Brucellosis in Animals and Man in Egypt

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In Egypt, brucellosis has been reported and recorded as early as 1939. However, attention was directed to the disease during the 1960s. With the importation of Friesian cows the incidence of brucellosis in cattle on some farms became very high. The disease was reported also in buffaloes, sheep, goats, swine, camels, horses, donkeys, dogs and rats. Various serological tests were used for the serosurveillance and the prevalence rates varied markedly from one author to the other. Nevertheless, the data published indicate clearly that brucellosis is endemic in animals in Egypt and the number of human cases is increasing. Various workers made isolations of *Brucella abortus* from cattle as early as 1943. Since 1970, *Brucella melitensis* has been isolated from sheep and goats and also from cattle. The biotyping of *Brucella isolates* revealed that *Brucella abortus* biogroup 1 was isolated from cattle and buffaloes, while *Brucella melitensis* biogroup 3 was isolated from cattle, buffaloes, sheep, goats and camels. *Brucella abortus* biogroup 7 was isolated from camels and *Brucella suis* biogroup 1 was recovered from swine. National Brucellosis Control Programme started in 1981 adopting the test and slaughter policy and vaccination of young female calves with the reduced dose *Brucella abortus* S19 vaccine. Recently, RB51 vaccine is used in some farms.

Brucellosis is a zoonotic disease caused by organisms of the genus *Brucella*. The disease in animals causes abortion during the last third of pregnancy, retained placenta after birth, and weak newborn at birth. The udder is a very important predilection site for *Brucella*. Infection in lactating non-pregnant animals is likely to lead to colonization of the udder with excretion of *Brucella* in the milk. Greatly reduced milk yield follows abortion, and infection of the udder following a normal birth also leads to a considerable reduction in milk yield. In humans, *Brucella* species cause undulant fever, a disease characterized by intermittent fever, headache, fatigue, joint and bone pain, psychotic disturbances, and other symptoms. It is contracted mainly through exposure to *Brucella*-contaminated milk and infected organs from infected animals. (Refai & Kopek, 1988).

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In Egypt, brucellosis in animals was reported for the first time in 1939. However, attention was directed to the disease during the 1960s. With the importation of Friesian cows for the establishment of governmental farms with large numbers of animals, the incidence of brucellosis in cattle on some farms became very high. In the Touch Tamasha farm in Menofia, for example, it reached up to 38%. Such a high incidence was observed only in farms with large numbers of animals concentrated on relatively small amounts of land.

Parallel with the open door policy in the late seventies and early eighties, there was a marked increase in the number of intensive breeding farms, both governmental and private. This was based on the importation of large numbers of Friesian cows from different countries. As an example 8136 breeding animals were imported in the year 1983. The appearance of brucellosis at high rates among these newly established farms in most governorates was alarming. It was in fact a dilemma for the owners as well as the veterinary authorities. The load on the diagnostic laboratory in Dokki was great. The quarantine measures applied on farms having positive reactors have created great problems to the owners, who sought advice from all possible sources with the result that several types of vaccines were used, sometimes in the same animal. This resulted in very high reactor rates that reached in a farm in Sharkia for example to 71% positive and 14% suspicious cases. In such cases, the policy of test and slaughter was a burden on the government because of the high costs of compensations (Refai et al., 1990).

Diagnosis of brucellosis

The methods of diagnosis of brucellosis comprise tests for isolation and identification, tests for direct demonstration of the agent, its antigens or DNA, tests for detection and estimation of antibodies induced in response to the agent, as well as the allergic test, Brucellin, particularly in small ruminants.

Diagnosis by serological tests

Antibodies have a great role in the diagnosis of brucellosis, as the serological diagnosis of brucellosis is the main tool for the rapid recognition of infected herd and individual animals, though it is not 100% reliable. A positive serology means that we are dealing with field strain infection, vaccination infection, residual vaccination titre, cross-reactivity with other organisms, like *Yersinia*, *Salmonella*, *Pasteurella*, etc or human errors. Although much information is available on serological tests used for diagnosis of brucellosis, most of it concerns *Brucella abortus* in cattle. In contrast, no specific serological test for *Brucella melitensis* infection in small ruminants has been developed and until now antigens prepared from *Brucella abortus* and tests used for diagnosis of *Brucella abortus* infection in cattle are used for diagnosis of *Brucella melitensis* in small ruminants, buffaloes, camels, swine and other animals (Refai, 2003).

The incidence of brucellosis in the different animal species in Egypt, as determined by serological tests, are reviewed in the following, based on the literature available to the author. The serological tests used were Tube Agglutination Test (TAT), Rose Bengal Plate Test (RBPT), Buffered Acidified Plate Antigen Test (BAPAT), Mercapto-Ethanol Test (MET), Rivanol Test (RT), Complement Fixation Test (CFT), Enzyme-Linked Immunosorbent Assay (ELISA) and Abortus Bang Ring Test (ABRT). The latter is also called milk Ring Test (MRT).

**Cattle:** Brucellosis in cattle in Egypt has been reviewed by Refai (1989) and updated by Refai (1994). The disease was reported in Egypt for the first time (11.2%) by Ahmed (1939). Gohar et al. (1940) tested 600 cattle and found positive reactors in 20% of them. The incidence later increased to 23.25% (Kamel & Abdel-Fattah, 1961) in some farms, while in others it was 9.2% (Alton, 1963). Variable results were published in the subsequent years by several authors. The testing of 1248 cows on several farms by El-Gibaly (1969) revealed, however, an overall incidence of 2.63%. Fahmy & Bendary (1970) reported rates of reactors of 30.6% among local breeds and 50.0% among imported animals. Other data were that of Barsoum (1980) 4.5%, Shalaby (1986) 22.2%, Salem (1987) 19% and Hamdy (1989) 38.4%.

