# FREQUENCY OF SALMONELLA ANTIBODIES AMONG FEBRILE PATIENTS WITH SUSPECTED ENTERIC FEVER [+]

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**Abstract:**

**Iraq is an endemic area with Salmonella, hence most population are with high Salmonella-specific antibody titer due to their continuous exposure to this bacterium.**

**This study was conducted from June 2010 to May 2011 on outpatients in public hospitals in Baghdad city to evaluate Widal test as a diagnostic kit for enteric fever using serum of 200 febrile patients at ages from 9 to 66 of both sexes. The overall seroprevalence of anti-O for *Salmonella typhi* was 56% (35% for females and 21% for males). Out of this rate, 3% were with only anti-O for *S typhi*, 9% were with anti-O and anti-H for *S typhi*, 27% were with anti-O (with or without anti-H) for *S typhi* plus anti-H for *S paratyphi* A or B, and 17 % were with anti-O (with or without anti-H) for *S typhi* plus anti-O (with or without anti-H) for *S paratyphi* A or B. The frequency of anti-O for *S paratyphi* was 1% for group A only, 24% for group B only and 1% for both A + B. For the old infections that were presented with only anti-H antibodies, the seroprevalence rate was 3% for *S typhi* only, and from 1-4% for other types of *Salmonella* or mixed infections. Concerning the seasonal variations, the highest rate of seroprevalence (40%) was in spring season and the lowest one (10%) was in winter season. In conclusion, using *one sample* Widal serology with a cut-off positive value of 160 and more is a valuable test for the diagnosis of enteric fever in highly endemic areas as the city of Baghdad. Worth to mention that most cases with confirmed enteric cases results (using *one sample* Widal serology) were of mixed origin of infection with *S typhi* and *S paratyphi* serotypes.**

## تواتر اضداد السالمونيلا بين المرضى المحمومين المشتبه باصابتهم بالحمى المعوية

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المستخلص:

العراق منطقةُ مستوطنةُ بالسالمونيلا، لِذلك أكثر السكانِ في تعرّض مستمر إلى هذه البكتيريا وبمعيار عالي للاضداد في امصالهم. هذه الدراسةِ أجريتْ مِنْ حزيران 2010 إلى ايار 2011 على المرضى الخارجيين في بعض المستشفيات العامّة لمدينة بغداد لتقييم فحص ويدال لعدة تشخيص للحمى المعوية في امصال 200 من المرضى المحمومين من عمر 9 الى 66 سنة من كلا الجنسين الذين يشك في اصابتهم بالحمى المعوية. كانت نسبة الانتشار المصلي الكلية لاضداد سالمونيلا تايفي نوع (O) والتي تمثل اصابة حديثة فعالة هي 56% (35% في الاناث و21% في

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**الذكور) بغض النظر عن وجود اي اضداد اخرى للانواع الاخرى من السالمونيلا. من مجمـــوع نـــسبة الانتـــشار الكليـــة المذكورة اعلاه كان توزيع النتائج الايجابية كما يلي: 3% لاضـــداد (TO) فقـــط, 9% لاضـــداد (TO) و(TH), 27% لاضداد (TO) مع او بدون اضداد (TH) مع وجود اضداد (AH) او (BH), و17% لاضداد (TO) (مـــع او بـــدون اضداد TH) واضداد AO او BO (مع او بدون اضداد AH او BH). نسب الانتشار المصلي لاضداد (O) للـــسالمونيلا باراتايفي كانت 1% للمجموعة A, 24 % للمجموعة B, و 1% للاصابة المختلطة (A زائدا B) اما بالنسبة للانتـــشار المصلي المقتصر على اضاد (H) فقط من دون اضداد (O) والتي تمثل اصابة قديمة غير فعالة فقد كانت 3% للسلمونيلا تايفي فقط, وتراوحت ما بين 1% –4% لانواع السالمونيلا الاخرى او الاصابات المختلطة. اعلى نسب الانتشار المصلي سجلت في موسم الربيع (40%) وادنى النسب سجلت في موسم الـــصيف (10%) للاصـــابات الفعالـــة لجميـــع انـــواع السالمونيلا. من النتائج اعلاه, يتبين ان كفائة فحص ويدال جيدة لتشخيص الحمى المعوية باستخدام عينة مصل واحـــدة في المناطق الموبوئة مثل مدينة بغداد كما ان معظم النتائج الموجبة كانت من اصل مختلط لانواع السالمونيلا المختلفة.**

