BRUCELLOSIS IN ANIMALS IN EGYPT AND ITS CONTROL

by

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INTRODUCTION

Reviewing the literature concerning brucellosis in Egypt indicates that the disease had already been diagnosed in animals as early as 1939. However, the incidence became high with the importation of cattle, particularly in the late seventies and early eighties with the "open door" policy where a marked increase in the numbers of intensive breeding farms was recorded following the importation of large numbers of Friesian cows from different countries. The occurrence of brucellosis cases in the herds bred in these newly established farms in most governorates was alarming. This attracted the attention of the authorities to the disease and placed it amongst the top priorities in the programmes of control.

The present article is a review of the work done on brucellosis in animals in Egypt and the control programme adopted by the veterinary authorities.

BRUCELLOSIS IN CATTLE

Ahmed (1939) was the first to report brucellosis in cattle (11.2%) in Egypt. Gohar (1940) tested 600 cattle and found positive reactors in 20% of them. The incidence later increased to 23.25% (Kamel and Abdel-Fattah, 1961) in some farms, while in others it was 9.2% (Alion, 1963). The testing of 1248 cows on several farms by El-Gibaly (1969) revealed, however, an overall incidence of 2.63%. In a farm tested by Fahmy and Bendary one year later, they reported rates of reactors of 30.6% among local breeds and 50.0% among imported animals.

This alarming incidence has initiated the application of test, slaughter and compensation policy which has been clearly reflected on the incidence of the disease in the seventies (Matter, 1974; El-Olmany, 1974; Nashed, 1977).

The increasing importation of animals and establishment of big farms in the eighties was accompanied with increased rate of brucella reactors. This has been documented by Barsoum (1980) 4.5%, Shalaby (1986) 22.2%, Selim (1987) 19% and Hamdy (1989) 38.4%.

In the farms of the Egyptian-American project on control of brucellosis (Refai et al., 1989) 15875 cattle on 16 farms in 6 governorates were tested during 1985 - 1987. The rate of positive reactors reached 34% in some farms. The application of the test and slaughter policy and calfhood vaccination with the S19 vaccine resulted in a drastic drop in the seropositivity. In 1988 only 1.6% of 29823 cows and 2.0% of 3355 calves on 29 farms were serologically positive.

The Tube Agglutination Test was commonly used for the diagnosis of brucellosis in cattle. During the eighties, tests such as Rose Bengal Plate, Mercapto Ethanol and Complement Fixation Test were applied. During the last 3 years the battery of tests was expanded to include besides the Tube Agglutination, Rose Bengal, Mercapto Ethanol and Complement Fixation as well as the Buffered Acidified Plate Antigen and Rivanol tests. (Salem et al., 1987; Abdel-Aal, 1987; El-Sheery, 1987; Hamdy, 1989; Refai et al., 1989).

The first trial for isolation was done by Roushy (1944) who could recover Brucella abortus from one sample of milk. Brucella abortus as well as Brucella melitensis were

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isolated from cattle (Kamel and Abdel-Fattah, 1961; El-Gibaly, 1969; Sayour et al., 1970; Hosney et al., 1977; Abdel-Aal, 1987; Salem et al., 1987; Refai et al., 1988). The biotyping done by Sayour et al. (1970), revealed the predominance of *Brucella abortus* biovar 3 followed by *Brucella melitensis* biovar 3. The later biovar was the most frequent among the isolates recovered by Abdel-Aal (1987) and Salem (1987). These authors reported also the isolation of *Brucella abortus* biovar 7 from cattle.

**BRUCELLOSIS IN BUFFALOES**

The incidence of brucellosis in buffaloes was always low during the last 50 years. The high incidence (37.5%) recorded by Zaki (1948) was an exception. Most of the authors report an incidence of either less than 1% (Hamada et al., 1963; Matter, 1974; El-Olemy, 1974; Nashed, 1977) or under 5% (El-Ahwal et al., 1968; El-Gibaly, 1969; Ismail, 1975; Bansum, 1980; Zagloul and Kamel, 1985; Abdel-Aal, 1987).

Only *Brucella abortus* biovar 3 was isolated from buffaloes (Sayour et al., 1970; Abdel-Aal, 1978; Salem, 1987).

**BRUCELLOSIS IN 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% (of 454) in 1963, 4.7 (of 403) in 1964, 0.0% (of 953) in 1965, 2.6% (of 435) in 1966 and 0.0% (of 2958) in 1967. Shawkat (1973) tested blood serum samples of 530 sheep and reported positive reactor rates of 2.86%, 2.26% and 2.44% by the Tube Agglutination, Rose Bengal and Complement Fixation Tests respectively. El-Olemy (1974) reported an incidence of 4.92% among sheep while Nashed (1977) found only 0.74% of 806 sheep to be positive. A high incidence (19.9% of 925 sheep) was reported by El-Bauomy (1989) when he used the Buffered Acidified Plate Antigen. This result was almost similar to that of Rose Bengal test (19.1%) but much higher than that of Agglutination (13.3%) or Rivanol test (15.0%). The highest rate of positive reactors (31.7%) was reported by Refai et al. (1989).

