

Serological Detection of Rickettsioses with Underlying Meningeal Syndrome in Algiers

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Our study aims to detect the serological positivity for *Rickettsia* spp with underlying Meningeal Syndrome at The National Centre of Infectious Diseases El-HADI FLICI Hospital in Algiers. A total of 55 sera were obtained from patients of different ages and sex, the mean age of the population study was 24.03 (2 to 50 years old). The IFA for *Rickettsia* ssp came back positive four 07 sera (07/55, 12.72%), within we diagnosed 04 cases of Spotted Mediterranean Fever (MSF) caused by *R. conorii*, and 02 cases of Murine Typhus caused by *R. typhi*, and only one (01) case of the Flea-borne Spotted Fever caused by *R. felis*. These serological results associated to the underlying Meningeal syndromes confirmed previously in all these cases in a hand, and the various clinical manifestation of Rickettsial and Meningeal symptoms among these patients in other hand, lead us to suggest the competitive physio-pathogenicity of the clinical expression between the two pathogens. These findings would provide more attention for the infectious disease specialists front of all confirmed Meningeal syndrome which may have a clinical similarity with the Rickettsial diseases especially in countries where the arthropod-borne zoonosis is highest.

Keywords: Rickettsia spp; Mediterranean Spotted Fever; Meningeal Syndrome; IFA Serology; Algeria Zoonosis**Introduction**

Rickettsioses are considered as emerging or re-emerging arthropod-borne zoonosis since cases of human infection are continually described in worldwide, and due to widely geographic distribution to the ecology of their vectors [5,7]. The causative agent of Rickettsial is an intracellular bacterium from the genus *Rickettsia*, which are phylogenetically classified into four groups: Spotted Fever Group (SFG), Typhus Group (TG), Transitional group and ancestral group [15]. Serologically, the SFG *Rickettsia* are similar to the transitional and ancestral groups, but all are distinguishable from the TG *Rickettsia*. Within the SFG, *R. conorii* is the etiological agent of Mediterranean Spotted Fever (MSF), *R. rickettsia* the etiological agent of the Rocky Mountain Spotted Fever. Among the TG, *R. typhi* is the causative agent of Murine Typhus (endemic typhus) [8]. Based on its high sensitivity and specificity, the Immunofluorescence Assay (IFA) is the Gold Standard for serological diagnosis

of Rickettsial infections [16]. The Rickettsioses are transmitted by various arthropod vectors, as ticks, fleas, body lice and mites [3]. The host animals of these vectors are also important as they transport vectors to environment in and around places where humans live. When the Rickettsial host-vectors animals are autochthonous, a positive serology is related directly to the circulation of the bacteria in this region [1]. Different rickettsial infections tend to cause similar symptoms: fever, severe headache, a characteristic rash (Maculopapular, Purpuric), a general feeling of illness (malaise). The Mediterranean Spotted Fever is the most common rickettsioses occurring in the Mediterranean region, this disease is transmitted by the bites of the brown dog ticks; *Rhipicephalus sanguineus* [2]. Algeria is a Mediterranean basin country, where the warm climate covers a significant part. Consequently, several human-vector Rickettsioses have been studied, namely *R. conorii conorii* and *R. massiliae* detected in *Rhipicephalus sanguineus*, *R. aeschlimannii* de-

tected in *Hyalomma marginatum* [2,14]; Also 63 sera-human cases came back positive for *Rickettsia conorii conorii* in Western Algeria and other 14 human cases were diagnosed in eastern Algeria [2,3].

In other respects, Meningococcal Syndromes are also one of the most common motives for consultation in the summer period at the Infectious Diseases Emergency in Algeria. It is necessary to know that Meningeal Syndrome is the set of symptoms associated with irritation pathological of the meningeal envelopes of the central nervous system. Its detection requires emergency hospitalization for lumbar puncture and Cerebro-Spinal Fluid (CSF) analysis for diagnosis etiological. The positive diagnosis of meningeal syndrome is defined by the triad: Headache, vomiting, stiff neck. In fact, lumbar puncture remains the exclusive criterion for diagnosis of acute meningeal syndrome [20].

