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LABORATORY DIAGNOSIS OF CLINICALLY SUSPECTED MEASLES AND RUBELLA CASES BY SEROLOGY (IgM ANTIBODY DETECTION BY ELISA) IN RAJASTHAN

Microbiology

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ABSTRACT

Introduction: Measles is among the foremost cause of child morbidity & mortality in India which is a vaccine-preventable, highly communicable acute febrile illness. While the Rubella virus causes a mild febrile rash disease in children, maternal infection with Rubella can have serious consequences for the developing fetus. **Objective:** To confirm the clinically suspected Measles and Rubella cases in the laboratory by detecting Immunoglobulin M (IgM) antibodies to Measles & Rubella viruses in patient's sera by ELISA and to find out the correlation of immunization status with the disease. **Material And Method:** This observational study was carried out in the WHO national Measles and Rubella Laboratory, Department of Microbiology, SMS Medical College, Jaipur, (Rajasthan). Samples from 217 suspected Measles & Rubella cases for case-based surveillance from October 2019 to September 2020 were received and tested for Measles and Rubella IgM antibodies using WHO-approved ELISA kits. **Results:** On laboratory confirmation, only 17.5% were found to be Measles positive, 10.6% were Rubella positive. Most cases were seen in the months of November to March. Most Measles & Rubella cases were reported from few selected districts. **Conclusion:** Though most of the cases are due to Measles, Rubella cases are also common in Rajasthan; hence laboratory diagnosis is essential for both Measles and Rubella especially when targeting elimination and indicates the inclusion of the Rubella vaccine is needed in the National immunization schedule.

KEYWORDS

Measles, Rubella, Surveillance, ELISA, Immunization.

INTRODUCTION

Measles is a globally occurring extremely communicable acute febrile illness characterized by fever, malaise, rash, cough, coryza, and conjunctivitis caused by Measles virus, (single-stranded, negatively sensed RNA virus) a member of the genus Morbilli-virus, family Paramyxo-viridae for which humans are the only reservoirs[1]. The major transmission of Measles is person-to-person via aerosolized droplets or by direct contact with nasal and throat secretions of infected persons. When the Measles virus infects a non-immune population, almost 100% of non-vaccinated individuals will develop clinical disease [2]. Widespread immunization activities promoting Measles vaccination had a foremost impact on reducing Measles deaths. Measles vaccination prevented approximately 20.3 million deaths, throughout 2000-2015. With a high Measles case-fatality rate (CFR) in malnourished children, India accounts for a significant proportion of Measles deaths in the world. There were 61255 cases of Measles reported from India in 2015 and therefore the respective figure from Rajasthan from an equivalent period was 1769 [3].

Rubella is an acute, contagious viral infection that usually causes a low-grade fever and rash illness in children and adults. Rubella infection during pregnancy, especially in the first trimester, can result in miscarriage, stillbirth, fetal death, or infants with congenital malformations, known as Congenital Rubella Syndrome (CRS). Ocular defects including cataracts, deafness, congenital heart disease, and developmental delay are common manifestations of CRS. The severity and nature of these defects depend upon the fetal age at the time of infection. The Rubella virus is transmitted by airborne droplets, once an infected person sneezes or coughs. Humans are the only identified host[4].

Deaths and disabilities caused by Measles and Rubella are completely preventable with safe and inexpensive vaccines and timely & accurate diagnosis & treatment. The Measles and Rubella initiatives (M & R initiative) was launched in 2001 as a global partnership programme led by the American Red Cross, United Nations Foundation, Centre for Disease Control and Prevention (CDC) UNICEF (United Nations International Children's Emergency Fund) WHO (World Health Organization). The M & R initiative is committed to ensuring that no child dies from Measles or is born with Congenital Rubella Syndrome; reducing Measles death by 95% by 2015, and achieving Measles and Rubella elimination in at least 5 WHO regions by 2020[5].

As clinical diagnosis can be unreliable, laboratory confirmation of Measles and Rubella cases becomes a vital aspect of surveillance at all stages of control programme. Detection of Measles/Rubella specific immunoglobulin M (IgM) antibodies in serum samples is the mainstay of laboratory confirmation. IgM antibodies appear in serum within the initial few days of the rash onset and decline rapidly after about one month. Their presence indicates current or recent Measles infection [6].

WHO continues trying to reinforce the worldwide laboratory network to make sure timely diagnosis of Measles and trying to track the global spread of the Measles viruses to allow a more coordinated national approach in targeting immunization activities and decrease Measles deaths from this vaccine-preventable disease[5].

