

# Management of Human Brucellosis in Al-Shirqat City/ Salah-Al-Din Governorate/Iraq

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## ABSTRACT

**Aim:** To determine the method of managing brucellosis in Al-Shirqat city, this city located to the west of the Tigris in Saladin Governorate, Iraq.

**Methodology:** this study enrolled 130 brucellosis patients during a period between September 2018 and March 2019, any patient diagnosed as brucellosis was chosen in this study from primary health care centers and Al-Shirqat hospital, Three questionnaires were performed, the first one including demographic data and clinical data and the antibiotics used for treatment, the second questionnaire about the actions of doctors in management of brucellosis; the third one about the outcome after treatment and any additional measures in management.

**Results:** The combination of Doxycycline & co-trimoxazole & Streptomycin was the most frequently used treatment (30%) followed by doxycycline and co-trimoxazole (27%), the least combination used was doxycycline & co-trimoxazole & gentamicin (0.7%). Rose Bengal test was performed to follow up the patient and an antibody titer of 1/320 was regarded positive and need additional treatment.

**Conclusion:** In Al-Shirqat city, Brucellosis is diagnosed depending on Rose Bengal test or rapid cassette for anti-brucella antibody. Six types of antibiotics are prescribed for brucellosis, these are: co-trimoxazole, doxycycline, streptomycin, rifampicin, amoxicillin, and gentamicin. Most of patients receive triple or dual antibiotics for 40 days, especially the combination of doxycycline, co-trimoxazole & streptomycin or doxycycline and co-trimoxazole. There is no case reporting or continuous contact with the patients by the doctors.

**Keyword:** Brucellosis, Brucella Abortus antigen, Rose Bengal test, rapid antibody cassette method

## INTRODUCTION

Brucellosis, is a frequent zoonotic communicable disease, it is also known as undulant or Mediterranean fever. Although it infects animals like sheep, cattle, goat, or pigs the disease can also transmitted to human<sup>1</sup>. The human get infection by direct interaction with infected domestic animals or their discharges, ingestion of the infected milk or its products, or infected meat<sup>2</sup>. Transmission from infected human to non-infected human can happen through blood transfusion, sexual transmission; mother to baby transmission is possible through trans-placental route or by breast milk, or by bone marrow transfusion<sup>3</sup>. The human brucellosis occurs world-wide especially in Middle East, Mediterranean countries, India, and South America<sup>4</sup>. Human brucellosis is a bacterial disease caused by organism belongs to genus *Brucella*, these are gram negative, aerobic, coccobacilli bacteria; four species in this genus are the etiological agents of human brucellosis, these are: *B. melitensis* in goats and sheeps, *B. abortus* in cattle, *B. suis* in swine and *B. canis* in dogs<sup>5</sup>. It is a systemic disease, with diverse clinical features, that begin usually at least fourteen days after inoculation. The clinical manifestations are often nonspecific and these include headache, asthenia, joint pain, fever, anorexia, and hepatosplenomegaly. The disease has the tendency to be prolonged and may relapse after a period of remission in part due to its ability to live and replicate in reticulo-endothelial system<sup>6</sup>.

The diagnosis of human brucellosis depends on clinical suspicion and serological investigations like agglutination test (Rose-Bengal test and 2-mercaptoetanol), lateral flow immunoassay for antibody

detection, enzyme-linked immunoassay to detect IgM and IgG antibodies specific to brucella antigens. Molecular techniques that targeting the brucella genes and culturing of blood or bone marrow are confirmatory test to diagnose human brucellosis<sup>7</sup>. In 1986, the World Health Organization recommended a treatment protocol for acute brucellosis in adults composed of dual antibiotics: rifampicin 600-900 mg plus doxycycline 100mg for 6 weeks<sup>8</sup>. Some researchers recommended the use of oral doxycycline for 45 days with intramuscular gentamicin for 7 days; they had the same effect as doxycycline for 45 days with streptomycin for 14 days<sup>9</sup>.

The aim of this study is to determine the method of managing brucellosis in Al-Shirqat city, this city located to the west of the Tigris in Saladin Governorate, Iraq.