Nowadays in Algeria, we have to consider the balance between the large Rickettsial symptoms and the lack of biological diagnostic means which are not widely available. Consequently, in most cases the infectious disease specialists rely on the clinical diagnosis. For instance, face to a clinical picture in favour of Rickettsioses, with or without an underlying infection (Meningeal Syndrome or others), due to the lack of biological diagnostic tools for rickettsiosis, aiming to confirm the clinical diagnosis provided, creates a critical diagnostic challenge and also a crucial therapeutic approach. In light of these considerations, our study aims to highlight the relationship between clinical suspicion of Rickettsioses and its serological confirmation in patients with an underlying meningeal syndrome, especially when we consider the common clinical signs between the two infections; as fever, headaches. Our study was conducted at the Algerian National Centre of Infectious Diseases EL-HADI FLICI Hospital.

Material and Methods

Study design

In order to show evidence, the relationship between clinical suspicion of Rickettsioses and its serological confirmation in patients with an underlying meningeal syndrome, we considered wise to focus our study at the National Reference Centre for Infectious Diseases in Algeria; named ELHADI FLICI Hospital-Algiers, which admits patients from all the Algerian departments. Our study was conducted between July and September 2017. knowing that Algeria is a warm country of the Mediterranean basin, this period coincides with summer season where vector-borne and tropical diseases are highest. A total of 55 patients coming from

different departments of Algiers, were admitted to our hospital and included in the study.

Inclusion criteria

In order to select the patients who well respond to our study, we considered only patients who were diagnosed for a Meningeal Syndrome, based on a clinical confirmation and a positive biological findings of the Cerebrospinal Fluid (CSF) study; these patients should present other strange clinical signs which are not associated to the Meningeal Syndrome and they are clinically in favour of Rickettsial infections by more than three from the following signs: sudden onset, high fever, severe headache, arthralgia, myalgia, ticks bits, a characteristic rash (maculopapular or purpuric rash), taches noire and a general feeling of illness. A total of 55 patients were enrolled in the study, 14 adult males (25.45%), 22 adult females (40.00%), and 19 children (under 15 years old, 34.54%: 11 males and 9 females), the mean age was 24.03 (2 to 50 years old). In parallel, for each patient we completed a questionnaire in order to have more information about them; such as epidemiological data, housing area (rural or not), contact with ticks/animals, profession, the notion of bite of the ticks.

Sample's collection

A total of 55 sera samples were collected aseptically in suitable dry tube. The samples were conserved at -20°C to handle them at the URMITE (Emerging Tropical Infectious Diseases Research Unit at the Institut Hospitalo-Universitaire (IHU) Marseille; for IFA serology and q PCR for the rickettsial spp.

Serological assays

Serologic tests were performed using an indirect immunofluorescent antibody (IFA) assay, which is the reference method for the serodiagnosis of Rickettsioses. At the serological service of the IHU Marseille, we test a panel of Rickettsial antigens; as *R. conorii*, *R. felis*, *R. typhi*. Immunoglobulin G (IgG) antibody titers of 1:128, seroconversion in paired serum specimens, or IgM antibody titers of 1:32 against any species were considered evidence of recent *Rickettsia* infection [2].

DNA extraction and Real time PCR

A total of 200 µL of DNA was extracted from the positive sera by IFA, using the QIAamp Tissue Kit by QIAGEN-BioRobot EZ1, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Extracted DNA from sera was stored at -20°C under sterile conditions until it was used in PCR assays. Sera extracted DNA

was used in qPCR amplifications the *gltA* gene of *Rickettsia* species [19]. Positive samples are further amplified by PCR targeting the outer membrane protein (*ompB*) [17] and citrate synthase (*gltA*) genes [18]. The final qPCR reaction mixture consisted of 5 µL DNA with 15 µL of mix from the Roche PCR Kit (Roche Diagnostics, Meylan, France). The PCR cycling parameters for the qPCR were 5 minutes at 95°C followed by 39 cycles each consisting of 5 sec of denaturation at 95°C and 30 seconds of annealing at 60°C.