In the frame of the Egyptian-American Project on control of brucellosis, 15815 cattle on 16 farms in 5 governorates were tested during 1985-1987 (Refai et al., 1989). The rate of positive reactors reached 34% in some farms. The application of test and slaughter policy and calving vaccination with the S19 vaccine resulted in a drastic drop in the seropositivity, so that in 1988, only 1.6% of 29323 cows and 2.0% of 3355 calves on 29 farms were serologically positive. On the other hand, Refai et al. (1989) tested blood samples from 1832 cows by TAT, RT and RBPT. The incidence of positive reactors were 37.9, 32.8 and 32.8 in the three tests, respectively. The testing of other group of cows (471) by TAT and RBPT gave positive results in 61.8% and 59% of the samples in the two tests, respectively. The high incidence of brucellosis in this year was coupled with a sudden increase of importation of animals from countries known to have the disease.

El-Enbaawy et al. (1995) tested 386 cattle serum samples collected from 4 farms using cELISA, TAT, RBPT and RT. They reported rates of positivity of 17.5%, 12.7%, 25.9%, and 9.9% in the four tests, respectively. Montasser & Melad (1999) found Brucella reactors in 73 out of 11659 (0.62%) cows tested in El-Fayoum Governorate. Montasser et al. (1999) reported an incidence rate of 3.65% in cattle as revealed by CFT. In the year 2001, Montasser et al. examined 800 cattle blood samples, collected from areas with history of brucellosis in 8 governorates, namely, El-Menofia, El-Kalyobia, El-Gharbia, E-Behera, El-Dakahlia, Damietta and Port-Saied, serologically using CFT, BAPAT, RBPT, TAT, MET and RT. The incidences of reactors by these tests were 7.5%, 10%,

7.75%, 9%, and 7.1%, respectively. In the same year, Abdel-Hafeez et al. (2001) tested blood serum samples collected from 17456 cattle in Assiut and reported an incidence between 0.8% and 1.2%.

**Buffaloes**: The incidence of brucellosis in buffaloes was always very low during the last 50 years (Refaei, 1990). The high incidence (37.5%) reported by Zaki (1948) was an exception. Most of the authors reported an incidence either less than 1% (Hamada et al., 1963; Matter, 1974; El-Olemy, 1974; and Nashed, 1977) or under 5% (El-Ahwai et al., 1968; El-Gibaly, 1969; Ismail, 1975; Barsoum, 1980; Zagloul & Kamel, 1985; Abdel-Aal, 1987 and El-Sheary, 1987). Refai et al. (1989) reported rates of positive reactors in buffaloes of 10.2% and 7.6%, when their blood sera (118 samples) were tested by TAT and RT, respectively. Examination of 54 buffaloes by TAT ABRT revealed positive rates of 22.2% and 18.5%, respectively. The incidence of brucellosis in buffaloes in El-Fayoum Governorate was found to be 0.16% of 10006 animals tested (Montasser & Melad, 1999). Montasser et al. (1999) revealed an incidence rate of 2.27% in buffaloes by the CFT. The testing of 320204 buffaloes by El-Taweel (1999) revealed a positive rate of 0.24%. Abdel-Hafeez et al. (2001) tested blood serum samples collected from 19818 buffaloes in Assiut and reported an incidence between 0.43% and 0.48%.

**Sheep**: Brucellosis in sheep was recorded for the first time by Zaki (1948). Alton (1963) tested 999 sheep and found positive reactors in 3.7% of them. Ismail (1971) recorded an incidence of 0.17%. Shawkat (1973) tested blood serum samples of 530 sheep and reported positive reactor rates of 2.86%, 2.26% and 2.44% by the TAT, RBPT and CFT, respectively. El-Olemy (1974) reported an incidence of 4.92% among sheep, while El-Gibaly et al. (1977) recorded 2% positive reactors and Nashed (1977) found only 0.74% of 806 sheep to be positive. Salem (1981) recorded an incidence of 5.6%, Kholeaf (1978) 2.31% and Nada (1982) 2.31%. Abdel-Wahab (1985) found only 0.4% of tested sheep to be positive, while Zagloul & Kamel (1985) failed to detect any positive sheep in Assiut. The highest rate of positive reactors (31.7%) was reported by Refai et al. (1989). A high incidence (19.9% of 925 sheep) was also reported by El-Bauonry (1989), when he used the BAPAT. Kaldes (1990) mentioned that the rate of brucellosis in sheep in El-Menia Governorate was 0.42%, while Mahmoud (1991) reported reactors in only 0.84% and Gadalla (1991) found the same reactor rate of 0.84% in Assiut Governorate. El-Gohary & Hattab (1992) reported a high incidence (10.7%) in El-Behera Governorate and Nada (1994) reported an incidence of 5.0%.

Montasser (1995) indicated that the rates of sheep positive reactors were 4.38%, 2.89%, 2.02%, 2.01% and 2.006% by BAPAT, TAT, RBPT, MET and RT, respectively. Ali (1997) tested 21776 sheep blood sera collected from different localities in Assiut by BAPAT and RBPT. Positive samples were retested by TAT and RT. The results indicated that the incidence of positive

reactors varied between 0.77% and 2.21%. Montasser (1999) tested 12300 sheep blood serum samples collected from fixed flocks, mobile flocks and small numbers owned by individual farmers in Alexandria, El-Behera and El-Fayoum, in addition to animals slaughtered at Cairo abattoir. The highest rate of reactors (14.28%) was reported in a fixed flock in El-Behera Governorate. However, the mean reactor rate in all fixed flocks was 0.91%. The reactor rate in mobile flocks varied from 0.0% to 9.33%, with a mean of 5.22%. The reactor rate among sheep owned by individual farmers was 1.5%. The incidence of brucellosis in sheep in El-Fayoum Governorate was found to be 2.4% of 5591 animals tested (Montasser & Melad, 1999). Using CFT, Montasser et al. (1999) revealed an incidence rate of 0.28% in sheep and El-Taweel (1999) reported an incidence of 2.63%. Abdel-Hafeez et al. (2001) tested blood serum samples collected from 32939 sheep in Assiut and reported an incidence between 2.01% and 4.13%.