## MATERIALS AND METHODS

The study enrolled 130 brucellosis patients from Al-Shirqat city/Salah Al-Din governorate; sampling method was designed so that any person diagnosed by the physician as a case of brucellosis was enrolled in the study, which extended between September 2018 and June 2019. The blood sample was collected and stored at Al-Shirqat Hospital.

The patients were selected by non-probability sampling method, in that all patients with brucellosis were chosen in the study. Three categories of data collection were designed, and all of them were administrated by the researcher, the first category is a questionnaire included five demographic data (age, gender, residency, occupation, and marital status) and four risk factors records involved history of brucellosis, contact with similar conditions, and

history of eating raw milk presence of other diseases; an additional four questions related to diagnosis and treatment of brucellosis included. The second category of data collection included three questions, the result of diagnostic procedures after the initial treatment, the clinical features, and the possible of administration of extra-medications. The third category included questions for the doctors who prescribe treatment, these questions included their specialties, the investigations they request, knowledge of laboratory investigations, combinations of antibiotics which are prescribed for treatment, duration of treatment, follow up of patients and writing case report for health authorities.

About 5 ml of peripheral blood sample was collected aseptically into a sterile test tube with anticoagulant from each person in study group and stored in refrigerator at 4°C.

The Brucella Abortus Antigen Rapid Test Cassette /All test Company / China (Whole Blood /Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Brucella abortus antigen in human whole blood serum or plasma specimen.

The membrane is pre-coated with mouse anti-Brucella abortus. During testing, the Brucella abortus or Brucella melitensis antigens in whole blood, serum or plasma specimen reacts with the dye conjugate, which has been pre-coated anti-Brucella abortus on the conjugate pad. The mixture then migrates upward on the membrane by capillary action reacts with mouse anti-Brucella abortus antibodies on the membrane on test line region. To serve as a procedure control, a colored line will always appear in the control line region. The test cassette contains anti-Brucella abortus antibodies coated particles and anti-Brucella abortus antibodies coated on the membrane.

**Data Processing & Analysis:** Categorical data were described using percentages and quantitative data using either medians with range or means with 95% confidence intervals. Cross-tabulation with Pearson's  $\chi^2$  test will be used to analyse differences between categorical variables. A p-value  $\leq 0.05$  was considered statistically significant. All data were analysed by SPSS Version 21.

## RESULTS

In the year 2017, the recorded cases of brucellosis in health authorities of Al-Shirqat city was 708, 490 cases in Al-Shirqat hospital and 218 in the primary health care centers; in the next year, 2018, there was decline in the registered cases to 428, 242 cases recorded in Al-Shirqat hospital while 186 were recorded in primary health care centers; the differences between the two years were statistically significant, p value  $\leq 0.05$ , as shown in figure 1. The total number of Al-Shirqat population is 234,000. This case registration did not include the cases who consult the doctor in the private clinics.

**Recorded cases of Brucellosis during the period of the study:** The period of cases registration in the current study is extended about 7 months including the last 4 months of the year 2018 and the first 3 months of the year 2019, during this period the registered cases of brucellosis in governmental health authorities of Al-Shirqat city was 130 cases of brucellosis.

**Gender of patients in the study:** In this study, the infection among males (n=72) was more commonly recorded than in females (n=58), as clarified in figure 2. The male: female ratio is 1.2:1. The patients were most frequently encountered at age groups 21-30 years old and 31-40 years old, in each of these groups 37(56.9%) cases of brucellosis were recorded (Fig.3).

The current study revealed that most of patients, 126(95%), are living in the rural areas of Al-Shirqat city while few of them, 4(5%), reside in urban regions (Fig.4).

The current study showed that that vast majority of brucellosis patients are married 112 (86%) (Table 1).

The current study showed that two techniques are used for laboratory diagnosis; these are Rose Bengal test (qualitative and semi-quantitative test) and rapid cassette method a qualitative technique for brucella antibodies (immunochromatography technique). The Rose Bengal test is positive if either one or both IgG or IgM anti-brucella antibodies are positive, test was used for diagnosis of 123 patients, while rapid cassette detects these antibodies separately was performed for diagnosis of 7 cases depending upon the availability of the test in nearby lab.

The researcher used a new rapid cassette method for detecting brucella antigen in 25 cases with clinical features suggestive of brucellosis and it was positive in 9 cases who were also positive by Rose Bengal test with titer of 1/320 while the other 16 were negative for brucella antigen but have Rose Bengal titer equal to 1/160 and they were considered as negative.

