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Serological evidence of rickettsial infections in Delhi

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***Background & objectives*: Rickettsial infections remain under-diagnosed due to lack of diagnostic facilities in developing world. Here we present our experience at National Centre for Disease Control, Delhi, about a serosurvey done in Delhi for rickettsial disease with easy to perform low cost, low expertise Weil Felix test.**

***Methods*: On the basis of cut-off titre obtained in healthy population, Weil Felix test results were interpreted along with clinical data. Entomological investigation was also carried out in select areas of Delhi. Rodents were trapped from houses and gardens and vector mites were collected.**

***Results*: When serum samples were collected during initial 5 yr period from patients with fever of unknown origin, seropositivity was 8.2 per cent whereas when rickettsial infection was kept as one of the differential diagnosis by clinicians seropositivity increased to 33.3 per cent. Rickettsial infections detected were scrub typhus (48.2%) followed by spotted fever group (27.5%) and typhus group (6.8%) during 2005-2009. In preliminary entomological survey vector mite *Leptotombidium deliense* was found on rodents.**

***Interpretation & conclusions*: Our findings showed that results of Weil Felix test should not be disregarded, rather clinically compatible cases should be treated to save lives.**

Key words Delhi - *Proteus vulgaris* - rickettsia - rodents - Weil Felix test

Rickettsial diseases may pose a serious threat to public health if not diagnosed or misdiagnosed. Rickettsial infections are one of the important causes of fever of unknown origin (FUO) and this needs to be differentiated from other febrile illnesses like enteric fever, malaria, dengue, leptospirosis, infectious mononucleosis, *etc*. The National Centre for Disease Control (NCDC, formerly National Institute of Communicable Disease) has played important role in providing serological evidence of rickettsial diseases in India in various States like Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Haryana, Rajasthan, Assam, West Bengal, Maharastra, Tamil Nadu, Kerala, Sikkim, and Manipur in the last decade[1]. The Zoonosis Division under NCDC initially conducted serosurvey for six year (1999-2004) for rickettsial diseases using Weil Felix test in FUO cases in the State of Delhi. Subsequently, from 2005 to 2009 samples were received at NCDC from various referral government hospitals in Delhi from suspected cases of rickettsial

infection. NCDC also conducted preliminary entomological investigation in a few affected areas in Delhi. This paper reports the findings of the serosurvey done during 1999-2004, and also the information on received samples during 2005-2009.

Material & Methods

This study was conducted by the Zoonosis Division of the NCDC, Delhi. The study protocol was approved by the ethics committee of NCDC, under Directorate General of Health Services, Government of India.

A total of 700 blood samples were collected from healthy blood donors from government hospitals of Delhi to ascertain baseline titre of Weil Felix test in Delhi.

During 1999 to 2004, 737 serum samples from cases of FUO in whom common illnesses including enteric fever and malaria were ruled out, were collected by NCDC from various government hospitals in Delhi *viz.*, Hindu Rao hospital, Lok Nayak hospital, Aruna Asaf Ali hospital, *etc*.

During 2005-2009, 87 serum samples were received from cases of FUO sent by physicians of medicine department of govt. hospitals where routine investigations were unremarkable and rickettsial disease was kept as one of the differential diagnosis. Clinical findings were recorded on pre designed proforma supplied by NCDC. All serum samples were stored at -20^{0}C till tested.

Weil Felix test: Antigens *Proteus vulgaris* OX2, *P. vulgaris* OX19 and *P. mirabilis* OXK were obtained from Central Research Institute (CRI), Kasauli, India. Weil Felix test was done using standard protocol with doubling dilution of 1:20 to 1:160 for initial screening[2] followed by further dilutions (from 1:20 to 1:1280) to achieve end tire.

Entomological investigations: Entomological investigations to detect the vector were carried out in Sadiq Nagar, Pushp Vihar, M.B. Road, Sainik Farm and Munirka of South Delhi since number of cases were more from these areas. The rodent trappings were also undertaken to know the prevalence of rodent species, vector mites and to determine chigger infestation rate (CIR) and extent of infestation by vector mite. The houses were mainly multi-storied government quarters having parks and kitchen gardens. The traps were laid in the houses and kitchen gardens adjoining the houses. A total of 196 wire cage rodent traps were laid and the rodents were trapped by wire mesh cage, wonder traps and break back traps. Vector mite was collected by combing of rodents and rodents were dissected for taking tissue smears of liver, spleen, lung and heart. Vector mites retrieved from the trapped rodents were preserved in 70 per cent alcohol for detection/ identification of vector mite.