Derbala and Ghazi (2001) examined 2 groups of sheep for brucellosis. The first group of 412 ewes, infested with P. sororitis mites, showed seropositivity rate of 18.93%. The flock could be cleared of the infection within one year following application of test and slaughter policy. The second group of 375 ewes, free from P. sororitis ovis, showed positive reaction at rates of 5.1%, 5.8% and 4.8% by RBPT, TAT and RT, respectively.

Goats: The first study of brucellosis in goats was published by El-Nahas (1951), who reported an incidence of 21.5% among 400 goats. Kamel (1953) found 6.7% of 200 goats and Kamel et al. (1961) 5.8% of 4618 goats as positive reactors. The rates of positives among 480 goats tested by Shawkat (1973) were 7.1% by TAT, 5.2% by RBPT and 5.6% by CFT. Almost similar results were obtained by El-Olemi (1974). The results of El-Bausomy (1989) demonstrated marked differences in the results of different tests. Nada (1994) mentioned that 3.3% and 2.2% of goats were positive, when tested by TAT and CFT, respectively. Montasser (1999) tested blood serum samples from 181 goats collected from Alexandria, El-Behera, Damietta, Cairo and El-Fayoum. The reactor rates, as confirmed by CFT, varied from 1.44% to 3.0% in living animals, while it was 11.3% in animals slaughtered at Cairo abattoir. The incidence of brucellosis in goats in El-Fayoum Governorate was found to be 2.17% of 2496 animals tested (Montasser & Melad, 1999). Using CFT, Montasser et al. (1999) revealed an incidence of 3.35% in goats, while in the same year, El-Taweel found only 0.34 of goat samples positive for brucellosis. Abdel-Hafeez et al. (2001) tested blood serum samples collected from 20241 goats in Assiut and reported an incidence between 0.58% and 1.32%.

Camel: As mentioned by Refai (1992), serological testing of camel sera was carried out by Ahmed (1939); Zaki (1943), Hamada et al. (1963); El-Nahas (1964), Ayoub et al. (1978); Salem et al. (1978); Fayed et al. (1982); Nada (1984), Zagloul and Kamel (1985); Nada (1990), Abo El-Hassan et al. (1991);

El-Miligy (1993) and Thabet et al. (1993). They reported rates of reactors that varied from 3.5 to 24.2%. El-Savalhy et al. (1996) tested camel sera for brucellosis using a battery of tests. They reported reactors rates of 14% in BAPAT, 7% in RBPT, 4.4% in RT, 2.93% in MET and 2.29% in cELISA. As reported by El-Taweel (1999), the results of a sero-survey study applied on camels in Cairo abattoir during 1998 showed that the incidence of brucellosis was 7.02%. Montasser et al. (1999) revealed an almost similar incidence rate of 7.48% in camels. Atwa et al. (1999) tested the sera of 1258 imported camels for brucellosis by BAPAT, RBPT, TAT and RT before and after the application of heat inactivation of the sera. The non-heated samples revealed positive reaction in 9.14%, 4%, 1% and 3% by the used tests, respectively. The corresponding figures after heat inactivation were 1.8%, 1.5%, 0.87% and 3%, respectively. Hamdy (2000) reported an incidence of positive reactors of 12.05% among 365 camels tested by the BAPAT. This percentage dropped to 7.87% when tested by the RT. Ghazi et al. (2001) tested sera from 123 apparently healthy camels at Berkash Village market for Brucella antibodies. The incidences of positive reactors were 30(24.4%), 23(18.7%), 26(21.3%) and 29(23.6%) with RBPT, TAT, MET and RT, respectively.

Equines: Brucellosis in donkeys was reported by Fahmy & Salem (1974), Salem et al. (1975) and El-Bohy (1979). They recorded incidences of 46%, 16.5% and 27%, respectively. Abdel-Kader et al. (1995) tested donkey’s blood serum samples by TAT, RBPT, BAPAT and Coomb test. They recorded reactor rates of 20.64%, 12.97%, 13.35% and 13.33% in these tests, respectively. The incidence of brucellosis in horses was reported to be 5.78% (Ahmed & Munir, 1995), 5.88% (Abdel-Kader et al., 1995) and 8.01% (Esmat, 1996).

Hamoda & Montasser (1998) evaluated the various serological tests in diagnosing brucellosis in donkeys. They tested sera from 258 donkeys found in areas where brucellosis is reported in ruminants. Some donkeys had fistulous withers and others had slight enlargement of the testes. The TAT was positive in 7.3%, BAPAT in 19.42%, RBPAT in 10.2%, RT in 10.2%, CFT in 16.5% and cELISA in 7.3%. A sero-survey in Assuit governorate using different serological tests proved that the tube agglutination test was the most sensitive one in equines and revealed a sero-reactors of 20.61%, 5.88% and 71.42% in donkeys, horses and mules, respectively (El-Taweel, 1999). Ghazy et al. (2000) examined 400 horses during the years 1998-1999. Sero-prevalence of brucellosis using RBPT, TAT, MET and CFT was 30(7.5%), 66(16.5%), 20(5.0%) and 13(3.3%), respectively.

Swine: Ibrahim (1996) tested sera from swine and reported 29.2% reactors in TAT, 24.6% in MET, 35.7% in BAPAT, 29.0% in RBPT and 27.4% in RT. Montasser et al. (1999) revealed an incidence rate of 9.64% in swine. As reported by El-Taweel (1999), the incidence of reactors in swine was about 9.72%.

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Dogs: Brucellosis in dogs has been reviewed by Refai (1994). Salem et al. (1975) tested the blood sera of 135 stray dogs. The TAT and CFT revealed incidences of 28.8% and 29.6%, respectively. Abdel-Aal (1987) reported an incidence of 6.48% of 108 dogs tested. In Upper Egypt, Gadalla (1991) recorded 4 out of 50 dogs to be positive by TAT and RBPT. Recent data on brucellosis in dogs were reported by Hosein et al. (2001) and El-Sherif & El-Sheary (2002). The latter authors recorded an incidence of 41.79% among 67 dogs found around infected cattle farms.