Six types of drugs are reported as medication prescribed by doctors (according to their frequent prescription), these are: co-trimoxazole, doxycycline, streptomycin, rifampicin, amoxicillin, and gentamicin, as described in table 2.

The vast majority of the patients were taking combinations of antibiotics for treatment, either triple or dual antibiotics, only few of them (3%) were receiving single drug for treatment (Fig. 5).

Four cases received single antibiotic, two of them were pregnant women given amoxicillin 500 mg four times daily for 15 days, while the others were children less than 7 years given oral (syrup) co-trimoxazole 480 mg twice daily for 15 days. Patients with brucellosis were receiving initial course of antibiotics for different durations (Table 3).

Most of patients (30%) received a combination of antibiotics include doxycycline, co-trimoxazole, and streptomycin, while the other patients received different combinations for different durations as illustrated in table 4.

The brucellosis patients were followed up after finishing the course treatment, agglutination test was used for follow up, those who had an antibody titer of 1/320 was regarded as positive and still in need for a second course of treatment, while patients who received treatment for 40 days (n=60) were regarded as cured after testing and there was no extra-medication. Those patients who received treatment for 30 days (n=40), eight of them were regarded as cured, the remaining 32 patients were given additional 10 days treatment with the same antibiotics received previously. Patients who were given treatment for 21 day (n=9) and 28 days (n=9) were tested and all of them received a second course of treatment for a total duration of 40 days. For those who received treatment for 20 days

(n=8), seven of them were given a second course of treatment, while the residual patient refuses the repetition of laboratory testing or additional treatment. The last four patients who given treatment for 15 days, the medications were repeated for 30 days to three of them, the fourth patient give treatment for only 15 days. Thus, a total number of 120 (92.3%) patients received the antibiotic treatment for a total duration of 40 days, while 10(7.7%) patients received the treatment for shorter durations.

The results of this study revealed that 35/130 (26.9%) of brucellosis patients were having history of contact with human brucellosis cases. In the current study, a questionnaire was designed to collect data from doctors in Al-Shirqat city; Nine (31%) of doctors said that they usually prescribe two types of antibiotics for treatment of brucellosis while the other 20 (69%) physicians prescribe three antibiotics, the duration of treatment, 12 of them said for one month while 17 of them prescribe for 40 days. The results revealed that different doctors prescribe different medications for each patient, usually the same doctor give repeatedly the same combination of drugs as illustrated in table 5.

All the doctors said that they did not insist for another visit by the patients for follow up, they made this optional for the patients according to clinical features of brucellosis.

Figure 1: The Frequency of Brucellosis in governmental health authorities in Al-Shirqat City in the years 2017 and 2018

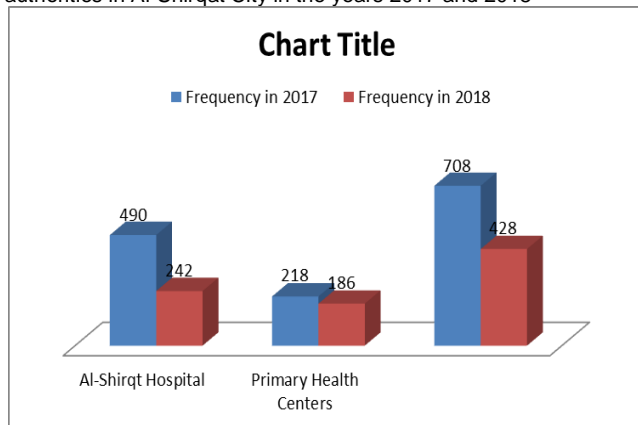


Table 1: Distribution of brucellosis patients according to their marital status

Marital status	Frequency	%age
Married	112	86
Singles	8	6
Children	6	5
Widow	2	1.5
Divorced	2	1.5

Table 2: The antibiotics prescribed by doctors in AL-Shirqat city for treating brucellosis

Antibiotic	Frequency	%age of use per patient
Co-trimoxazole	96	73.8
Doxycycline	74	56.9
Streptomycin	69	53
Rifampicin	47	36
Amoxicillin	2	1.5
Gentamicin	1	0.07