The biovar prevalent in sheep was *Brucella melitensis* biovar 3 (Sayour et al., 1970; Salem, 1987; El-Bauomy, 1989).

**BRUCELLOSIS IN GOATS**

The first study of brucellosis in goats was published by El-Nahhas (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 Shankat (1973) were 7.1% by Tube Agglutination, 5.2% by Rose Bengal and 5.6% by Complement Fixation Tests. Almost similar results were published by El-Olemy (1974). The results of El-Bauomy (1989) demonstrated marked differences in the results of different tests.

*Brucella melitensis* was recovered from goats by El-Nahhas (1951), El-Olemy (1974) and El-Bauomy (1989).

**BRUCELLOSIS IN CAMELS**

Brucellosis in camels was reported for the first time by Ahmed (1939) who published an incidence of 3.5%. Hamada et al. (1963) reported an incidence of 10.29%, while Fayed et al. (1982) found 8.3% of 350 camels to be positive. Zagloul and Kamel (1985) tested 37 camels and found 8.1% of them as positive reactors.

**BRUCELLOSIS IN OTHER ANIMALS**

Salem et al. (1975) tested the blood sera of 135 stray dogs. 39 (28.8%), 26 (19.2%) and 40 dogs (29.6%) were positive as revealed by the Plate Agglutination, Tube Agglutination and Complement Fixation Tests respectively. They could isolate *Brucella abortus* biovar 3 from one dog and *Brucella melitensis* biovar 1 from two dogs. Three from thirty-eight wild rats (7.8%) were positive serologically and *Brucella abortus* biovar 3 was recovered from one rat.

Abdel-Aal (1987) made similar studies on dogs and rats. He found that 9.2% of 130 rats and 6.48% of 108 dogs were positive reactors. *Brucella melitensis* biovar 3 could be isolated from 3 out of 40 dogs tested while all 15 dogs tested were negative.

**CONTROL**

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, when we started practicing this type of vaccination several problems emerged:

a. Differences in opinion of veterinary officials regarding the policy of brucellosis control (e.g. vaccine to be used, age of vaccination, type of tests to be used).

b. Lack of exact information about the incidence of brucellosis and the prevailing brucella species and biotypes among animals in Egypt.

c. Confusion among field veterinarians and herd owners about brucellosis control.

d. Shortage of vaccines and diagnostic reagents.

Accordingly, a National Brucella Committee was established representing the General Organization of Veterinary Services, the Animal Health Research Institute, the Animal Reproduction Research Institute, the Serum and Vaccine Research Institute and the Universities. Through this Committee the following decisions were made:
without any history of brucellosis, a titre of 1/80 (approx. 160 I.U.) is highly indicative of the presence of the disease. In rural or endemic areas, or in a patient with a previous history of brucellosis, the result must be carefully interpreted. In this case low titres should be complemented by the Coombs test or the same test repeated after a week. In the case of an acute phase of the disease the agglutination test would show a high titre (>1/320).

In the case of chronic brucellosis the agglutination test mostly gives low or negative titres. Therefore, a negative agglutination test does not exclude the presence of the disease and should be supplemented by the Coombs test which detects non-agglutinating antibodies.

However, the Coombs test while being more complicated than the agglutination test, it is nonetheless as specific. Normally the titre is at least the same or higher than in the agglutination test, but presents the same difficulties in the definition of the significant values. A titre of 1/160 is highly significant in urban and non-endemic areas as well as for patients without previous history of infection. In the case of an active brucella infection with a long evolution, the Coombs titres can be very high (between 640 and >10,240). In these patients the persistency of antibodies is very long.

Rose Bengal is a plate test for rapid agglutination and is utilized for screening. It is very simple, but it is recommended to confirm the results by the agglutination test. Especially for acute brucellosis, an early treatment can be initiated whilst waiting for the results of the blood culture and of other tests for confirmation.

Further serologic tests of great theoretic importance like the ELISA test which can detect the response of various Ig are not yet standardized.

For brucellosis diagnosis the following conclusions of practical importance can be drawn:

- Due to its accuracy and other advantages, blood culture should be included where possible in the diagnosis of brucellosis.
- The epidemiology and the clinical history of possible previous brucella infection are very important for the correct interpretation of the serologic results.
- The use of the agglutination test in conjunction with the Coombs test will enable the detection of most brucellosis cases. Negative results of these two tests can exclude the presence of a brucella infection except during the very early stages of the disease.
- There are no serologic criteria for the cure of brucellosis or for its recidivity. Only in conjunction with the clinical history can these stages be judged.
- The maintenance of positive serologic titres in the absence of clinical symptoms do not justify an antibiotic therapy.