Ethics statement

All the patients gave us permission to include in the study, by interview information, blood samples. Clinical data were obtained through a standardized questionnaire with clinical information, contact with animal, health history. These data were analysed retrospectively when the serological analysis or molecular test were positives.

Results

IFA Serology

A total of 55 sera were collected from 55 patients who were hospitalized in the Algerian National Centre of Infectious Diseases EL-HADI FLICI Hospital, for clinical suspicion of Rickettsioses with an underlying confirmed meningeal syndrome. The percent distribution of signs and symptoms of all patients included in the study are summarized in table 1.

Signs and Symptoms	Number	Percentage
Fever	32	58.18%
Headache	27	49.09%
Arthralgia	44	80.00%
Myalgia	21	38.18%
Maculopapular Rash	17	30.90%
Purpuric Rash	09	16.36%
Taches noire	08	14.54%

Table 1: Percentage distribution of signs and symptoms of all patients included in the study.

The results shown in table 1, lead us to point that Fever and Arthralgia were the most common symptoms for all the patients in-

cluded in the study (58.18%, 80.00% respectively). However, only 14.54% of the population study have Taches noire, and 16.36% of them have a purpuric rash. Maculopapular rash is the most noticeable cutaneous symptom for the Rickettsial diseases, despite this we only find that in 30.90% patients. This distinction in symptoms from one patient to another, it would make challenging to diagnose certainty.

The screening for all the sera for the antigens against *R. conorii*, *R. felis* and *R. typhi*, came back positive for only 07 sera (07/55, 12.72%).

Detection of *Rickettsia* spp by quantitative q PCR

q PCR was used for the detection of *Rickettsia* spp in sera by employing *Rickettsia* specific primers and a probe designed to amplify the *gltA* gene. All the sera q PCR came back negative for the Rickettsial gene *gltA*, even those came back positive by IFA for any Rickettsial species. This negativity may be explained by the low quantity of bacteria DNA in the sera which makes difficult the DNA amplification and their detection

The IgG and IgM titers against *R. conorii*, *R. felis* and *R. typhi*, and q PCR results and other signs belonging to positive IFA are summarized in table 2.

Using IFA against *Rickettsia* spp antigens, we obtained only 07 positive samples from 55 ones (12.72%). Among of these positive samples, we note that 04 of them are Female and the 03 left are male. However, the Dogs/Ticks contact didn't reflect the real infectious status, because even with no Dogs/Ticks contact we found positive rickettsial titers. Otherwise, according to these IFA results, we diagnosed 04 cases of Spotted Mediterranean Fever (MSF) caused by *R. conorii*, and 02 cases of Murine Typhus caused by *R. typhi*, and only one (01) case of the Flea-borne Spotted Fever caused by *R. felis*. In parallel, the IFA of the patient number 06, came back positive four two (02) Rickettsioses, *R. conorii* and *R. felis*. These cross-reactive antibodies have to be confirmed by Western-Blot test, which makes the result more sensitive.

The four cases of MSF and the one case of Flea-borne Spotted Fever, they belong to the SFG of Rickettsial diseases; However, the two cases of Murine Typhus, belong to the TG of Rickettsioses.

Patient	Sex/age	Contact with dogs/Ticks	Titers against <i>R. conorii</i>		Titers against <i>R. felis</i>		Titers against <i>R. typhi</i>		q PCR <i>gltA</i> gene
			IgG	IgM	IgG	IgM	IgG	IgM	
01	F/08	Yes	n	n	n	n	1:64	1:256	n
02	F/11	No	1:64	1:32	n	n	n	n	n
03	F/44	Yes	1:256	1:32	n	n	n	n	n
04	M/48	No	n	n	1:128	1:64	n	n	n
05	M/50	Yes	1:64	1:32	n	n	n	n	n
06	F/19	Yes	1:4096	1:128	1:4096	1:64	n	n	n
07	M/47	No	n	n	n	n	1:32	1:64	n

Table 2: Epidemiological history and IgG/IgM titers against *R. conorii*, *R.felis*, *R.Typhi*, and also the q PCR results in patients with IFA positive samples.

