C for 20 min) was used. Reactions were initiated by adding 0.5 U of *Taq* polymerase (Appligene, Illkirch, France). The reaction mixture was covered with 15 μl of mineral oil (Sigma) to prevent evaporation. Following hot start treatment at 95°C for 3 min, PCR was performed with an Eppendorf Thermocycler (Eppendorf, Hamburg, Germany) as follows: 35 cycles of PCR, with 1 cycle consisting of 20 s at 95°C for DNA denaturation, 1 min at 50°C for
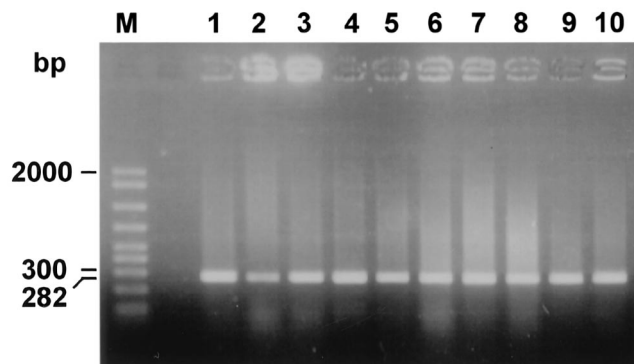
FIG. 1. Agarose gel electrophoresis of PCR-amplified *omp2* gene fragments from *Brucella* prototype strains. The figure shows a single band, a 282-bp DNA fragment. Lanes: M, molecular size ladder (in base pairs); 1, *B. abortus* strain 2308; 2, *B. melitensis* strain B115; 3, *B. abortus* strain Tulya; 4, *B. abortus* strain 544; 5, *B. abortus* vaccine S19; 6, *B. melitensis* strain 16M; 7, *B. suis* vaccine S2; 8, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 9, *B. melitensis* vaccine strain Rev.1 (ocular lot); 10, *B. melitensis* strain Ether.

DNA annealing, and 1 min at 72°C for polymerase-mediated primer extension. The last cycle included incubation of the sample at 72°C for 7 min. Ten microliters of the amplified product was analyzed by electrophoresis in 1.5% agarose gels in TEA buffer (20 mM Tris-acetate, 1 mM EDTA [pH 8.0]).

DNA digestion. Restriction enzymes were used according to the manufacturer's instructions (Boehringer GmbH, Mannheim, Germany). The digested DNA was separated by electrophoresis on either 1.5% agarose gels (wt/vol in Tris-acetate buffer) or 10% polyacrylamide gels (wt/vol in Tris-borate buffer). DNA fragments were visualized by staining with ethidium bromide (1.5 μg/ml).

RESULTS

Validation of the method with prototype strains. The PCR was first performed to test specificity by comparing *Brucella* species DNAs to the DNAs from several other bacteria, including the taxonomically closely related *Agrobacterium* and *Rhizobium* strains (29). A single band with the expected size of 282 bp (19) was obtained only when *Brucella* DNA was used as a template. All other bacterial strains and a water sample failed to produce an amplified fragment (data not shown).

The PCR test was studied with *Brucella* prototype strains from two of the three important species, namely, *B. melitensis* and *B. abortus* (field and vaccine strains). In addition, *B. suis* vaccine strain S2 was included as a representative of this species. As shown in Fig. 1, the DNAs from all the strains produced a 282-bp band. As shown in Fig. 2, *Pst*I digestions of the amplified fragments from the strains gave different bands on agarose gels. *B. abortus* (lanes 1, 3, 4, and 5), *B. suis* S2 (lane 8), and *B. melitensis* biovar 3 (lane 12) digests revealed a single band, a 238-bp band. Other possible smaller fragments are not shown on the gel. In comparison, *Pst*I digestion of *B. melitensis* strain 16M (prototype for *B. melitensis* biovar 1 virulent strain [lane 9]) and strain Rev.1 (a vaccine strain from two different producers administered by a subcutaneous and an ocular route [lanes 10 and 11, respectively]) amplified DNAs, included two visible bands: a large band, which was uncut DNA (lanes 6 and 8), and another band, a 238-bp *B. abortus* fragment. Other possible smaller bands are not shown on the gel. The *Pst*I digestion pattern of the amplified fragment obtained from *B. melitensis* strain B115 (a stable rough form obtained from an infected

goat in Malta in the early 1970s) (lane 2) was similar to those for strains 16M and Rev.1 (lanes 9, 10, and 11, respectively).