Results

Of the 700 blood samples tested by Weil Felix test from healthy blood donors in Delhi, 667 (95.2%) showed titre of 1:20 or less in OX2, OX19 and OXK antigens. Twenty one (3.0%) samples showed titre of 1:40 and 12 (1.7%) showed tire of 1:80 or more in one or more Weil Felix antigens. Hence, cut-off titre was taken as 1:80 for Delhi population and results in FUO cases were interpreted accordingly.

During the initial serosurvey of rickettsial diseases in FUO cases in Delhi from 1999 to 2004, a total of 737 serum samples were tested by Weil Felix test, of which 640 (86.8%) showed titre 1: 20 or less; 36 (4.8%) had titre 1:40 and 61 (8.2%) had titre 1:80 or more (Table I). Suggestive clinical findings along with titre of 1:80 were taken as positive. Of the 61 positives, 26 (42.6%) were positive for OXK suggestive of scrub typhus, 24 (39.3%) were positive for OX2 suggestive of spotted fever group (SFG), and five (8.1%) were positive for OX9 suggestive of typhus group (TG) with titre of >1:80. The remaining six samples were positive for two antigens *viz.*, four were positive for OX2 and OX19 (6.5%) and one each for OX19 and OXK, and OX2 and OXK with titre of >1:80 (Table I).

From 2005 to 2009, 87 serum samples from suspected cases of rickettsial infections were tested by Weil Felix test, of whom, 29 (33.3%) were positive (Table I). Of these 29 positives, 14 (48.2%) were positive for OXK suggestive of scrub typhus, eight (27.5%) for OX2 suggestive of SFG and two (6.8%) for OX19 suggestive of TG. Remaining five were positive for two antigens. Paired serology was possible in only six cases (five scrub typhus & one Indian tick typhus); however, all of these demonstrated more than four fold rise in titre in Weil Felix test 7-14 days apart. Later, all 29 serum samples were tested by ELISA based test for scrub typhus and Indian tick typhus. Almost 100 per cent correlation was obtained in OXK 1:160 titre or more and ELISA test (unpublished data). Maximum number of cases of rickettsial infections (79.3%) were in age group of 11-60 yr. Adequate clinical response was obtained in most of the cases after initiation of therapy with doxycycline within 2-3 days, however antibiotic was continued for 10-14 days. Important clinical findings are presented in Table II.

Table I. Weil-Felix test results from serosurvey in Delhi (January, 1999 to December, 2009)

Year	Samples tested	No. of positive samples (%)	Titre = 1:80			Titre > 1:160			Titre > 1:80		
			OX2	OX 19	OXK	OX2	OX 19	OXK	OX2 & OX 19	OX 19 & OXK	OX2 & OXK
1999	110	24 (21.8)	8	3	7	1	0	4	1	0	0
2000	200	17 (8.5)	10	1	4	0	0	2	0	0	0
2001	136	6 (4.4)	4	0	1	0	0	0	0	0	1
2002	168	11 (6.5)	1	1	5	0	0	3	0	1	0
2003	73	0 (0.0)	0	0	0	0	0	0	0	0	0
2004	50	3 (6.0)	0	0	0	0	0	0	3	0	0
Total	737	61 (8.2)	23	5	17	1	0	9	4	1	1
2005	23	2 (8.6)	1	0	0	0	0	0	0	0	1
2006	14	3 (21.4)	0	1	1	0	0	0	0	0	1
2007	10	4 (40.0)	0	1	0	1	0	1	0	0	1
2008	19	12 (63.0)	1	0	2	2	0	6	0	1	0
2009	21	8 (38.0)	2	0	0	1	0	4	0	0	1
Total	87	29 (33.3)	4	2	3	4	0	11	0	1	4