Table 1

Advantages and limitations of a diagnosis through isolation of brucella organisms

- Diagnostic evidence
- Very efficient in acute forms and at an early stage
- Permits the identification of the species and biotype
- Easily performed in hospitals and health centres
- Important for the documentation of recidives
- Specificity
- Not easily accessible for home visits and in rural areas
- Moderate efficiency during advanced stages of the disease

Table 2

Circumstances and conditions that influence the positivity of the blood culture

- More reliable in early stages of the disease
- More reliable in the acute forms
- Positive also during asymptomatic (feverless) moments of the disease
- Should be performed before antibiotic treatment is initiated or after it had been suspended for 48 hours
- A single blood culture (5-10 ml) is normally sufficient, during chronic evolution of the diseases. However, it is recommended that three blood samples be taken at 30 minute intervals.
- The use of a biphasic bottle of Castaneda with an atmosphere with 10% CO₂
- Incubation during 30-45 days

Table 3

Advantages and limitations of serologic tests

- Technically easy
- Accessibility to any medical environment
- Highly efficient in the diagnosis of:
  * acute stages
  * without previous history
- Highly indicative if negative
- Possibility of fast screening (Rose Bengal test)
- Applicable for sero-epidemiological investigations
- Difficult interpretation and low positive predictive value in:
  * advanced stages
  * presence of a clinical history
- There are no criteria for cure
- Low reproducibility of the tests
- Does not identify the specific brucella species involved

Editor's comments: If more detailed information on the individual techniques is required by the reader, we advise him/her to refer to "Techniques for the brucellosis laboratory" by G.G. Allison, published by Institut National de la Recherche Agronomique, 147 rue de l'Université, 75007 Paris, France.
It was decided to use US origin reduced dose S19 vaccine (5-10 billion organisms per dose) in serologically negative 3-7 month old calves. The adult vaccination (0.5 billion) was not approved; instead the adults are allowed to be vaccinated with the killed 45/20 vaccine. The application of S19 was planned initially to be used in selected farms in 5 governorates and the vaccination was expanded in the first year to 28 farms and in the second year to 37 farms. All other vaccines are officially banned at present.

It was decided to use the Buffered Acidified Plate Antigen (BAPA) as a presumptive test. Positive samples are then tested with the Tube Agglutination and Rose Bengal (card) tests. For confirmation Rivanol and, if possible, CFT may be used.

A tighter follow up was suggested for the evaluation of the vaccine. Challenge experiments were not accepted. Calves should be negative before breeding age.

In dairy farms, the Milk Ring Test (MRT) is to be applied to bulk milk tank samples every 3-4 months and positive herds are to be subjected to blood testing of individual animals.

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 help in carrying out the screening tests.

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 were printed and distributed.

All imported animals are to be kept in quarantine for at least 30 days. Pregnant imported animals should be negative when tested 14 days after calving. Herds containing even one positive animal are put under quarantine and all animals are to be subjected to periodical testing every 21 days. Quarantine measures are lifted if the animals show negative in three consecutive tests taken at 21-day intervals.

In our opinion, all these measures, namely the periodical testing, slaughtering of positives, calfhood 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 a drastic drop of incidence of brucellosis in cattle and buffaloes in some farms. In order to get better results, this system needs to be expanded to cover all governorates in Egypt.

Brucellosis in sheep and goats, mainly due to Brucella melitensis remains a considerable problem. Brucella melitensis was the predominant species isolated from samples originating from diseased herds of cattle and buffaloes examined in the laboratories during the last three years.

This situation requires that more attention is paid to sheep and goats with regard to testing, slaughtering and vaccination in order to control the disease among them and to eliminate the hazards of Brucella melitensis infection among cattle and buffaloes and possibly humans.

More studies are needed to determine the efficiency of S19 vaccine in protecting cattle from Brucella melitensis infection and test whether melitensis vaccine such as Rev. 1 should be used.

A full set of References and supplementary Tables are available on request from:

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BRUCELLOSIS: DIAGNOSTIC CRITERIA FOR BRUCELLOSIS

Information from the regional laboratory for brucellosis and the medical faculty of Valladolid, Spain.  
(Boletín Microbiológico Semanal 44-45/88)

Human brucellosis can be diagnosed through blood culture and serologic tests, e.g. agglutination, Coombs anti-brucella and Rose Bengal tests. Blood culture is considered the most suitable method for the direct isolation of brucella organisms; its advantages and limitations are given in Table 1. The conditions and circumstances that influence the positivity and reliability of this diagnostic tool are summarized in Table 2.

If brucellosis infection is suspected on the basis of clinical observations, the laboratory should be informed, so that it can carry out the necessary tests, which might differ from the routine bacteriological examinations.

Although indirect diagnosis through serological methods is extremely useful it has nevertheless some limitations (see Table 3). At present serologic diagnosis is carried out through Agglutination and Coombs antibrucella tests. This can be complemented by the Rose Bengal test for rapid diagnosis. Agglutination tests are quite specific. A positive titre indicates a previous contact of the person with brucella bacteria. In order to define a significant titre for the diagnosis of an active brucella infection some difficulties are being encountered. In an urban or non-endemic area, a patient...