This study aimed to confirm the diagnosis of clinically suspected cases of Measles and Rubella & initiate prompt treatment and avoid further complications. Accordingly, appropriate measures for immunization can be taken in the field.

MATERIAL AND METHODS:

A Descriptive type of observational study was conducted at WHO National Measles and Rubella Laboratory, Department of Microbiology in SMS Medical College, Jaipur (Rajasthan). Serum samples of 217 suspected cases of Measles and Rubella from various districts in Rajasthan from October 2019 to September 2020 collected for case-based surveillance of Measles & Rubella were studied. Samples collected from patients fulfilling Measles case definition according to WHO guidelines "any person with generalized maculopapular rash lasting 3 or more days with a temperature of 38.3°C (101°F) or higher, and cough, coryza, or conjunctivitis" [7] & with properly completed laboratory requisition form (LRF) were included and sample received in poor conditions such as visibly lysed, inadequate collection and cold chain not maintained during transport or contaminated samples were excluded in this study.

Serum samples were tested for the presence of IgM antibodies against Measles & Rubella by using ELISA kits (EUROIMMUN Anti Measles virus NP ELISA IgM Kit & EUROIMMUN Anti Rubella virus Glycoprotein ELISA IgM Kit) as approved by WHO. The test was performed as per the directions given in the kit leaflet supplied along with the kits. Samples were first tested for Measles IgM antibodies and the result equivocal or negative for Measles IgM antibodies, was further tested for Rubella IgM antibodies. Ethical

clearance was obtained from Institutional Research Review Board before initiation of the study.

RESULTS

A total of 217 serum samples were tested and basic demographic information, date of rash onset, vaccination status and date, age & sex, and IgM ELISA results were analyzed. Out of 217 samples tested, 38 (17.5%) were confirmed to be IgM positive for Measles. A total of 179 samples which were Measles IgM negative, were further tested for Rubella IgM antibodies and 19 (10.6%) were found to be positive (Table 1).

Table 1: Laboratory Confirmation Of Suspected Serum Samples

Result	Cases (n) (%)
Total sample tested for Measles IgM ELISA	217
Measles IgM positive	38 (17.5%)
Total sample tested for Rubella IgM ELISA	179
Rubella IgM positive	19 (10.6%)

Most cases of Measles and Rubella were seen in the months of November to March with the peak occurring in December & February months (Figure 1). The maximum number of samples was collected from Jaipur, Kota, Chittorgarh, and Rajsamand. The positivity for Measles was found more in samples collected from Barmer, Bharatpur, Bundi, Chittorgarh, Dausa, Ganganagar, Jaipur, Jhalawar, Kota, and Swai Madhopur districts while positivity for Rubella was found more in samples collected from Kota, Alwar, Barmer, Bharatpur, Swai Madhopur, Tonk, Udaipur, Chittorgarh, Bhilwara, and Rajsamand districts (Figure 2).

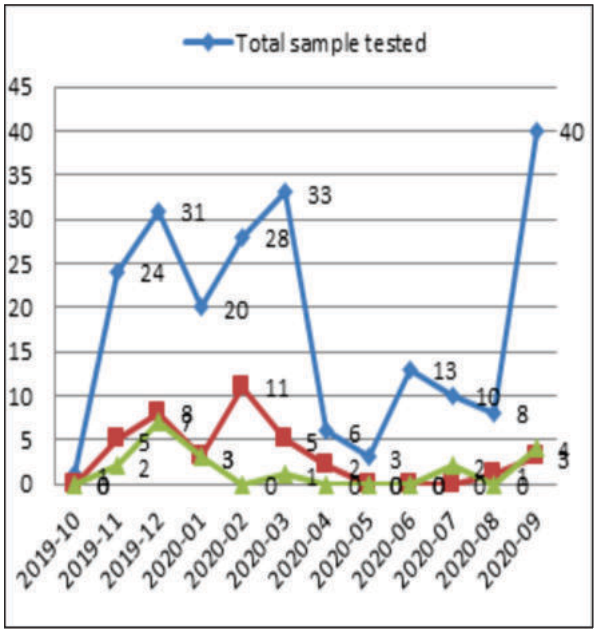

Figure 1: Time Trend Of Measles And Rubella Cases

Age of Measles cases ranged from <1 year to 14 years. 18.4% of children infected with Measles were less than 9 months of age; among them, 7.9% of children were less than 6 months of age. The majority of the affected children were below 3 years (55.3%). 94.7% of Rubella cases were in this age group, only 5.3% of Rubella cases were below 1 year age (Table 2). The male to female ratio of Measles cases was 1.4:1, 57.9% of male children and 42.1% of female children developed the disease while out of total 19 Rubella cases, 11 (57.9%) cases were male & 8 (42.1%) cases were female (Table 3).