Figure 2: Gender distribution of brucellosis patients

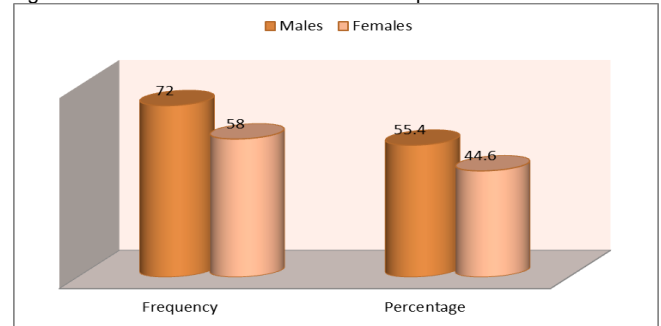


Figure 3: The distribution of patients according to age groups.

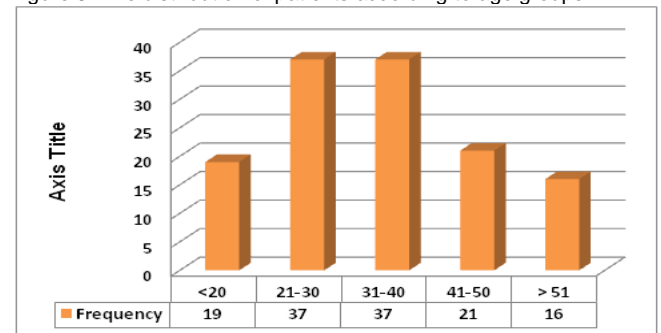


Figure 4: Residence of brucellosis patients.

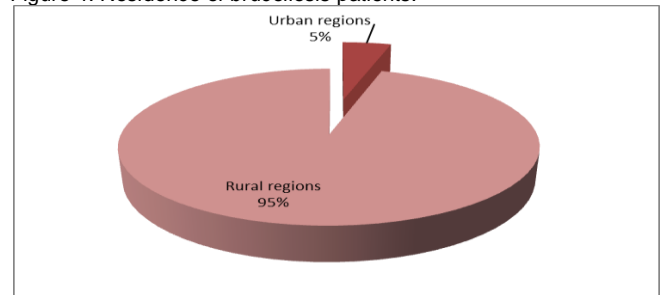


Figure 5: The number of antibiotics given for each patient

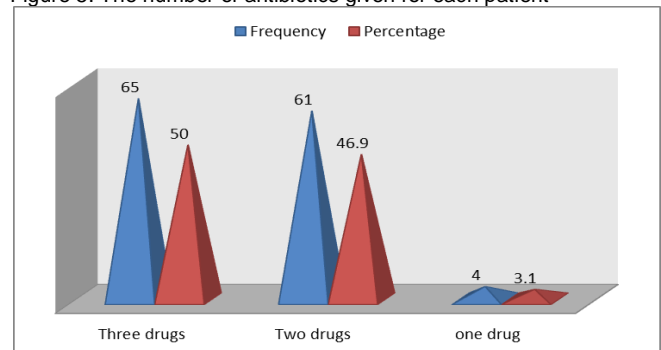


Table 3: Duration of treatment of brucellosis

Duration of treatment (days)	Frequency%
15	4 (3.1%)
20	8 (6.2)
21	9 (6.8%)
28	9 (6.9%)
30	40 (30.8%)
40	60 (46.2%)

Table 4: The antibiotic therapy for treatment of brucellosis

Monotherapy	Frequency	Combination of two drugs	Frequency	Combination of three drugs	Frequency
Amoxicillin	2 (1.5%)	Doxycycline & Co-trimoxazole	35 (27%)	Doxycycline & Co-trimoxazole & Streptomycin	39(30%)
Co-trimoxazole	2 (1.5%)	Rifampicin & Co-trimoxazole	26 (20%)	Rifampicin, Co-trimoxazole & Streptomycin	25(19.3 %)
				Doxycycline & Co-trimoxazole & Gentamicin	1(0.7%)

Table 5: The types of drugs prescribed by different doctors

No. of doctors	Type
11 (37.9%)	Streptomycin, co-trimoxazole, and doxycycline
9 (31%)	Streptomycin, co-trimoxazole, and rifampicin
5 (17.2%)	co-trimoxazole and doxycycline
3 (10.4%)	co-trimoxazole and rifampicin