The digestion profiles of the same DNAs were analyzed by polyacrylamide gel electrophoresis, as shown in Fig. 3. The purpose of this analysis was to identify possible smaller fragments that were not shown by agarose gel electrophoresis. As can be seen in Fig. 3, besides the 282- and 238-bp DNA bands, all samples produced an additional identical smaller fragment which was calculated to be 44 bp. It was calculated that the two smaller bands together were the same size as the uncut DNA, confirming the expected *Pst*I restriction pattern for *B. abortus* biovar 1 (19).

***Pst*I digestion profile of the *omp2* gene amplified fragment from atypical *B. melitensis* biotype 1 strains.** Atypical *B. melitensis* biotype 1 isolates from a human source (isolate 6012) and sheep source (isolate 9413) were described previ-
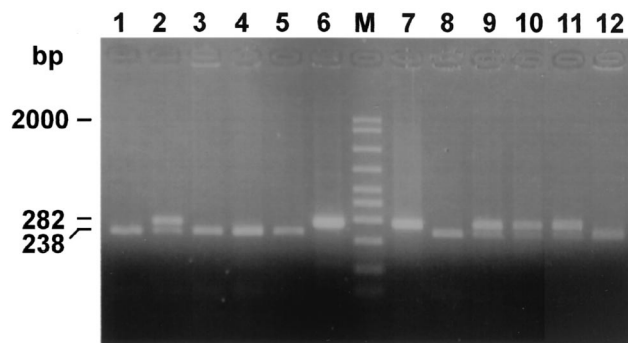

FIG. 2. Agarose gel electrophoresis of *Pst*I digests of amplified *omp2* gene fragments from *Brucella* prototype strains. The figure shows the uncut 282-bp DNA and the larger, *Pst*I-digested DNA fragments. The smaller, 44-bp DNA fragment is not shown. Lanes: 1, *B. abortus* strain 2308; 2, *B. melitensis* strain B115; 3, *B. abortus* strain Tulya; 4, *B. abortus* strain 544; 5, *B. abortus* vaccine S19; 6, *B. melitensis* strain 16M (uncut); M, molecular size ladder (in base pairs); 7, *B. abortus* strain 544 (uncut); 8, *B. suis* S2; 9, *B. melitensis* strain 16M; 10, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 11, *B. melitensis* vaccine strain Rev.1 (ocular lot); 12, *B. melitensis* strain Ether.
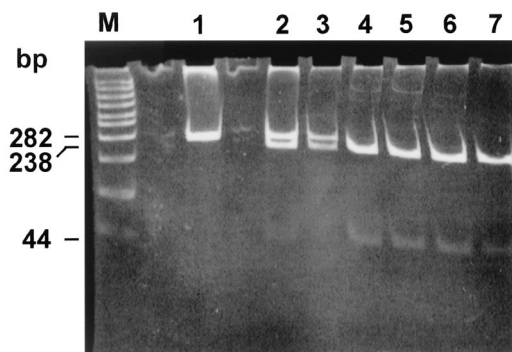