*n: negative. *All sera are screened as first-line with Total Immunoglobulin. if the serum is positive at 1/100 dilution, then the antibodies present in this sample are differentially quantified (IgG, IgM).

Discussion

Aiming to showcase the relationship between clinical suspicion of Rickettsioses and its serological confirmation in patients with an underlying confirmed meningeal syndrome, we focused our study at the National Centre of Infectious Diseases EL-HADI FLICI Hospital in Algiers. During the study period, from July to October 2017, high temperatures were recorded in Algiers (till 39.5°C); knowing that Algeria is warm country, in the summer season the vector-borne infectious diseases are highest. It is well known that the distribution of vectors and associated pathogen transmission rates can be affected by changes in the ambient temperature and climate [5]. Therefore, other species ticks are getting in the Rickettsial cycle, thus they increase potentially the risks of human infections.

Meningeal syndrome is the set of symptoms associated with irritation pathological of the meningeal envelopes of the central nervous system, this disease was confirmed clinically and biologically with all patients included in our study. Furthermore, a panel of clinical symptoms in favour of Rickettsial infections, was required for the inclusion criteria, such as fever, severe headache, a characteristic rash (maculopapular, purpuric), a general feeling of illness (malaise). A total of 55 sera belonging to 55 patients, were collected aseptically, and test by IFA Rickettsial serology and molecular detection of *Rickettsia spp* in the positive sera. Rickettsial IFA serology came back positive four 07 sera from the 55 tested (12.72%), whereas, the molecular detection of *Rickettsia spp* targeting the *gltA* gene was negative for all the sera. The positivity of

Rickettsial IFA varies from patient to patient, precisely 04 cases of Spotted Mediterranean Fever (MSF), 02 cases of Murine Typhus, and only one (01) case of the Flea-borne Spotted Fever. The positive patient’s sera for Rickettsioses and the diagnosed underlying Meningeal syndromes in all these cases in a hand, and the various clinical manifestation of Rickettsial and Meningeal symptoms among these patients in other hand, lead us to suggest the competitive physio-pathogenicity expression between the two pathogens. All cases develop symptoms with Fever or more clinical findings. The most common finding was the presence of Maculopapular Rash and arthralgia. This different expression of clinical symptoms for the both pathogens (Meningeal and Rickettsial), probably depend on the severity of the disease and may range from mild to more severe ones. It is noteworthy to assess these results concerning the co-infections between the Meningeal pathogen and the *Rickettsia* spp.

In addition, further studies took also in account the Rickettsial diseases. In Western Algeria, Oran, the first cases of MSF have been clinically diagnosed for the first time in 1993 and since that time the number of cases has steadily increased to reach 134 in 2004 [2]. Mouffok, *et al.* in 2006, conducted a study in Western Algeria in order to present the descriptive clinic and epidemiology, to identify more severe forms, the presence of the multiple eschars, and different rickettsial strains caused the disease, where they found 63 positive IFA Rickettsial serology from out 104 sera, and they also reported that all the patients included in the study had fever, and 44% of them presented underlying diseases, und 72% of the

population study have a Maculopapular rash. All the same, Mouffok., *et al.* in 2009, reported 24 positive IFA serology for *R. conorii* (24/36), belonging to Algerian children patients. These results are in concordance with ours in term of clinical expression of Rickettsial disease and specially the presence of an underlying infection. In addition, Mouffok., *et al.* in 2011, evaluated the advantage of skin swab samples (Eschar) for diagnosis of Rickettsial diseases in Algeria, by a molecular detection (q PCR) of the *RC0338 gene* which is specific of *Rickettsia spp*, they obtained 63.4% of positive samples, these result confirmed that the efficiency of the skin swab sampling to diagnose the Rickettsioses, and also confirmed the presence of the Rickettsial pathogen in Algeria. Another study was occurred by Mokrani., *et al.* in 2012, aiming to have a prevalence of Rickettsioses in Febrile Exanthemas in Eastern Algeria, where they calculated 12.96% of positive IFA serology confirmed by Western Blot, within 5 cases of *R. conorii*, 2 cases of *R. felis* and 4 cases of *R. typhi*, the left 03 cases belonged to other *Rickettsia species* (*R. aeschlimanii*). In a common epidemiological study of *Rickettsia felis* and Malaria in Africa, Mediannikov., *et al.* in Africa, they worked for a cohort of African patients, within 266 patients from Western Algeria, 183 patients from Tunisia and 48 patients from Morocco, and also 400 patients from Marseille as control patients. They reported 02 positive