## DISCUSSION

The human brucellosis is endemic in Middle East including Iraq; the brucellosis is frequently reported in Iraq with annual incidence rate of 278.4 per million <sup>(10)</sup>. In 2004, for five months (July-November), 28 patients consulting Tikrit Hospital were having brucellosis diagnosed as brucellosis cases using Rose Bengal agglutination test. In the year 2000, 253 brucellosis cases were reported in Ibn-Saif hospital at Al-Musaib district<sup>11</sup>. In the current study, the data collected from records revealed high incidence of human brucellosis in Al-Shirqat city which belong to Salah Al-deen governorate in the middle of Iraq, located to the north of the capital, Baghdad. Records revealed incidence of 708 cases in 2017 and 428 in the next year, 2018, both are higher than those recoded from the above two previous studies in Tikrit and A-Musaib, these differences may be due to involvement to primary health care center in this study, however, even when we exclude the PHC centers it stills high incidence.

In the current study, the infection among males was more frequently recorded than in females, and most of patients were young adults, living in rural areas, farmers, and married. These results reflect the natural history of brucellosis which is zoonotic disease affecting the sheep and cattle which are domestic animals and if they are infected the disease can easily transmitted to humans especially those in direct contact with these animals like farmers who are mostly living in villages and young adults and usually they are males, though young females in Al-Shirqat city are also engaged in farm work thus many of affected patients in this study were females and married.

The number of children recorded to have brucellosis in this study was low, and this is unexpected result as children are the most consumers of milk which is the most common route of transmitting the brucella to human; Jawad K. recorded more prevalence of brucellosis among children in Babel city which is against our results, one possible explanation of our data is that adults are handling or dealing with infected animal products like milk, milk products and meat while upon using these products for their children, they will process these products in a manner make these products more hygienic like heating of the milk before giving it to children. Two previous studies<sup>10,12</sup> done in Tikrit city registered less prevalence of brucellosis among children, which is in accordance to our data.

Rose Bengal test and rapid cassette method are the only techniques that are used in health care facilities in Al-Shirqat city for the diagnosis of brucellosis by detecting anti-brucella antibodies in serum. Other serological tests like ELISA technique for determination of IgM and IgG anti-Brucella antibodies in serum are not used in Al-Shirqat city as a diagnostic technique; the ELISA technique is not requested by doctors in Al-Shirqat city and only few of them have scientific knowledge about this technique. Moreover, none of the doctors have knowledge about the availability of the new brucella antigen test, which detects the brucella antigen in whole blood, plasma, or serum.

Worldwide, the available specific laboratory tests are microbiological (culture), serological (e.g. slide or tube agglutination, Coombs test, immunocapture agglutination, Brucellacapt, immunochromatographic lateral flow, enzyme-linked immunosorbent assays and the indirect fluorescent antibody test) and molecular (e.g., PCR). Each of these tests has advantages and limitations, and thus requires careful interpretation<sup>13</sup>.

The current study tried to introduce a new laboratory technique for diagnosis of brucellosis by detecting brucella antigen using immunochromatographic lateral flow by commercial available cassette, this was done to compare the results of the new antigen detection method with the available antibody detection method, the results were comparable, but still there is need for future assessment of the new technique by comparing it with standard techniques like culturing methods or molecular assays. Most of the patients in this study were taking either triple or double antibiotics for treatment of brucellosis in a duration ranged between 15-40 days; the triples of doxycycline, co-trimoxazole & streptomycin was the most frequently used followed by the combination of doxycycline and co-trimoxazole, then the combination of rifampicin & co-trimoxazole with or without streptomycin.

in 2006, M.J. Corbel, write a WHO report about animal and human brucellosis, he marks that authorities advice the use of a combination of antibiotics like tetracycline (500mg every six hours orally) or doxycycline (100 mg every 12 hours orally) administered for a period of six weeks and the aminoglycoside streptomycin (1g/day intramuscularly) administered for two to three weeks; the streptomycin is added to prevent relapse<sup>14</sup>.