Rats: Salem et al. (1975) reported 3 out of 38 wild rats (7.8%) to be serologically positive for brucellosis. Abdel-Aal (1987) found 9.2% of 130 rats to be positive. A high rate of brucellosis reactors (19.37%) was recorded in rats found in brucellosis positive cattle farms. In brucellosis-free farms the rate of reactors among rats was 9.18% (El-Sherif and El-Sheary, 2002).

Humans: Although human brucellosis is a notifiable disease in Egypt, it is often unrecognised and unreported. The awareness of medical specialists in relation to brucellosis is very weak and, in most of the cases, public health laboratories are not carrying out diagnostic tests for brucellosis. Cases of brucellosis very often remain unrecognised and are treated as another disease. They are often labelled "fever of unknown causes". For these reasons, the actual number of cases of brucellosis is unknown and is believed to be far more than the officially reported figures (Nawar et al., 1992). On the other hand, Wasif et al. (1992) considered human brucellosis to be endemic in Cairo, Alexandria and El-Menofia.

In general, human brucellosis in Egypt has received little attention until the WHO strengthened the Zoonosis Centre in Imbaba Fever Hospital in 1990. Before this date only few cases of brucellosis were recorded although the disease is notifiable, e.g. in 1988 only 45 cases were reported. In 1991, a survey was done in 4 governorates with a total population of 6.34 million. The serological examination of 2720 serum samples revealed positive reactors in 10.5% of the samples. The examination of serum samples from 747 cases admitted to the Imbaba Fever Hospital and diagnosed as cases of fever of unknown causes were positive for brucellosis in 323 cases (43.23%). In 1994, 309 cases were confirmed by isolation in Imbaba Hospital.

Handy (1989) examined serologically 73 blood samples collected from persons with previous history of dealing or handling infected animals. He reported positive results in 45 cases (5 veterinarians, 10 milkers, 22 animal attendants and 8 agriculture engineers). Handy (1992) examined 1133 human blood samples collected from Imbaba Fever Hospital (795), out-patients (206) and farm workers (132). The total incidence of positive reactors was 20.9%. Amal (1994) considered 21% of farm workers in 3 farms in Assiut as seropositive, though their antibody titres were not correlated with clinical findings. Abdel-Hafeez et al. (2001) tested blood serum.

samples collected from 108 veterinarians and 51 animal attendants working in infected farms in Assiut and reported an incidence of 16.7% among veterinarians and 9.8% among animal attendants.

As reported by El-Taweel (1999), the rate of the disease in humans in Egypt is greatly affected by the rate of the disease in animals. The middle age group from 20-40 years represents the highest incidence of brucellosis, and infection rate in females was higher than that of males. Direct contact with infected animals was responsible for 67.9% of human patients, while the indirect means were responsible for 27.3%, and 4.8% of the patients were due to unknown means.

Raw milk and dairy products made from raw milk represent the main source of infection to the consumers. Roushdy (1944) has already isolated Brucella from market milk and Abd-Alla et al. (2000) isolated Brucella abortus biovars 1,2 and 7 and Brucella melitensis biovar 3 from Kastesh cheese and raw milk from which the cheese was prepared. Brucella can survive up to 5 days in raw milk, up to 10 days in yoghurt and up to 40 days in ice cream prepared from untreated milk (El-Gibaly et al., 1993, Ahmed & Montasser, 1999).

Isolation and identification of Brucella organisms

Isolation is the most definitive diagnosis when it is positive. Failure to isolate the organism does not mean a negative result. The sensitivity of culturing depends on the viability and numbers of the Brucella in the sample and the nature of the sample, which is commonly contaminated with other bacteria. However, isolation is important because it helps to complete the identification by biotyping, serotyping, phage typing, nuclear sequencing, restriction endonuclease fragmenting and hybridisation.

Isolations of Brucella abortus from cattle were made as early as 1940 (Gohar et al., 1940). Roushdy (1944) was the first to recover Brucella abortus from one sample of milk. Isolation and biotyping of Brucella abortus and Brucella melitensis were reported by Kamel and Abdel-Fatah (1961), Nawito et al. (1967), El-Gibaly (1969); Sayour et al. (1970); El-Gibaly (1975); Hosney et al. (1977); Abdel-Aal (1987); Salem et al. (1987); Refai et al. (1988); Hardly (1989); Hamouda (1989); Ibrahim (1990); Salem & Hosein (1990); Montasser (1991); Handry (1992); El-Gibaly (1993); Sayour (1995); Ibrahim (1996); Kadry (1996); Bassioni & Ibrahim (1997); Atwa (1997); Anwar (1999); Hardly (2001) and Hosein et al. (2002). Brucella abortus biovars 1 and 2 were isolated from cattle and buffaloes while Brucella melitensis biovar 3 was isolated from cattle, buffaloes, sheep, goats, camels and swine. Ibrahim (1998) reported the isolation of Brucella suis biovar 1 from 7 out of 87 pigs.

Doghiem et al. (1995) reported that the highest rate of isolation of Brucella microorganisms was obtained from the spleen (57%), followed by the liver (40%), lungs (34%), lymph nodes (33%) and kidneys (32%) of serologically positive sheep. All isolates were biotyped as Brucella melitensis biovar 3.

Montasser et al. (2001) examined bacteriologically the lymph nodes, spleen, and livers of 60 slaughtered reactor cattle in addition to the spleen and stomach contents of 12 aborted foeti. They succeeded in isolating 16 Brucella isolates from the slaughtered animals and 6 isolates from the foeti. Only one isolate was biotyped as Brucella abortus biivar 1. This was recovered from an animal in El-Dakahlia. All other isolates recovered from animals in various governorates were biotyped as Brucella melitensis biovar 3.


Bacteriological examination of human blood samples revealed the recovery of 19 Brucella isolates, of which 18 isolates were biotyped as Br. melitensis biovar 3 and one isolate as Br. melitensis Rev 1 vaccinal strain. These results were confirmed by NAMRU-3 (Abbassia-Cairo (Hamdy, 1992).