Table II. Clinical findings of rickettsial diseases in Delhi

1. Total number of cases (n) = 29	
2. Sex	
Males	13 (44.8)
Females	16 (55.2)
3. Age (yr)	
<10	5 (17.2)
11-60	23 (79.3)
>60	1 (3.4)
4. Important findings	
History of insect bite	3 (10.3)
Fever with chills	16 (55.2)
Headache	12 (41.4)
Rash	15 (51.7)
Vomiting	11 (37.9)
Pain abdomen	6 (20.7)
Diarrhoea	3 (10.3)
Constipation	1 (3.4)
Jaundice	3 (10.3)
Thrombocytopenia	13 (44.8)
Eschar	6 (20.7)
Lymphadenopathy	3 (10.3)
Hepatomegaly	13 (44.8)
Splenomegaly	8 (27.6)
Sepsis & multiorgan failure	2 (6.9)
Acute respiratory distress syndrome	2 (6.9)

Values in parentheses are percentages

From three different areas in Delhi, a total of 20 rodents comprising of three species *viz. Suncus murinus* (15), *Rattus rattus* (3) and *Bandicoota indica* (2) were trapped giving the over all trap positivity rate of 10.2 per cent. Results revealed the presence of vector mite *Leptotrombidium deliense* on *Rattus rattus* and *Suncus murinus*. The chigger infestation rate (CIR) was found to be 15.0 per cent and chigger (vector mite) index as 5.75 which was higher than the critical limit of 0.69/ rodent. All the rodent serum (18) and impression tissue smears (20) were found to be negative for *Orientia tsutsugamushi*.

Discussion

Weil Felix test is the oldest assay based on detection of antibody to various *Proteus* antigens that cross-react with rickettsiae. Although it lacks specificity and sensitivity, it may be used in developing countries as first diagnostic step in diagnosis of rickettsial diseases[3]. One of the major limitations of serology is the cross-reactivity that often exists among antigens of pathogen within the same genus and occasionally in different genera[4]. However, to deal with this issue it is important to collect acute and convalescent phase serum separated by several weeks to confirm the diagnosis. In reference laboratories, cross-absorption (CA) techniques and Western blotting may be used to differentiate rickettsial infection by antibody evaluation[5].

In developing countries like India where epidemiology and burden of rickettsial disease is largely

undiscovered, high cost of conducting investigations like immunoflourescence, Western blot or PCR based tests is additional hindrance in making accurate diagnosis of rickettsial diseases. However, simple, economical Weil Felix test as initial investigation can guide a clinician in instituting appropriate treatment. Weil Felix test detects IgM antibody detectable 5-10 days following the onset of symptoms. Whole cells of *P. vulgaris* OX2 react strongly with serum from person infected with spotted fever group (SFG) rickettsiae with the exception of those with Rocky mountain spotted fever (RMSF); and whole cells of *P. vulgaris* OX19 react with serum from person infected with typhus group rickettisial as well as with RMSF. Also, OXK strain of *P. mirabilis* agglutinates with serum from scrub typhus patients[6,7]. For a test to be useful in the diagnosis of acute rickettsial infection, the most important criteria are sensitivity and the length of delay between the onset and appearance of detectable antibody titres[5].

Overall significant titre in single antigen (OX2 or OX19 or OXK) were obtained in 79 of 90 (87.7%) cases during 11 years. Cross-reaction or significant titre in double antigen can be resolved by testing convalescent serum to demonstrate significant rise in titre. In our study, four-fold rise in titre in paired serum samples was demonstrated in only five cases of scrub typhus. In the remaining, paired serology could not be performed.

Our results showed that among rickettsioses, scrub typhus seems to be most common followed by Indian tick typhus in Delhi. In the entomological study poorly maintained kitchen garden and long grass attracted rodent population. At many places vector mites were collected from the rodents caught from active rodent burrows in kitchen gardens. Presence of vector mite above the critical limit indicates that during monsoon season these areas may act as potential sites for the transmission of rickettsial diseases. Therefore, proper entomological and epidemiological profile for rickettsioses in Delhi as well as in other States in our country can provide a clear view of louse or flea or mite or tick borne rickettsioses. Results of Weil Felix test along with other test like IgM antibody ELISA or immunofluorescence can improve the reliability of results barring cost per test for diagnostic purposes.

In conclusion, Weil Felix test can serve as initial but not sole method to recognize and diagnose rickettsial diseases, particularly if no rickettsioses have been previously isolated or detected in the considered area. Therefore, it is possible for most microbiology laboratories across the country to start Weil Felix test, assess the burden in their area and later on add other test like ELISA or immunofluorescence or Western blot depending upon the individual need and facilities available. Our results show that rickettsial infections are one of the important causes of FUO and active surveillance of rickettsial diseases is required to know exact magnitude and distribution of vector and diseases.

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