Table 2: Age Distribution Of Measles & Rubella Cases

Age group	Measles cases		Rubella cases	
	(n=)	%	(n=)	%
<1 year	8	21.1	1	5.3
1-4 years	19	50	10	52.6
5-9 years	7	18.4	7	36.8
10-14 years	4	10.5	1	5.3
15+ years	0	0	0	0.0
Total	38	100	19	100

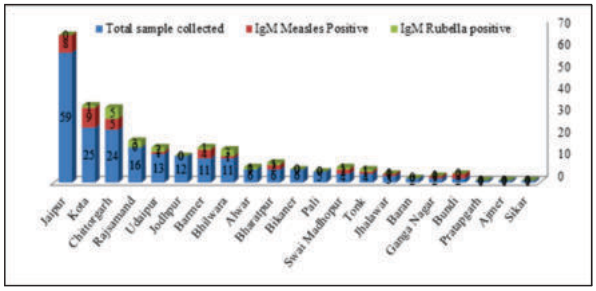

Figure 2: Geographical Distribution Of Measles And Rubella Cases

Table 3: Gender-based Distribution Of Measles & Rubella Cases

Cases	Male		Female		Total	
	(n=)	%	(n=)	%	(n=)	%
Measles	22	100	16	100	38	100
Rubella	11	100	8	100	19	100

Only 53 out of 217 suspected cases were found either not vaccinated or vaccination status not known. Out of 53 non-vaccinated cases, 15 cases were of age less than 9 months (below vaccination age). Total 164 cases were immunized. 78.7% male & 71.6% female suspected cases were immunized that shows immunization is slightly higher in males than females (Table 4).

Table 4: Vaccination Status Of Suspected Cases

Vaccination status	Total suspected cases		Male		Female	
	(n=)	%	(n=)	%	(n=)	%
Vaccinated	164	75.58	96	78.7	68	71.6
Non-vaccinated	53	24.42	26	21.3	27	28.4
Total	217	100	122	100	95	100

DISCUSSION

Measles remains a significant cause of fatality among under-five children. To a large extent of this persisting worldwide burden of Measles is to be found in Africa and Asia, notably in India. Cause-specific mortality assessment documented a 90% decline in 1–59 month Measles mortality rates in India from 2000 to 2015[8]. Deaths due to Measles are reduced from 106,000 in 2005 to 65000 in 2010 and 29336 in 2012. Still, India contributes to almost 47% of the worldwide Measles deaths, which reflects poor performance. With the highest birth cohort in the world, the maximum number of Measles deaths, and comparatively poor vaccine coverage, India poses a challenge for the Global Measles Eradication goal. The country needs sustained > 95% vaccination coverage to control Measles. A recent vaccination coverage survey in India showed overall 71% coverage for the Measles vaccine (given during 9 to 12 months of age). Accepting 85% vaccine efficacy for vaccination at 9 months, actual protection was offered to only 60% of annual birth cohorts (71% × 85% = 60%). In other words, 40% remained vulnerable to Measles. Among different states in India, there is a considerable variation in vaccination coverage. States like Kerala, Goa, Sikkim, and Punjab exhibit almost 90% coverage, whereas states like U.P., Bihar, M.P., and Rajasthan report less than 70% coverage. Changing epidemiologic trends has led to more children getting Measles less than 1 year of age as a result of low protective maternal Measles antibody titer in children[9].

According to NFHS 2015-16 survey data, only 54.8% of 12-23 months aged children of Rajasthan are fully immunized of which 60.9% were urban and 53.1% were rural. Out of total children population who were vaccinated under national immunization programme, 78.1% (86.5% urban & 75% rural) children had received Measles vaccine[10].

Misdiagnosis of Measles on a clinical basis has often been reported. The diagnosis of Measles cannot be easy, even for experienced practitioners, particularly in individuals with a pigmented skin. Several different infectious agents, including Parvovirus B19, human herpes virus type 6, Dengue virus, Epstein-Barr virus, *Mycoplasma pneumoniae*, and *Rickettsia conorii*, are known to cause symptoms that can easily be confused with Measles [11]. This is also one of the reasons why we need laboratory confirmation of clinically suspected Measles cases.