FIG. 3. Polyacrylamide gel electrophoresis of *Pst*I digests of amplified *omp2* gene fragments from *Brucella* prototype strains. The figure shows the three DNA fragments, the uncut DNA and the two *Pst*I-digested DNA fragments, with sizes of 282, 238, and 44 bp, respectively. Lanes: M, molecular size ladder (in base pairs); 1, *B. melitensis* strain 16M (uncut); 2, *B. melitensis* strain 16M; 3, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 4, *B. abortus* vaccine S19; 5, *B. abortus* strain 544; 6, *B. abortus* strain 2308; 7, *B. melitensis* strain Ether.
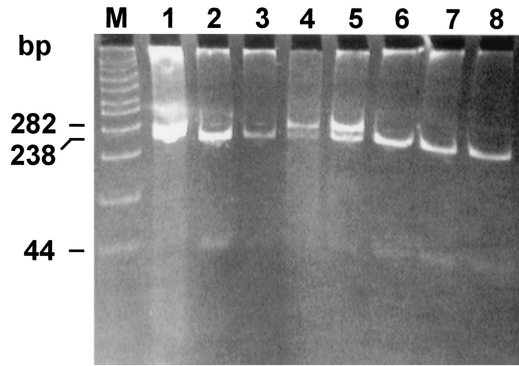
FIG. 4. Polyacrylamide gel electrophoresis of *Pst*I digests of amplified *omp2* gene fragments from atypical *B. melitensis* biotype 1 isolates from humans and animals compared to prototype strains. The figure shows the three DNA fragments, the uncut DNA and the two *Pst*I-digested DNA fragments, with sizes of 282, 238, and 44 bp, respectively. Lanes: M, molecular size ladder (in base pairs); 1, *B. melitensis* strain 6012 (human isolate) (uncut); 2, *B. melitensis* strain 6012 (human isolate); 3, *B. melitensis* strain 9413 (sheep isolate); 4, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 5, *B. melitensis* strain 16M; 6, *B. abortus* vaccine S19; 7, *B. abortus* strain 544; 8, *B. melitensis* strain Ether.
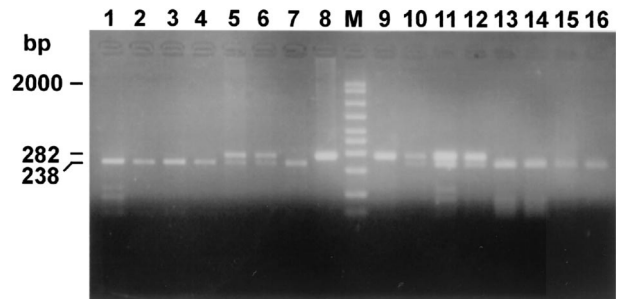

FIG. 5. Agarose gel electrophoresis of *Pst*I digests of amplified *omp2* gene fragments from *Brucella* field strains. The figure shows the uncut 282-bp DNA and the larger, *Pst*I-digested DNA fragment. The smaller, 44-bp DNA fragment is not shown. Lanes: 1, *B. melitensis* biotype 1 strain 117790; 2, *B. melitensis* biotype 1 strain 118762; 3, *B. melitensis* biotype 2 strain 118808; 4, *B. melitensis* biotype 2 strain 160621; 5, *B. melitensis* vaccine strain Rev.1 (ocular lot); 6, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 7, *B. melitensis* rough strain 119056; 8, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot) (uncut); M, molecular size ladder (in base pairs); 9, *B. melitensis* strain 16M (uncut); 10, *B. melitensis* biotype 1 strain 5000 (human Rev.1-like isolate); 11, *B. melitensis* strain 16M; 12, *B. melitensis* biotype 1 strain 204215 (sheep Rev.1-like isolate); 13, *B. melitensis* biotype 3 strain 119917; 14, *B. melitensis* biotype 3 strain 119919; 15, *B. melitensis* biotype 1 atypical strain 124386; 16, *B. melitensis* biotype 2 atypical strain 124906.

ously (2). We compared the *Pst*I digestion profiles of the *omp2* gene amplified fragment obtained from these strains and those obtained from prototype strains. All *Brucella* strains produced identical amplified 282-bp fragments (data not shown). Figure 4 depicts a polyacrylamide gel analysis of the digestion profile of these DNAs. As can be seen, *Pst*I digestion of the two *B. melitensis* atypical strains (lanes 2 and 3) produced a uniform pattern identical to that obtained for *B. abortus* strains S19 and 544 (lanes 6 and 7) and to that of *B. melitensis* biotype 3 (lane 8). The *Pst*I digestions of the amplified fragments from the commercial vaccine strain Rev.1 and the prototype strain 16M yielded a different pattern (lanes 4 and 5, respectively). The gel also shows the smaller, 44-bp band common to all digests.