blood q PCR for *R. conorii*, 01 positive blood q PCR for *R. conorii*, in Algerian and Moroccan patients respectively. However, even Tunisia and Marseille have no positive case to *R. conorii*. These results confirm the linear relationship between Rickettsioses and other underlying infection (Malaria in this case), as we reported in our study between Rickettsioses and Meningeal Syndrome. Nevertheless, Znazen., *et al.* in 2006, in Tunisia, they reported 86 positive IFA serology for *R. felis* confirmed by Western Blot from out 638 patient’s sera tested. In addition, in Greece, Chochlakis., *et al.* in 2017, conducted a study aimed to improve testing tick-borne disease, they got 28 positive IFA for *R. typhi* and 8 positive IFA for *R. felis*. Rickettsial diseases remain born-vector disease, mainly transmitted by arthropods, as ticks, fleas, body lice and mites.

In Algeria, several studies have been occurred in order to determine the various vectors and reservoirs of the Rickettsioses diseases, the table 03 summarized the results of studies in order to identify the borne-vector of *Rickettsia species* in Algeria.

The previous describing table 3, suggests the circulation of many *Rickettsia spp* in Algeria, transmitted mainly by ticks, which remain a very important vectors of the Rickettsial diseases, especially if they are transported by animal species where humans live.

Authors	Year	Vectors	q PCR gene targeted	Results	Rickettsia species
Bitam., <i>et al.</i>	2006	Ticks: - <i>Hyalomma marginatum</i> <i>Rhipicephalus sanguineus</i>	<i>gltA ,OmpA</i> genes	21 positive qPCR of out 78	<i>R.conorii</i> <i>R. aschlimanii</i> <i>R. massilia</i>
Kernif., <i>et al.</i>	2012	Ticks: - <i>Ixodes ricinus</i> - <i>Dermacentor,marginatus</i>	<i>gltA ,OmpA</i> genes	15 positive qPCR of out 32	<i>R. helvetica</i> <i>R. monacensis</i> <i>R slovacca</i>
Lafri., <i>et al.</i>	2015	Soft Ticks: Acari: Argasidae	<i>gltA</i> gene	41 positive qPCR of out 154	<i>Novel Rickettsia</i>
Bessas., <i>et al.</i>	2016	Ticks: <i>Rhipicephalus sanguineus</i>	<i>gltA</i> gene	01 positive qPCR of out 115	<i>R conorii</i>
Leulmi., <i>et al.</i>	2016	Ticks: - <i>Rhipicephalus sanguineus sensu lato</i> - <i>Rhipicephalus bursa</i> - <i>Hyalomma scupense</i> - <i>Hyalomma excavatum.</i>	<i>gltA ,OmpA</i> genes	29 positive qPCR of out 191	<i>R. aschlimanii</i> <i>R slovacca</i>

Table 3: Summarize of the study’s results conducted in Algeria to identify the borne-vector of *Rickettsia species*.

Conclusion

In conclusion, based on serological findings and the reported clinical and epidemiological antecedents, there is a clear evidence

of the existence of human Rickettsial diseases in Algiers, especially with underlying infection. As we explained previously, both the Rickettsial diseases and Meningeal syndromes, could infect simul-

taneously the human and the clinical expression reflect the severity of the pathogens in question. These findings would provide more attention for the infectious disease specialists front of all confirmed Meningeal Syndrome which may have a clinical similarity with the Rickettsial diseases, especially in countries where the arthropod-borne zoonosis is highest.

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