Diagnosis by modified and advanced methods

The tube agglutination test was the test commonly used for the diagnosis of brucellosis in animals in Egypt. During the eighties tests such as RBPT, MET and CFT were also applied. Then the battery of tests was expanded to include BAPAT and RV (Salem, 1982; Salem et al., 1987; Abdel-Aal, 1987; El-Sheary, 1987, Hamdy, 1989 and Refai et al., 1989).

Nada (1994) used brucelline as a field test for the diagnosis of ovine and caprine brucellosis. The allergic skin test was evaluated in experimental animals by Salem et al. (1995) and it was applied for diagnosis of brucellosis in sheep, goats, cattle and buffaloes by El-Gibaly et al. (1995).


Salem & Refai (1992) developed a modified RBPT. The method required mixing 50 ul of serum with 50 ul of 0.2 M2 mercapto-ethanol solution. Equal volumes (25 ul) of the treated serum and Rose Bengal antigen were then mixed and the test was read within 2 min. The test showed high specificity as compared to TAT.

Zaabal and Ghazi (2003) used dithiotheritol agglutination test (DAT) for diagnosis of brucellosis in cattle and camels. The dithiotheritol has the ability to


depolymerize IgM and positive agglutination will be thus due to IgG. The results of testing 140 cows and 30 she camels showed high concordance between DAT and MET of 93.57% and 100% in the 2 animal species, respectively.

During the last years ELISA was applied in the diagnosis of brucellosis (Salem et al., 1994; El-Enbaawy et al., 1995; Farid et al., 1995; El-Sawalhy et al., 1996) and Hamdy, 2000 and Abd El-Razik, 2001).

The problem of animals with low titre was discussed by Montasser et al. (2002). They tested 405 by various serological tests and found that CFT was a definite test in detecting animals at early and late stages of the disease, particularly those having low titres.

Robinson (2003) emphasized that no serological test is appropriate for all epidemiological situations. The buffered Brucella antigen tests (RBPT and BAPAT) are suitable for screening herds and individual animals. The reactivity of positive samples should be confirmed by the CFT and ELISA, both of which can be also used for screening and confirmation. The SAT is inferior to other tests in specificity and sensitivity, and it is not recommended if other procedures are available. The MRT and indirect ELISA performed on bulk milk samples are effective for screening and monitoring dairy cattle for brucellosis, but are less reliable in large herds and less sensitive with Brucella melitensis.

The reliability of serological tests can be much improved, however, by using specific diagnostic reagents prepared by genetic engineering techniques, such as monoclonal antibodies and specific immunodominant antigens. Further research is needed on the identification, isolation, characterisation and cloning of such immunodominant epitopes which could be used as diagnostic antigens that are more specific and sensitive. Several Brucella cell components have been prepared and tested as antigens, e.g. cell wall fractions, lipopolysaccharide (LPS), O-polysaccharides (OPS), outer membrane proteins (OMPs), ribosomal fractions or DNA. Major OMPs were initially identified in the early 1980s by selective extraction techniques and classified on the basis of their molecular mass, in kilodalton, as: 94-98 KDa OMPs (group 1), 36-40 KDa OMPs (group 2) and 26-34 KDa OMPs (group 3). The minor outer membrane proteins described were 10 KDa, 16.5 KDa, 19 KDa and 89 KDa. Several studies have been published on the immunogenicity of various OMPs of Brucella, e.g. those of Zygmunt et al. (1994), Cloeckaert et al. (1996) and Tibor et al. (1996).

Other authors (Beck et al., 1990; Tabatabai & Hennager, 1994; Tabatabai & Pugh; 1994 and many others) have studied the cloning, expression and occurrence of the Brucella CU-ZN superoxide dismutase (CU-ZN SOD) which is a salt-extractable protein detectable in all Brucella species and biovars except B. neotomae and Brucella suis 2. However, this protein is not immunodominant.
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in infected cattle and therefore is unsuitable as diagnostic reagent. Other proteins investigated included cytoplasmic P15, P17 and P39 proteins. These antigens have detected more than 80% of animals that reacted positively by conventional serological tests. However, when they were used as recombinant proteins they failed as antigens.

Recently, methods of molecular biology have increasingly been used in diagnosis of brucellosis. PCR is particularly useful in case of tissues and fluids contaminated with non-viable or low numbers of Brucella in diagnosis, as it can detect Brucella DNA. A high sensitivity of PCR was reported by Fekete et al. (1990 a, b), Baily et al. (1997) and Da Costa et al. (1996). Several techniques of PCR are used such as AP-PCR (arbitrary primed PCR), rep-PCR (repetitive extragenic palindromic PCR), ERIC-PCR (enterobacterial repetitive intergeneric consensus PCR), either alone or together with labelled probes to differentiate some Brucella species and biovars (Feketa et al., 1992, Romero et al., 1995; Matar et al., 1996). Bricker & Halling (1994) described a multiplex primer assay, designated as AMOS (Abortus, Melitensis, Ovis, Suis), that uses a five-primer cocktail and which can distinguish Brucella abortus biovars 1,2 and 4, Brucella melitensis biovars 1,2 and 3, Brucella ovis and Brucella suis biovar 1. Ewalt & Bricker (2000) reported on the successful application of AMOS PCR as a rapid screening method for differentiation of Brucella abortus field strain isolates and the vaccine strains, S19 and RB51.

PCR was also used in Egypt for diagnosis of brucellosis in cattle and buffaloes (Gabal et al., 1994, Amin et al., 1995; Hosein et al., 1996; Radwan & Ibrahim, 2000; Amin et al., 2001, Abd El- Razik 2001, and H. Hamdy & Amin, 2002). Amin et al. (1995) examined 20 field samples (14 and 6 from serologically positive cows and buffaloes, respectively) Brucella DNA was detected by PCR in all samples, while culture detected Brucella organisms only in 4 samples from cows and 2 from buffaloes. Radwan and Ibrahim (2000) examined 52 bovine foetal and maternal samples bacteriologically and by PCR. Amin et al. (2001) mentioned that PCR was more sensitive than the traditional cultural methods for the detection of Brucella melitensis in semen. The PCR detected Brucella-DNA in 12 (10%) out of 120 semen samples, while direct culture detected only 7 (5.8%). Abd El-Razik (2001) used the nested PCR technique for the detection of Brucella DNA in milk samples of obligatory slaughtered cattle, buffaloes and sheep suspected for brucellosis. 38 out of 92 milk samples (41.3%) gave the expected product (725 bp or 677 bp). This result proved that the nested PCR was 5 times more sensitive than the isolation method. Hamdy & Amin (2002) applied the PCR and culture method on milk samples from serologically positive 52 cows, 21 ewes, 18 goats, and 12 camels. The PCR was positive in 29 bovine milk samples, 10 samples from sheep, 13 from goats and one sample from a camel. The culture method recovered Brucella organisms from 24 bovine samples, 12 sheep samples, 10 goat samples and failed to detect the organism in any of the camel samples.