We then analyzed the digestion profile of the *omp2* DNA fragment of selected human isolates as shown in Fig. 5. The samples included biovar 1 strains (lanes 1, 2, 3, 4, and 14), a rough isolate (lane 7), atypical biotype 1 (lane 15) and atypical biotype 2 (lane 16) strains, and two Rev.1 human isolates (lanes 10 and 12). In addition, we included the prototype strain 16M (lane 11) and two uncut DNAs from strain Rev.1 (lane 8) and strain 16M (lane 9). Besides strain Rev.1 (lanes 5 and 6), Rev.1-like isolates (lanes 10 and 12), and *B. melitensis* strain 16M (lane 11), all other isolates had similar digestion profiles (as shown in Fig. 2 for *B. abortus* strains) and *B. melitensis* biotype 3 strain Ether.

PstI digestion profile of the *omp2* gene amplified fragment from *B. melitensis* Rev.1-like isolates. A total of 23 *B. melitensis* biotype 1 field isolates, 5 *B. melitensis* atypical biovar 1 field isolates (2), 13 *B. melitensis* biotype 2 field isolates including 3 atypical strains, and 13 *B. melitensis* biotype 3 field isolates were studied. All exhibited the pattern shown for *B. abortus* strains (data not shown). In contrast, 37 field isolates with Rev.1-like bacteriological characteristics produced an identical *Pst*I digestion pattern (as did the commercial vaccine, strain Rev.1). A representative selection is shown in Fig. 6, lanes 1, 2, 3, 8, 9, 10, and 11, compared to lanes 4 and 7, respectively.

DISCUSSION

The PCR technique has increasingly been used as a supplementary method in *Brucella* diagnosis (8, 14, 15, 21, 23, 25). Recently, a molecular biotyping approach has been proposed on the basis of restriction endonuclease polymorphism in the genes encoding the major 25- and 36-kDa outer membrane proteins of *Brucella* (9, 17, 20). The *omp2* gene exists as a locus of two nearly homologous repeated copies that differ slightly among *Brucella* spp. and biotypes (18). We used this information to design specific primers that amplify a 282-bp fragment
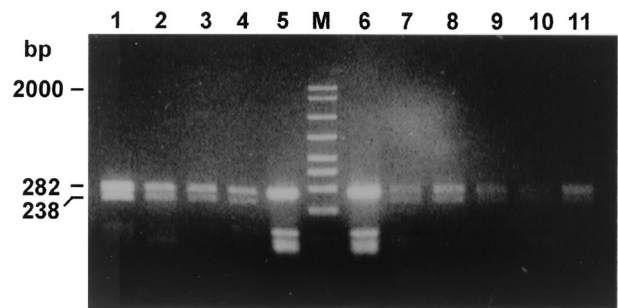

FIG. 6. Agarose gel electrophoresis of *Pst*I digests of amplified *omp2* gene fragments from *Brucella* field strains that resemble Rev.1 according to biochemical biotyping. The figure shows the uncut 282-bp DNA and the larger, *Pst*I-digested DNA fragment. The smaller, 44-bp DNA fragment is not shown. Lanes: 1, *B. melitensis* strain 134172; 2, *B. melitensis* strain 116375; 3, *B. melitensis* strain 124596; 4, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot); 5, *B. melitensis* vaccine strain Rev.1 (subcutaneous lot) (uncut); M, molecular size ladder (in base pairs); 6, *B. melitensis* vaccine strain (ocular lot) (uncut); 7, *B. melitensis* vaccine strain Rev.1 (ocular lot); 8, *B. melitensis* strain 225877; 9, *B. melitensis* strain 226390; 10, *B. melitensis* strain 226997; 11, *B. melitensis* strain 223713.