## Introduction:

Typhoid fever is estimated to cause 21 million illnesses and 200,000 deaths annually worldwide [1]. Ingestion of contaminated drinking water and food is the most common route of disease transmission [2]. Although typhoid fever is common in many regions of the world, the actual burden of disease is poorly defined in most endemic countries.

In the setting of clinical illness consistent with typhoid fever, the gold standard for diagnosis is isolation of *S. typhi* from blood, bone marrow, stool, urine or any other body fluid [3, 4]. In addition, other serological techniques as immunoblotting [5] and molecular biology techniques as PCR [6] were used recently for the diagnosis of typhoid fever with different degrees of success. However, in countries such as Iraq (in which typhoid fever is endemic like many other developing countries), isolation of the organism is often jeopardized by lack of facilities or inadequate and/or improper antibiotic use prior to culture [7, 8]. For these reasons, laboratory diagnosis of *S. typhi* infection relies heavily on serological tests such as the Widal test [9, 10].

Based on their O and H antigen composition, more than 2300 Salmonella serotypes (species) are described in the Kauffmann-White scheme. Salmonella are placed in groups by their O antigen (A, B, C, etc) and subdivided by their H (phase 1 and 2) antigens. Using Widal test as a serological test for detecting Salmonella antibodies in a patient's serum can be of value in diagnosing typhoid and paratyphoid fever in endemic areas when the facilities of culturing or antigen testing are not available and if the test is performed reliably and interpreted with care [11, 12]. However, the value of the Widal test in diagnosing enteric fever in endemic areas remains controversial [13]. Some authors express the view that the test lacks standardization and adequate sensitivity and specificity to be clinically useful, while others consider the test to have a diagnostic value when judged with clinical findings and knowledge of the normal O and H agglutinin titers in the local population (baseline titers) [14-16]. Regarding O- agglutinin and H-agglutinin titer, Huckstep have claimed that the level of H agglutinins is unhelpful in the diagnosis of typhoid, maintaining that the H-agglutinin titer remains elevated for a longer period than the O- agglutinin titer after an episode of typhoid fever and also may rise as a nonspecific response to other infections [17]. However Sommerville et al. and Pang & Puthucheary have proposed that the H-agglutinin titer is as useful as or more useful than the O-agglutinin titer [18,

19] while Parry et al., stated that H agglutinins were less sensitive but more specific than O agglutinins and yielded better positive predictive values [20] .In previous studies from different parts of the world, the baseline titers of TO and TH have revealed wide variations with different prevalence [14-17].

This study was conducted primarily to determine the seroprevalence of anti-Salmonella O and H antibodies among febrile patients with suspected enteric fever. It was also aimed to find out any existing correlation between the frequency of Salmonella antibodies and some demographic factors as age, gender as well as seasonal variations.

## Materials and Methods:

### Sampling and patient selection

About 5 ml of venous blood samples were collected randomly from 200 febrile patients who were attending the Outpatients Consulting Clinic of the Medical City in Baghdad during the period from June 2010 to May 2011. All patients were clinically presented with symptoms suspected to be enteric fever with an age range of 9-66 years of both sexes. Sera were separated and kept at -20 C° till the time of serology testing.

### Diagnostic kit

A commercial Widal test kit (SPINREACT, S.A. Ctra Santa Coloma, SANT ESTEVE DE BAS, SPAIN) was used in this study.