Control of brucellosis

As reported by Refai et al. (1990), the initiation of a control programme based on calfhood vaccination with the reduced dose of S19 vaccine was made possible through the American-Egyptian Project (EG-APHIS-217). However, on practicing this type of vaccination several problems emerged such as:

1. Differences in opinion of veterinary officials regarding the policy of brucellosis control, e.g., vaccine to be used, age of vaccination and type of tests used.
2. Lack of exact information about the incidence of brucellosis and the prevailing Brucella species and biovars among animals in Egypt.
3. Confusion among field veterinarians and herd owners about brucellosis control.
4. Shortage of vaccines and diagnostic reagents

Accordingly, a National Brucella Committee was established representing the General Organization of Veterinary Services, veterinary laboratories and universities. Through this committee the following decisions were made:

1. It was decided to use U.S. reduced-dose S19 vaccine (3-10 billion organism per dose) in serologically negative female calves, 3-7 months old. The adult vaccination (0.5 billion) was not approved; instead, the adults were allowed to be vaccinated with the killed 45/20 vaccine. The vaccine was used initially in selected farms in 5 governorates, expanded to 28 farms in the first year and to 37 farms in the second year. All other vaccines were not allowed.

2. It was decided to use the buffered acidified plate antigen Test (BAPAT) as a presumptive test. Positive samples were then tested with the tube agglutination and Rose Bengal tests. Rivanol and if possible, CFT were used as confirmatory tests.

3. In dairy farms, the milk ring test was to be applied to bulk milk tank samples every 3-4 months and positive herds were to be subjected to blood testing of individual animals.

4. Because of the increased volume of laboratory work in the central laboratory at Dokki, selected provincial laboratories were strengthened as far as possible with facilities and trained personnel so that they can carry out the screening tests.

5. To eliminate any confusion concerning brucellosis epidemiology and control, training courses for field veterinarians were conducted and a guide covering the most essential facets of brucellosis in cattle was printed and distributed (Refaï & Kopec, 1988).

6. All imported animals were to be kept in quarantines for at least 30 days. Pregnant imported animals should be negative when tested 14 days after calving. Herds containing even one positive animal were kept under quarantine and all animals were to be subjected to periodical testing every 21 days. Quarantine measures were released if the animals passed three consecutive negative tests at 21 days intervals.

Intensive surveillance programmes were however initiated after the approval of the National Brucellosis Control Programme in 1981, adopting the test and slaughter policy and vaccination of young female calves with the reduced dose Br. abortus S19 vaccine.

In our opinion, all these measures, namely, the periodical testing, slaughtering of positives, calfood vaccination with the reduced dose S19, adult vaccination with 45/20, strict quarantine measures and testing of imported animals and infected herds, have led to the drastic drop in incidence of brucellosis in cattle and buffaloes at some farms. The success achieved through this project has encouraged the FAO to support the continuation of the surveillance programme in 4 governorates in the Delta, namely Suez, Ismailia, Port Said and Sharkia and El-Menia Governorate in Upper Egypt. This project initiated Brucella Control Units in these governorates, which carried out field tests for brucellosis, namely Rose Bengal, Buffered Acidified Plate Antigen and Milk Ring tests. The positive samples were then confirmed in the regional laboratories using tube agglutination and rivanol tests. The American-Egyptian Project 416 supported the establishment of such Brucella Control Units, so that they are now 73 units covering all governorates. This project, which ended in 1997, has enabled the government to test almost 40% of the animals. In all these projects, the positive animals were slaughtered and the government has compensated the owners.

The present control programme of brucellosis in Egypt was well described by El-Taweel (1999) as follows:

1. The test and slaughter policy

All female cows and buffaloes over 6 months old and the valuable bulls are serologically tested - regularly every 6 months - through the Brucella Field Diagnostic Units newly established and distributed all over the country, where the field tests (RBPT, BABAT and MET) are used. Sero-reactors should be confirmed by the laboratory tests in the regional laboratories belonging to the Animal Health Research Institute. The confirmed cases, either positives or suspicious, should be isolated to be slaughtered in the nearest slaughterhouse, and their owners are compensated by the local veterinary authorities according to the Decrees and governmental regulations.
Infected farms or herds are quarantined and disinfected periodically, retested every 21 days and the quarantine measures are lifted only after 3 successive negative results. The regional public health authority should be informed about any infected quarantined herds to deal with the contact persons and the produced milk.

All the diagnostic antigens of *Brucella* tests are produced locally in Egypt by the Veterinary Sera & Vaccines Production Research Institute, restricted only for the GOVS use and not allowed for sale except through its permission. The budget is located annually to compensate the owners, and the rates of compensation are revised periodically to meet the market’s prices of animals.

2- **The Calfhood vaccination**

The calfhood vaccination policy is applied only to buffaloes and cattle calves (3-7 months old) using the imported live attenuated reduced dose of *Brucella abortus* S19 vaccine, which is authorized but its application is not compulsory. *Brucella melitensis* Rev 1, vaccine is used for lambs and kids of 3-6 months old. All vaccinations are free of charge. Calves, lambs and kids are examined serologically before vaccination and only the negatives are vaccinated. Re-testing of vaccinated ones should be done at the age of puberty before service or insemination. Reactors for the tests either before vaccination or before puberty should be isolated, slaughtered and the owners are compensated.