(Fig. 1), flanking upstream sequences of the 5′ terminus of the two genes and expanding downstream of the *Pst*I and *Kpn*I sites (17). We assumed that the sensitivity of the test would be doubled by selecting duplicated DNA sequences of the two genes. Moreover, we assumed that because of the existing *Pst*I site polymorphism between *B. melitensis* and *B. abortus*, the test would distinguish between the two species. According to the working hypothesis, DNA fragments obtained from *B. melitensis* strain 16M should produce three bands, an intact 282-bp fragment from the amplified *omp2a* gene that lacks the *Pst*I site and two smaller fragments of 238 and 44 bp, the products obtained from digestion of the *omp2b* amplified fragment (17). In contrast, *B. abortus* DNA should produce only the two smaller fragments from both genes, a 238-bp fragment and a 44-bp fragment, respectively (Fig. 2 and 3).

We used this method to study the stability of the *omp2* gene among local *B. melitensis* isolates derived from small ruminants, cattle, and humans and representing the current *Brucella* population in Israel. Our data confirmed the expected paradigm for *B. melitensis* strains B115, 16M, and the vaccine strain Rev.1 (Fig. 2, lanes 2, 9, 10, and 11), as well as for *B. abortus* (Fig. 2, lanes 3, 4, and 5) and *B. suis* strain 2 (Fig. 2, lane 8). The Israeli *B. melitensis* field isolates from the three biotypes, including the atypical biotype 1 strains, unexpectedly exhibited the *Pst*I digestion profile which occurs in *B. abortus*, i.e., two bands of 238 and 44 bp, respectively (Fig. 4, lane 3, and Fig. 5, lanes 1, 2, 3, 4, 7, 13, 14, 15, and 16; also data not shown).

In a comprehensive study, Meyer has shown that unlike *B. abortus*, *B. melitensis* lacked plasticity in the features characterized by the conventional biotyping methods (24). Our data indicated that in contrast to these findings, *B. melitensis* has undergone genetic diversions in a pattern similar to that previously shown to occur in other *Brucella* spp. From the data, one could infer that the prevailing Israeli biotype 1 strains have acquired a new *Pst*I site in the *omp2a* gene (compared to the sequences established for strain 16M). On the other hand, strains belonging to biotypes 2 and 3 acquired this change earlier, since all isolates demonstrated the same pattern, which was similar to the *B. melitensis* biovar 3 prototype strain Ether (Fig. 2, lane 12).

Results obtained by Cloeckaert et al. (9) confirmed these data, showing that *B. melitensis* isolates were split between those with a single *Pst*I site located in the *omp2b* gene and those with two *Pst*I sites, one in *omp2a* and one in *omp2b*.

Interestingly, from the list presented by Cloeckaert et al., it can be seen that even Israeli isolates from the 1970s all had two *Pst*I sites, one in *omp2a* and the second in *omp2b*, similar to the results presented above for isolates from the later period. This suggests that the Israeli *B. melitensis* biotype 1 strains emerged separately from 16M, a strain that originated in the United States.

It is interesting that recent field isolates in Mexico produced a digestion profile of the *omp2* amplified gene fragments similar to that of strain 16M and strain Rev.1 (T. A. Ficht, personal communication). Our data and those obtained by Cloeckaert et al. have further shown that the described phenomenon applied not only to strain 16M and Rev.1 but also to the rough strain B115 and H38 as well (Fig. 2, lane 2) (9). We could propose, therefore, that at least two separate *B. meliten-sis* biotype 1 lines have evolved, one represented by strain 16M and the other represented by the Israeli isolates. A similar conclusion was drawn by Cloeckaert et al. regarding the absence of a *Bgl*II restriction site in the *omp2b* genes of the Israeli isolates (9).