## Method :

Widal slide test was used to analyze serum samples for the presence of Salmonella Abs.

The bacterial suspension (stained antigen) is prepared specifically for the detection and identification of serum agglutinins develop during infection with *Salmonella typhi* and/or *Salmonella paratyphi* A and B. A direct slide agglutination technique was used in this study for qualitative determination of the agglutination ability of sera. The test procedure as it was instructed by the manufacturer was followed.

## Statistics :

Statistical analysis was done using SPSS version 17.0 computer software (Statistical Package for Social Sciences). A P-value 0.05 was considered significant at a confidence interval of 95% according to $X^2$ test.

## Results:

Table 0 shows the symbols keys according to the serological result profiles. Titer of 0-80 for anti-O and anti-H was considered as negative result, whereas titer of 160 and more was considered as positive result for all studied cases. Table 1 shows the general distribution of the active infection with *S. typhi* as it was estimated primarily by positive anti TO regardless to the other test results. The overall positive TO results was 112 cases representing 56% of the total tested 200 cases (including 35% females and 21% males) with a highest rate of positivity in the age group of 20-35 years for both genders. The details of seropositive results for the active infection with *S .typhi* as it was estimated by anti TO in association with other test results are

shown in Table 2. The highest rate (27%) was noticed among the cases which exhibited (in addition to positive anti TO) a positive titer of one or more anti-H Ag (TH, AH and BH) with or without seropositivity to other anti-O Ags (BO and/or AO). The lowest rate (3%) was noticed in cases which exhibited a positive titer to anti-TO only with no positive titer to any other Salmonella Ags. Gender variation for all groups is shown in the same table. Negative results for all Salmonella species are shown in Table 3. Table 4 shows the detailed distribution for seropositive results of active infections with *S. paratyphi* A and/or B. Fifty two cases representing 26% of the total 200 cases were positive for anti AO and/or BO for both genders. The rate of seropositivity for BO was higher in females (17%) than in males (9%). The presence of anti-H positive titer which represents an old or inactive infection is revealed in Table 5. The highest rate of positivity (4%) was noticed in one group in which an anti-H positive titer for all 3 Salmonella species was shown. Table 6 and Figure 1 show the distribution of Widal positive test results for active infection according to the season of the year. The highest percentage was noticed in spring season (40%) and the lowest was in winter season (10%).

**Table 0. Symbols key for test results.**

| Serological profile | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Anti-TO** | + | + | + | + | - | - | - | - | - | - | - | - | - |
| | **Anti-TH** | - | + | + | + | V | V | V | + | + | + | - | - | + |
| | **Anti-AO** | - | - | V | V | + | - | + | - | - | - | - | - | - |
| | **Anti-AH** | - | - | V | - | V | V | V | - | + | - | + | - | + |
| | **Anti-BO** | - | - | V | V | - | + | + | - | - | - | - | - | - |
| | **Anti-BH** | - | - | V | - | V | V | V | - | - | + | - | + | + |
| | **Symbols keys** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |

**TO= *S typhi* O-Ag, TH= *S typhi* H-Ag, AO= *S paratyphi* A O-Ag, AH= *S paratyphi* A H-Ag, BO= *S paratyphi* B O-Ag, BH= *S paratyphi* B H-Ag, V= Variable**

**Table 1. Positive TO regardless of other test results**

| Age group (years) | Studied cases (n=200) | | | | | | | | | Statistics |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive TO regardless of other test results | | | | | | | | | |
| | Females | | | | Males | | | | Total | |
| | № | % | Total | | № | % | Total | | № | % | |
| | | | № | % | | | № | % | | | |
| <20 | 22 | 11 | 70 | 35 | 6 | 3 | 42 | 21 | 112 | 56 | P>0.05 |
| 20-35 | 30 | 15 | | | 20 | 10 | | | | | |
| >35 | 18 | 9 | | | 16 | 8 | | | | | |