A total of 217 serum samples were tested for Measles IgM antibodies, out of which 38 (17.5%) were confirmed to be IgM positive for Measles. In another study done by Amita Raoot et al [12] from Delhi,

9.87% (132/1337) samples were laboratory-confirmed Measles. The age of the children suspected of Measles & Rubella in the present study ranged from 1 month to 14 years. 18.4% of Measles positive children were less than 9 months of age, the age at which the first dose of Measles vaccination is given in our country. Among them, 7.9% of children were less than 6 months of age, where maternal Measles antibodies are said to be protective against Measles. The majority of the affected children were below 3 years (55.3%). Around one-fifth of children are acquiring Measles before the age of vaccination. The age distribution was similar to the study done by Alphonsus N. Onyiriuka *et al* (2011)[13] in Nigeria. The male to female ratio of Measles cases was 1.4:1, 57.9% of male children and 42.1% of female children developed the disease. There was a slight male preponderance which was similar to Syed Tanwir Alam et al (2015)[14].

The present study was done over twelve months from October 2019 to September 2020. February month had the most number of Measles cases (28.94%). 84.21% of total Measles cases were between November 2019 to March 2020. Similarly, Ranjan Kumar Srivastava et al (2018)[15] reported a peak of Measles cases from January to March. Sunil R. Vaidya et al (2016)[16] reported two major seasons of Measles, *i.e.* first between January & April and second between September & December, Alphonsus N. Onyiriuka et al(2011)[13] reported a peak in the months of January to April, J.S. Thakur et al (2002)[52] found a peak was in November to February months in their studies.

India's immunization coverage still stands at 62 percent as per the National Family Health Survey 4 (2015-16). In December 2014, Mission Indradhanush was launched for a targeted approach to immunization in India, in a bid to alter the sluggish annual growth rate of 1 percent. The Government has identified 201 high focus districts across 28 states in the nation that have the maximum number of partially vaccinated and non-vaccinated children. Alwar, Barmer, Bundi, Dholpur, Jaipur, Jodhpur, Karauli, Swai Madhopur, and Tonk districts of Rajasthan were included in high focus districts [17]. To enhance the routine immunization coverage in the nation, the Ministry of Health and Family welfare introduced Intensified Mission Indradhanush 2.0 to make sure reaching the unreached with all available vaccines and hasten the immunization coverage of children and pregnant ladies in the identified districts and blocks from December 2019-March 2020. This aims to attain the Sustainable Development Goal of ending preventable child mortality by 2030. It aims at immunizing 272 districts in 27 States.[18]

In the present study, the samples were received from 24 districts of Rajasthan. The maximum numbers of the sample were collected from Jaipur, Kota, Chittorgarh, and Rajsamand. The positivity of Measles samples was found more in Kota, Jaipur, and Chittorgarh districts. Banswara, Churu, Dholpur, Hanumangarh, Jaisalmer, Jalore, Karauli, Nagaur, Sirohi, Ajmer, Baran, Bundi, Dausa, Dungarpur, Shri Ganganagar, Jhunjhunu, Pratapgarh, and Sikar districts did not report any suspected cases of Measles & Rubella or reported very few cases. It shows an awareness of surveillance activities were not sufficient in these areas and may need better surveillance activities. On the contrary, surveillance has been done more actively in Jaipur, Kota, Chittorgarh, and Rajsamand districts as maximum samples of suspected cases and maximum positive cases were from these districts. There is a large difference in immunization coverage in terms of socio-economic & demographic factors and gender. Among various socio-demographic factors, the low education status of the mother, high birth order, place of delivery, the standard of living, and media exposure are main the factors that influence immunization coverage. Between 2014 and 2018, India's annual immunization growth rate has risen to 4 percent, with an unprecedented 16 percent rise in the number of fully immunized children [18].

In the present study, only 53 out of 217 suspected cases were found either not vaccinated or vaccination status not known. Out of 53 non-vaccinated suspected cases, 15 suspected cases were of age less than 9 months (below vaccination age). Total 164 suspected cases were immunized. 78.7% male & 71.6% female suspected cases were immunized that shows immunization is slightly higher in males than females. Out of 53 non vaccinated suspected cases 13 (24.52%) developed Measles and among 164 vaccinated suspected cases 25 (15.24%) developed Measles. Vaccinated individuals were supposed to have a reduced risk of Measles infection. Vaccine efficacy was supposed to be 85% for the first dose when vaccinating before age one, 95% after age one, and 98% for two doses, as suggested by a meta-analysis. Vaccines were assumed to be "all or nothing": individuals receiving the vaccine were either fully protected or not at all [19]. Malnutrition, cold chain not maintained, SOP not followed properly, etc are the reasons due to which seroconversion does not take place or immune response develops inadequately after vaccination. 65.8% of Measles cases were vaccinated. Similarly, Muhammad Ali Raja et al (2016)[20] reported 62.5% of Measles cases were vaccinated.