This study included 37 isolates that according to conventional bacteriological methods were characterized as vaccine strain Rev.1. The *Pst*I digestion pattern of the *omp2* amplified gene fragments resembled that of strain 16M, the prototype strain for virulent *B. melitensis* biovar 1, and that of the vaccine strain Rev.1 (Fig. 2, lanes 9, 10, and 11). In contrast, the *Pst*I digestion profile of the *omp2a* gene amplified fragments from all other Israeli isolates, representing the three biotypes, depicted a reproducible and conserved pattern that was different from that shown for strains 16M and Rev.1 (Fig. 5, lanes 1, 2, 3, 4, 7, 13, 14, 15, and 16). This suggests that a genetic link might be established between the prototype strain 16M and the vaccine strain Rev.1. A few other geographically remote isolates may have shared the same ancestral strain.

Human infection with the vaccine strain Rev.1 in South Africa has been reported, following horizontal infection among sheep. Clonal selection of virulent colonies during the preparation of a working seed stock probably led to production of a vaccine lot with undesirable characteristics (22, 27, 28). It is interesting that, in Israel, we also identified a human case of infection with the vaccine strain Rev.1. The owner of an intensively managed sheep farm was infected with the Rev.1 vaccine strain 6 months after a series of abortions in ewes and isolation of Rev.1 from the fetal membranes (3). In a report from South Africa, the researchers biotyped the isolates by conventional *Brucella* biotyping methods, and no direct molecular linkage was shown between the field isolates and the commercial Rev.1 vaccine strain. To the best of our knowledge, our report is the first to associate animal and human infection with the vaccine strain Rev.1 based on molecular identification of the strain.

The resemblance of the phenotypic properties of the vaccine strain Rev.1 and the atypical strain characterized in Israel (2) regarding susceptibility to penicillin and dyes has raised the possibility that the atypical strain had originated from a mutation of the vaccine strain. The unique *Pst*I pattern described for strain 16M and the vaccine strain Rev.1 has allowed us to elaborate on this subject by comparing their *omp2* gene *Pst*I digestion patterns. If the atypical strain had originated from a Rev.1 mutant, its *omp2* gene *Pst*I digestion pattern should have matched that of strain Rev.1 and strain 16M. The contrary would be true if it had originated from a field strain mutant. The similarity between the *Pst*I *omp2* gene digestion profiles of the atypical strains and the virulent field isolates (Fig. 4) clearly implied that the latter was the case, lending a mutation in a virulent strain to render it susceptible to penicillin and dyes. The zoonotic competence of the atypical isolates (2) that caused human infection further supported the idea that these strains had originated from a virulent strain.

The results presented in this study have highlighted some of the potential hazards associated with use of the Rev.1 vaccine in national control programs. It has been argued that vaccine quality could be impaired if its production did not adhere to stringent standards (5, 7). Having encountered the adverse effects of the subcutaneous vaccine, we assumed that the commercial Rev.1 vaccine supplied to Israel originated from a

defective seed stock, similar to the events described in South Africa. To overcome these problems, Israel changed the vaccine source in November 1997, purchasing it from a company that had sustained its seed stock on a true Elberg strain (passage 101, 1970; M. Banai, personal communication). Other expected advantages of the new vaccine were the safety to adult animals, attributed to the lower dose (10^8 CFU instead of 10^9 CFU), and the method of inoculation as an ocular preparation (16). Implementation of the new vaccine in whole-flock vaccination, including vaccination of pregnant animals, led to outbreaks of abortions in several intensively managed flocks and isolation of the strain from the milk of the aborting animals. In this study, besides conventional biotyping, we also applied the new PCR method to confirm the Rev.1 identity of the isolates according to the *omp2a Pst*I digestion profile (Fig. 6). This new technique made it possible to associate a second human case of strain Rev.1 infection in a 15-year-old girl (Fig. 5, lane 12). Since then, the Israeli veterinary services changed the vaccination policy back to the consensus method of vaccination of only young females, and no additional problems have been encountered.

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