**Table 2. Positive TO according to genders in association with other test results.**

| Test results symbols | Studied cases (n=200) | | | | | | Statistics |
|---|---|---|---|---|---|---|---|
| | Positive TO in association with other test results | | | | | | |
| | Female | | Male | | Total | | |
| | № | % | № | % | № | % | |
| 1 | 6 | 3 | 0 | 0 | 6 | 3 | P= 0.05 |
| 2 | 6 | 3 | 12 | 6 | 18 | 9 | |
| 3 | 34 | 17 | 20 | 10 | 54 | 27 | |
| 4 | 24 | 12 | 10 | 5 | 34 | 17 | |
| Total | 70 | 35 | 42 | 21 | 112 | 56 | |

**Table 3: Negative results for all Salmonella species.**

| Studied cases (n=200) | | | | | | Statistics |
|---|---|---|---|---|---|---|
| Females | | Males | | Total | | |
| № | % | № | % | № | % | P= 0.056 |
| 4 | 2 | 6 | 3 | 10 | 5 | |

**Table 4. Positive AO and BO in association with other test results.**

| Test results symbols | Studied cases (n=200) | | | | | | Statistics |
|---|---|---|---|---|---|---|---|
| | Positive AO and BO in association with other test results | | | | | | |
| | Females | | Males | | Total | | |
| | № | % | № | % | № | % | |
| 5 | 0 | 0 | 2 | 1 | 2 | 1 | P= 0.005 |
| 6 | 34 | 17 | 14 | 7 | 48 | 24 | |
| 7 | 0 | 0 | 2 | 1 | 2 | 1 | |
| Total | 34 | 17 | 18 | 9 | 52 | 26 | |

**Table 5. Positive anti H Ab for all Salmonella species in association with other test results**

| Test results | Studied cases (n=200) | | | | | | Statistics |
|---|---|---|---|---|---|---|---|
| | Positive anti H Ab for all Salmonella Species in association with other test results | | | | | | |
| | Females | | Males | | Total | | |
| | № | % | № | % | № | % | |
| 8 | 4 | 2 | 2 | 1 | 6 | 3 | P= 0.005 |
| 9 | 2 | 1 | 0 | 0 | 2 | 1 | |
| 10 | 2 | 1 | 4 | 2 | 6 | 3 | |
| 11 | 2 | 1 | 0 | 0 | 2 | 1 | |
| 12 | 2 | 1 | 0 | 0 | 2 | 1 | |
| 13 | 4 | 2 | 4 | 2 | 8 | 4 | |
| Total | 16 | 8 | 10 | 5 | 26 | 13 | |

**Table6: Distribution of positive test results for Salmonella antibodies according to seasons.**

| Antigen | Studied cases (n=200) | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Distribution of positive cases of titer 160-320 according to seasons | | | | | | | | | |
| | WINTER | | SPRING | | SUMMER | | AUTUMN | | | |
| | № | % | № | % | № | % | № | % | № | % |
| TO | 12 | 6 | 52 | 26 | 28 | 14 | 20 | 10 | 112 | 56 |
| AO | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 1 |
| BO | 6 | 3 | 28 | 14 | 12 | 6 | 2 | 1 | 46 | 24 |
| AO + BO | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| Total | 20 | 10 | 80 | 40 | 40 | 20 | 24 | 12 | 164 | 82 |

## Discussion:

The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other febrile illnesses [12]. Serodiagnosis of typhoid fever has been attempted since the late nineteenth century by Widal and Secard [21]. The test was based on demonstrating the presence of agglutinins (antibodies) in the serum of an infected patient, against the H (flagellar) and O (somatic) antigens of *Salmonella enterica* serotype Typhi (*S. Typhi*). While the definitive diagnosis of typhoid fever depends on the isolation of *S. Typhi* from blood, stools, urine or other body fluids [10], the role of the Widal test has been to increase the index of suspicion for the presence of typhoid fever by demonstrating a positive agglutination during the acute and convalescent period of infection with evidence of four fold rise in antibody titer [22]. Over 100 years since its introduction as a serologic means of detecting the presence of typhoid fever, the Widal test continues to be plagued with controversies involving the quality of the antigens used and interpretation of the result, particularly in endemic areas [23]. Hoffman et al. stated that the results of single Widal test, tube dilution or slide agglutination test are virtually un-interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known. Many authors stated that the recommended definitive interpretation of the Widal test is four fold rises in agglutinins in sera taken 7 to 10 days apart [23,24].