3. **Reporting of aborted animals**

Any aborted animals should be reported to the veterinary authorities and specimens should be sent to the laboratory for the isolation and identification of the causative organism and quarantine measures should be implemented.

4. **Market-Control**

Control of brucellosis is carried out also by controlling milk, butter, cream, cheese, ice cream and other animal products in the markets by bilateral controllers of both the Veterinary and Public Health sectors. Tracing back of the positive samples to their origin is the responsibility of the veterinary epidemiologists.

5- Imported pregnant heifers are controlled by the quarantine officers and VPH & Zoonoses employees with two testing for brucellosis directly on arrival and after parturition, reactors should be slaughtered. Importation is authorized only from Brucella-free countries, and steers must be castrated in its origin-country.

The extensive application of *Brucella abortus* S19 vaccination of young females, either with the full dose or the reduced dose together with slaughtering of positive animals, has drastically decreased the overall rate of positive reactors among cattle (Refai et al., 1990). On the other hand, the incidence of brucellosis *Egypt. J. Vet. Sci. Vol.* 37 (2003)
in sheep and goats, though reported for many years, is still high and sporadic. Efforts are still made to control the disease. This situation has resulted in the transmission of *Brucella melitensis* to cattle, buffaloes, and camels. This might explain why *Brucella melitensis* is now the predominant cause of brucellosis in animals and humans in Egypt. The question frequently arises whether we should continue vaccinating cattle, subjected to *Brucella melitensis* infection, with *Brucella abortus* S19 vaccine or should we use the *Brucella melitensis* Rev. 1 vaccine in these cases. Experimental studies have shown that *Brucella melitensis* Rev. 1 provides immunity to *Brucella melitensis* infection equal to or superior than that induced by *Brucella abortus* S19 vaccine and at lower dose. Despite these encouraging results, the use of *Brucella melitensis* Rev. 1 vaccine in cattle has been very limited (Nicoletti, 1993).

*Advances in Brucella vaccine development*

Efforts were concentrated on improving the available vaccines or developing other better attenuated vaccines. The use of reduced dose S19 vaccine in young female calves or the conjunctival route in case of Rev. 1 vaccine in small ruminants have reduced the seroconversion and degree of vaccination infection but did not completely eliminated these disadvantages. Several studies were concerned with development of safer mutants from the *Brucella abortus* strain19. S19 mutants in which certain virulence factor has been deleted, namely, the gene for a 20-KDa Cu-Zn superoxide dismutase (SOD) protein or the gene for a 31-KDa protein, proved to be protective against infection and abortion in cattle (Cheville *et al.*, 1993). Efforts should be continued in this direction until a better vaccine is obtained.

To overcome the problem of seroconversion of animals vaccinated with the smooth *Brucella abortus* S19 or *Brucella melitensis* Rev 1 vaccines, and hence their positive reaction in conventional serological diagnostic tests, attention was directed to the rough vaccines. This approach was encouraged by the experiences obtained earlier from the *Brucella abortus* rough vaccine 45/20 which proved to be protective, but it does not induce antibodies detectable by smooth antigens used in conventional diagnostic tests. This would allow the differentiation between vaccinated and infected positive reactors (Edwards *et al.*, 1945). However, because of the instability of its roughness, it was used as killed vaccine. Schurig *et al.* (1991) produced the rough *Brucella abortus* strain (RB51) by selecting for a rough mutant using monoclonal antibodies against *Brucella abortus* Oclain and by adaptation (resistance) to rifampicin in vitro. *Brucella abortus* strain RB51 proved to have diminished virulence in comparison with the parent *Brucella abortus* strain 2308 and the vaccinal *Brucella abortus* strain S19 (Tobias *et al.*, 1992) and moreover did not induce the formation of OPS-specific antibodies that are commonly used for serodiagnosis of brucellosis in infected animals. Research on bio safety, vaccine efficacy and field applications rapidly

established that *Brucella abortus* strain RB51 is protective in cattle at doses comparable to those of *Brucella abortus* strain 19.

Jimenez de Bagues *et al.* (1994) performed experiments in mice that indicated the possibility of using RB51 as an alternative vaccine against *Brucella melitensis* infection. Preliminary experiments suggested that RB 51 vaccine could be an effective prophylaxis against *Brucella melitensis* infection in goats (Suarez *et al.*, 1998). Adams (1990) stated, however, that homologous *Brucella* spp. are usually more protective than heterologous *Brucella* spp. within a given livestock species but, in some cases, heterologus *Brucella* spp. may induce protective immunity equal to homologous *Brucella* spp. He proposed a hierarchy of the immunizing capacity of vaccines or field strains of *Brucella* spp. for cattle, goat and sheep to be *Brucella melitensis* > *Brucella Suis* > *Brucella abortus*. Perhaps this is why Winter *et al.* (1996) prepared the rough mutant, VTRM 1, by transposon mutagenesis from the smooth *Brucella melitensis* 16M to be used in vaccinating animals subjected to *Brucella melitensis* infection. Like RB51, VTRM1 does not induce antibodies against S-LPS (smooth lipopolysaccharides) and does not interfere with the classical serological tests. However, it failed to confer adequate protection against *Brucella melitensis* infection in goats (Elzer *et al.*, 1998). On the other hand, preliminary experimental studies on Rbbk, which is another live rough strain obtained by transposon mutagenesis from smooth *Brucella abortus* 2308, suggested that this mutant can be protective against *Brucella melitensis* infection (Suarez *et al.*, 1998).

In Egypt, RB51 was introduced since few years and is used in small scale in some cattle farms, so that it is still early to judge its efficiency in the field. Handey *et al.* (2002) made comparison between immune responses and resistance in mice vaccinated with RB51 and Rev. 1 vaccines and challenged with *Brucella melitensis* biovar 3. They concluded that Rev. 1 was superior to RB51 vaccine in protection of mice against *Brucella melitensis*. They stated, however, that in view of its advantage in not eliciting diagnostic antibodies, it could be used for the control of *Brucella melitensis* infection. Refai & Mahmoud (2003) studied RB51 in Guinea pigs. Vaccinated animals did not shed the vaccinal strain, cleared it from the spleen at the 9th week post vaccination and 60% of the animals were protected, when challenged by the virulent *Brucella abortus* strain 2308.