A total of 179 samples which were Measles IgM negative, were further tested for Rubella IgM antibodies and 19 (10.6%) were found to be positive. Similar results were reported by Jitendra Sharma et al (2014) [21] that 11 (14.28%) out of 77 suspected cases were Rubella IgM positive. Sunil R. Vaidya et al (2016)[16] and Ranjan Kumar Srivastava et al (2018) [15] also reported that 3 (8.57%) out of 35 suspected cases & 73 (11.28%) out of 647 suspected cases were Rubella IgM positive respectively. Maximum positivity of Rubella cases was observed from November to January. Similar results were reported by Surender N. Gupta et al (2013) [22] that a mixed outbreak of Rubeola-Rubella was confirmed in District Kangra of Northern India from September 2006 to January 2007. Similar to the occurrence of Measles, Rubella too occurs in the age group of 1 to 15 years. 94.7% of Rubella cases were in this age group, only 5.3% of Rubella cases were below 1 year age. Jitendra Sharma et al (2014) [21] reported that 73% (8/11) Rubella-positive cases were children. Out of the total 19 Rubella cases, 11 (57.9%) cases were male & 8 (42.1%) cases were female. Similar results were reported by Surender N. Gupta et al (2013) [22] that males were more affected than females while Jitendra Sharma et al (2014) [21] reported that both males and females were affected equally.

In the present study, sample collection was case-based instead of outbreak-based sample collection. Clinical diagnosis of Measles in many cases can be difficult and unreliable, especially in individuals with pigmented skin. Previous studies like EL Mubarak et al [11] have reported misdiagnosis of Measles on clinical grounds. Differentiation of Rubella from Measles is also difficult on clinical grounds. In the present study, only 17.5% of suspected samples were lab-confirmed as Measles positive and 73.7% of clinically suspected samples were negative for both Measles and Rubella IgM antibodies. **Implementation of various programme like mid-day meal scheme/programme, National Immunization Plan (NIP), and Mission Indradhanush are key factors that improve vaccination coverage & nutritional status so positivity of vaccine-preventable Measles and Rubella is decreasing.**

The timely diagnosis and reporting of Measles & Rubella are the key factors in controlling outbreaks & immunization of susceptible. The data of the present study showed that 99.1% of samples were collected within 28 days of rash onset. 78.4% samples were received in the laboratory within 7 days of sample collection and test results of 96.3% samples were reported within 3 days of sample receiving.

CONCLUSION

Improved vaccination coverage, timely investigation, and application of specific control measures can control Measles & Rubella infection. By strengthening routine immunization activities and cold chain monitoring in the districts where more cases were reported, can prevent spread in susceptible communities. Special attention is required for high-risk areas like urban slums, migratory populations, minority settlements, etc. The self-help groups should be involved in motivating the communities to improve vaccination coverage. In the present study, more vaccination coverage was found in male children compared to female children, so special attention is required for those communities where there is discrimination against the girl child.

For efforts toward Measles elimination, laboratory confirmation of suspected cases becomes vital for accurate diagnosis. IgM antibody ELISA offers an accurate lab diagnosis method for Measles and Rubella surveillance. Reinforcement of case-based surveillance activity and reporting system is required especially in low reporting districts. Refresher training to the workers of the affected areas for proper cold chain maintenance, enhancement in routine immunization coverage, and inclusion of Rubella vaccine in routine immunization is required to achieve the national goal of Measles elimination and Rubella CRS control. Information, education, and communication activities should be targeted towards modifying the help-seeking behavior of the mother, especially in the Measles affected areas.

WHO recommends Measles elimination strategy "catch up, keep up

and follow up" the immunization programme;

- Catch up – This is a national campaign to vaccinate all children between the ages of 9 months and 14 years regardless of Measles history or immunization status.
- Keep up – This is the routine vaccination schedule in which the aim is to vaccinate at least 95% of children in each consecutive birth cohort.
- Follow up – It is conducted every 2 to 4 years following the catch-up phase. All children born after the catch-up phase are vaccinated in the follow-up phase.

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