Regarding O-agglutinin and H-agglutinin titer, Huckstep have claimed that the level of H agglutinins is unhelpful in the diagnosis of typhoid, maintaining that the H-agglutinin titer remains elevated for a longer period than the O-agglutinin titer after an episode of typhoid fever and also may rise as a nonspecific response to other infections [17].

The overall positive cases with a titer of 160 and more for TO Ag of *S. typhi* were 112 representing 56% of the total 200 studied cases (Table 1). This very high rate of prevalence for *S. typhi* looks unusual and if it is true, it would represent a very high epidemic. However, many factors should be taken in consideration when we discussed such very high results including: Firstly, the low specificity and sensitivity of Widal test. Secondly, the test was done using a single sample which might not reflect the precise state of infection. For a better and actual results, two samples (7-10 days apart) should be taken which must reveal an increasing titer of 4 folds before making a decision of actual infection. Thirdly, Iraq is an endemic area with *Salmonella*, and most population has a continuous exposure to this bacterium that produces a high titer against this bacterium in the sera of the exposed people [25]. Finally, the presence of cross reactivity with many other pathogens the patient might have exposed to [25, 26].

In the same table, the rate of prevalence rates for females and males were 35% and 21% respectively. No logic explanation for such gender variation as Salmonella infection has no gender variation; however, using a small sample of 200 cases in this study may support the factor of coincidence in such results. Concerning the age factor, the highest rate of positive results was within the age group of >20-35 years for both genders.

Table (2) shows the detail of table (1). The highest rate of positivity (27%) was in patients with positive TO, AH, with or without positive titer for BH, TH, AO and BO. Such result is difficult to be interpreted, however, a base line of positive TO titer indicating a current active *S. typhi* infection. Seropositivity to other O Ags (AO or BO) is almost a cross reaction due to the common epitopes between *S. typhi, S. paratyphi* A and *S. paratyphi* B. On the other hand seropositivity to TH Ag indicating that the infection with *S typhi* is of at least of 2 weeks earlier than the time of performing the test as anti-TH antibodies are developing after the anti-TO antibodies. The presence of other anti H antibodies (AH or BH) may indicate a previous (old) infection with *S. paratyphi* A or B, or a previous vaccination. If all agglutinins (TO, TH, AH, BH) are raised, it is most likely due to previous vaccination or an anamnestic reaction - seen in individuals who have suffered from enteric fever in the past, and developed anti-*Salmonella* antibodies (agglutinins) during an unrelated or closely related infection. This includes non-enteric fever salmonella infections, infections due to other Enterobacteriaceae, malaria, dengue, typhus, or immunological disorders [16]. These can be differentiated from true infection by repeat testing after a week or 10 days, which will show no rise in titer. Theoretically, another reason for raised agglutinins could be recurrent or repeated infections due to different *Salmonella* from the enteric fever group, but this is extremely unlikely, especially in the absence of symptoms. In the same table, 17% of cases show seropositivity to TO Ag in the presence or absence of anti-TH positive titer plus positive titers for other O Ags (AO or BO) in the absence of any positive titer to other H Ags (AH or BH). Such result can be interpreted as an active infection with *S. typhi* with cross reactivity with other Salmonella and the absence of a previous infection or vaccination with *S. paratyphi* A or B. The result of having a seropositive titer for TO Ag only or for both TO and TH Ags is easy to be interpreted as an active *S. typhi* infection without cross reactivity or a previous infection or vaccination with other *Salmonella* species. In most results listed in table 2, the rate of infection is higher in females than males.