Attention has been directed recently to genetic engineering to produce alternative vaccines that are safe and do not induce antibodies, which interfere with serodiagnosis of field infections. Subunit vaccines have been prepared but when used they proved not to be effective in protecting animals from subsequent infection (Confer *et al.*, 1987 and Winter & Rowe, 1988). On the other hand, recombinant *Brucella abortus* proteins when used as immunogens have induced an increased humoral immune response but did not protect *Brucella*-challenged mice (Pugh and

Tabatabai, 1994). Oliveira & Splitter (1996) immunized mice with recombinant L7/L12 ribosomal protein and showed that this conferred protection against Brucella abortus infection. Onate et al. (1999) reported on the protection of mice vaccinated with live Escherichia coli expressing Brucella abortus Cu/Zn superoxide dismutase. Vemulpalli et al. (2000) induced immunity in mice by using vaccinia virus expressing the 18 kDa outer membrane protein. They also reported on the characterization of specific immune responses of mice inoculated with this recombinant vaccinia vaccine. On the other hand, Baloglu et al. (2000) failed to protect mice against a Brucella abortus challenge by a vaccinia virus recombinant expressing Brucella abortus GroEL heat shock protein.

The failure to obtain an effective subunit or recombinant monovalent Brucella vaccine is attributed to the difficulty in answering the question: which antigen or antigens are responsible for eliciting protective immunity in brucellosis. This problem is related to the antigen processing and presentation events which are rather complex. Moreover, microorganisms do not express the same antigens at all times (Schurig, 1994 and Yura et al., 1993). This is why the best immunity is commonly achieved by live microorganism. Bang (1906) had already realized that infection of cattle with virulent Brucella abortus often leads to long-life solid immunity. It is, however, impossible to use virulent organisms for vaccination. Therefore, the best approach to the development of protective immunity is to use attenuated live Brucella which can express all or most antigens necessary for the induction of protective immunity. The attenuation should, however, leave some degree of virulence to allow the microorganisms to remain alive for sometime to stimulate the immune system. The attenuated Brucella abortus S19 and Brucella melitensis Rev. 1 vaccines have been, therefore, used extensively and, in most cases, successfully in protection of large and small ruminants, irrespective of their residual virulence and induction of antibodies (Refai et al., 1990 and Elberg, 1996). Several studies have been concerned with development of safer mutants derived from the Brucella abortus strain 19. Brucella abortus S19 mutants in which a virulence factor has been deleted, e.g., the gene for a 20-KDa Cu-Zn superoxide dismutase (SOD) protein or the gene for a 31-KDa protein, proved to be protective against infection and abortion in cattle (Cheville et al., 1993). Efforts should, therefore, be continued in this direction until a better vaccine is obtained.

The knowledge obtained about the full genome of Brucella melitensis strain 16M (Sanchez et al., 2001) should open a new era in the study of the disease since local strains may now be compared molecularly to the reference strain and biological changes and diversions of the local strains may be characterized. For this purpose the region should develop monitoring procedures to identify newly evolving strains that might not respond to the current vaccination program.

Regional control of brucellosis

The FAO/WHO/OIE guidelines for a regional control programme for the Middle East, based basically on vaccination using Rev.1 vaccine to control animal brucellosis in all relevant species primarily through a comprehensive vaccination programme, discussed at the Workshop in Amman, Jordan, 1993, was adopted by some countries in the Region. Following publication of the guidelines some reservations were expressed concerning the safety of Rev.1 vaccine when used on large scale. In an attempt to overcome these reservations, the above-mentioned organizations have organized a Round-Table on the use of Rev.1 vaccine in small ruminants and cattle in France (1995). It was concluded in this meeting that Amman guidelines are still valid, the reduced dose of Rev.1 vaccine has no comparative advantage over the full dose in terms of innocuity, whereas the protection it confers is questionable and the conjunctival route is superior to the subcutaneous route. Consequently, some countries of the region began to apply the mass vaccination policy. This policy was not accepted by the veterinary authorities in Egypt, though the policy of test and slaughter for control of brucellosis is considered impractical due to various limitations, of which the presence of carriers, latent infections, difficulty of application of hygienic measures in most farms, difficulty to impose measures to control animal movements etc. (Hosein et al., 2002).

Vaccination of pregnant animals with the full standard dose administered subcutaneously is in most of these cases followed by abortion. Blasco and Diaz (1993) stated that the degree of attenuation of Rev. 1 strain is, in fact, not enough to allow its use without restriction. Due to residual virulence it may induce abortions and also leads to persistent immune response, which could interfere with classical methods of serological diagnostic tests. On the other hand, Rev. 1 can cause infection in human. B osseray (1991) compared five commercial Brucella melitensis Rev. 1 vaccines from different sources with the standard original Elberg strain and confirmed variations in residual virulence and immunogenicity among them. When tested in a mouse model, differences in residual virulence and immunogenicity have been demonstrated among the different Rev. 1 vaccines produced worldwide. Similar variations in residual virulence were confirmed by Grillo et al. (2000) in some commercial Rev. 1 vaccines in Spain.

These differences may account for the variable safety results obtained in mass vaccination trials in different countries. The induction of abortion when vaccinating pregnant animals means that there is no entirely safe strategy for Rev. 1 vaccination. Although, conjunctival vaccination is safer than the subcutaneous one, it is not safe enough to be applied regardless of the pregnancy status of the animals and should be used only under restricted condition (Blasco, 1997). Also, Brucella abortus S19 vaccine is not recommended for use in pregnant animals as it can induce abortion. Both Brucella melitensis Rev.1 and

Brucella abortus S19 vaccines are human pathogens and can induce persistent infections of vaccinated animals, in addition to the diagnostic problem of seroconversion, as they produce antibodies similar to that produced by field strain infection, and hence, it becomes difficult to differentiate between infected and vaccinated animals.

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