In our study, the tetracycline was not used and it was exchanged with doxycycline which is a long acting

tetracycline analogue, used once or twice daily, and associated with fewer gastrointestinal side effects; The physicians in Al-Shirqat city did not use the standard di-therapy combination (doxycycline and streptomycin), instead of this combination, they either added co-trimoxazole to become a triple therapy, or instead of streptomycin they used co-trimoxazole in this di-therapy combination (Co-trimoxazole plus doxycycline). Another regimen was recommended by the WHO involves the use of combination of doxycycline (200 mg/day orally) plus rifampicin (600–900 mg/day orally), with both drugs administered for six weeks; which has overall treatment failure and relapse rates nearly close to that of doxycycline and streptomycin; this combination of doxycycline plus rifampicin was also not prescribed by doctors in Al-Shirqat city, instead one of them was replaced by co-trimoxazole.

Although gentamicin, in a dose of 5mg/kg/day intravenously or intramuscularly, administered for 7 to 10 days in combination with doxycycline administered for six weeks, yielded good results in one study, experience with this regimen is too limited to justify its use over doxycycline plus streptomycin. In Al-Shirqat city, gentamicin was only used one time in combination with doxycycline & Co-trimoxazole, thus gentamicin, in an infrequent antibiotic for treatment of brucellosis in AL-Shirqat city. The WHO, mentioned the trimethoprim/sulfamethoxazole (TMP/SMZ, co-trimoxazole) in a fixed ratio of 1:5 (80 mg TMP/400 mg SMZ) as an alternative therapy but should always be used in combination with another agent, such as doxycycline, rifampicin or streptomycin. Moreover, the Fluoroquinolone antibiotics have greater activity in vitro against *Brucella* species, though quinolones should always be used in combination with other drugs, such as doxycycline or rifampicin.

In Al-Shirqat city, the physicians prescribe monotherapy for treatment of brucellosis in very low occasions and we record this only for pregnant women or children and the drug of choice, they used, is amoxicillin or co-trimaxazole. In a systematic review done by Seyed Mohammad, et al for 25 years (1993-2012) found that Cotrimoxazole plus rifampin for six weeks may be the regimen of choice for the treatment of patients younger than 8 years old. Gentamicin for 5 days plus cotrimoxazole for six weeks may be a suitable alternative regimen<sup>15</sup>, none of these two regimens were not used in Al-Shirqat city. The main feature of brucellosis is the tendency for relapse after finishing the treatment, this relapse may occur few weeks, months, or even, more than one year after finishing the treatment, this is because the *brucella* bacterium has the ability to stay alive dormant in cells and evading the host defense responses, since the majority of relapses happened in the first six months after treatment, it is important to follow up the patients during this period<sup>16</sup>.

In this study we watched the routine techniques and time which are used by of the physicians in Al-Shirqat city for follow up of brucellosis patients after starting the treatment with antibiotics, most of them used the clinical features and laboratory methods as criteria of improvement, the Rose Bengal serology test was the main laboratory technique applied for predicting the response to treatment. The time of follow up is immediately after finishing the treatment and it is only one time. The Rose

Bengal test used for follow up may remain positive even after cure from the infection because the elevated antibody titer may continue to be high and there is need to assess this technique as a post-treatment technique for follow up. Some researched recommended the use of blood culture and PCR as a follow up techniques instead of serological techniques<sup>(17, 18)</sup>. In Iraq none of these two techniques are used in clinical laboratory settings. Unfortunately, we couldn't find any Iraqi study that reviewed the post-treatment follow up of brucellosis patients by physicians, our study may be the first to do this, however, we did follow up for a sort period after finishing the treatment, and there is a need for a future study to do follow up for longer time.

## CONCLUSION

the results of this study revealed that 26.9% of brucellosis patients were having history of contact with brucellosis cases; this high percentage with contact might represent one source of infection, Rose Bengal test is the major technique that is used in Al-Shirqat city for the diagnosis of brucellosis; immunochromatographic lateral flow for antibody detection is the only available alternative technique. The triples of doxycycline, co-trimoxazole & streptomycin were the most frequently used. The follow up procedures are not appropriate for controlling and prevention of brucellosis.

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**Ethical clearance:** The researchers already have ethical clearance from College of Medicine, University of Baghdad, Iraq, and the Technical College of Health Sulaimani Polytechnic University, Sulaimani City, Kurdistan Region, and Saladin Health Directory, Ministry of Health- Iraq

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