Table (3) shows the negative cases for all *Salmonella* Ags which was 5% of the total studied cases.

In table (4), positive anti AO and/or anti BO with negative TO and variable results for other antigens are shown. The highest frequency of 24% was noticed in the group with positive titer for anti-BO with negative titer for other O Ags (TO and AO) and positive or negative titer for H Ags (TH, AH and BH). The interpretation of such results is an active infection with *S. paratyphi* B. Most cases were female (17%) compared to 7% for males. Very few positive titers (1%) were noticed for AO (active infection with *S. paratyphi* A) or BO+AO (active mixed infection with *S paratyphi* A and B).

Table (5) shows the inactive infection (old) with different species of *Salmonella*. The total cases with such serological profile were 13% (8% for females and 5% for males). The distribution of such cases for different species of *Salmonella* was variable and ranged from 1% for a previous exposure to *S. typhi* + *S. paratyphi* A, *S. paratyphi* A only, and *S. paratyphi* B only, to 4% for a mixed previous exposure to the three species of *Salmonella*.

An important point relating to the titer of anti O or anti H antibodies in the patient serum at which the case is considered as true Salmonella infection should be discussed thoroughly especially when one single sample is considered for the diagnosis. In fact, the actual titer for true infection

is not an absolute matter as it is associated with many factors [1] including: the degree of endemicity in the targeted population, the presence or absence of the clinical manifestation that is specific to the disease, the degree of specificity and sensitivity of the diagnostic method, the patient's age, the presence or absence of other confirmatory test as stool culture which considered as the gold standard diagnostic test, the exposure to other non-typhoid Salmonella and non-Salmonella pathogens that might cross react with typhoid Salmonella species, the immunocompetente state of the patient, the presence or absence of standardization of the antibody levels for Salmonella in the normal population in order to decide the upper limits of normal [20, 21], and last, any previous history of immunization.

In a study conducted in Vietnam it was found that with a cutoff titer of ≥200 for O agglutinin or ≥100 for H agglutinin, the Widal test would diagnose correctly 74% of the blood culture-positive cases of typhoid fever. However, 14% of the positive results would be false-positive, and 10% of the negative results would be false-negative [20]. In the absence of vaccination, an elevated level of H and/or O agglutinin titer of 1: 320 is of diagnostic value for typhoid fever especially in that study setting where a single sample of serum is relied on for the diagnosis of typhoid fever.

In India [27] 'O' or 'H' liters of 1:160 or more were indicative of typhoid fever. Similarly, when both titers were considered together, either 'O' or 'H' titers of1:160 or more were suggestive. In other countries, the cut-off titer for typhoid fever infection is variable like 1: 80 in Philippines [28], 50 in Hong Kong [29], 1:160 in Jordan [30], 1:160 in Peru [31], and 1:160 in Ethiopia [32]. In Iraq, there is no established level for a significant cut-off value for typhoid fever infection; however medical field workers, on the basis of their field experience, believe that a titer equal to or more than 160 is the cut-value for typhoid infection [25, 26].

Finally, the distribution of the active infection with different Salmonella species according to the season of the year is shown in table (6) and figure (1). The highest rate of infection was noticed in spring season which was 40% and the lowest one was noticed in winter season which was 10%. The high rate of infection in spring season is expected as in this season the Iraqi weather tends to be hot rather than moderate and accordingly people would drink iced water and juices and eat ice cream of different origin which could be contaminated with the bacteria. In addition, these bacteria would be more active in the moderate seasons than in the extreme seasons of the year.

In conclusion, using *one sample* Widal serology with a cut-off positive value of 160 and more is a valuable test for the diagnosis of enteric fever in highly endemic areas as the city of Baghdad. Worth to mention that most cases with confirmed enteric cases results (using *one sample* Widal serology) were of mixed origin of infection with *S typhi* and *S paratyphi* serotypes.

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