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# Measles and rubella IgM positivity and case classification accuracy in Mozambique's national case-based surveillance system, 2016–2021

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## Abstract

# Introduction:

Mozambique adopted case-based surveillance for measles and rubella in 2016. This study aimed to characterise clinically suspected cases and assess serological and epidemiological data quality.

# Methods

We conducted a cross-sectional study using data from the national surveillance system, 2016–2021. Per national protocol, all clinically suspected cases (fever and generalised rash) are tested for measles and rubella IgM. Analyses used cases with valid (adequate specimen and clear result) measles IgM results (n = 12,674), rubella IgM results (n = 12,177), or both (n = 12,143). Multivariable logistic regression identified factors associated with IgM positivity.

# Results

Of 15,782 suspected cases, 12,708 (81%) had at least one valid IgM result (12,674 for measles and 12,177 for rubella). Among those tested for both (n = 12,143), IgM was positive for measles in 1,581 (13%), for rubella in 993 (8.2%), and for both in 424 (3.5%). Among those with any IgM result (n = 12,708), 4,368 (34%) had specimens collected in the optimal collection window (4–28 days for measles; 6–28 days for rubella) after rash onset, and 597 (4.8%) had received measles-containing vaccine ≤ 28 days before sample collection. Among confirmed measles cases (n = 1,697), 424 (25%) were also rubella IgM-positive; among confirmed rubella cases (n = 996), 424 (43%) were also measles IgM-positive. All provinces consistently reported cases and submitted samples. Positivity was higher in the northern and central regions versus the southern region, among those vaccinated 8–28 days before sample collection, and among those sampled in the optimal window versus ≤ 3 days after rash onset (measles: 16% vs 12%; adjusted odds ratio [AOR] = 1.2 [1.0–1.3]).

# Conclusion

The high proportion of co-positive cases, together with elevated IgM positivity among recently vaccinated individuals, may reflect vaccine-induced IgM or cross-reactive serology. IgM serology alone is insufficient for accurate case classification without integration of vaccination history and epidemiological context. Surveillance systems that achieve higher completeness of documentation, appropriate sample timing, and consistent application of classification criteria are better positioned to generate accurate incidence estimates and detect outbreaks promptly.

## What is already known on this topic

- Mozambique experiences recurrent measles and rubella outbreaks across all provinces.
- Robust case-based surveillance for measles and rubella is essential for monitoring transmission patterns and enabling timely outbreak detection and response.

**What this study adds**

- This study identifies potential misclassification of cases in Mozambique's measles and rubella surveillance system, arising from suboptimal sample collection timing, incomplete vaccination documentation, and post-vaccination IgM responses.
- Findings can inform targeted improvements to Mozambique's measles and rubella surveillance and offer transferable insights for other countries implementing similar surveillance strategies.

# Introduction

Measles and rubella are highly contagious airborne viral diseases that are preventable through vaccination.[1–4] Each year, measles is estimated to infect about 10 million people and cause more than 100,000 deaths globally.[1] Rubella, while generally milder, is an important public health concern due to its potential to cause congenital rubella syndrome, which is estimated to affect approximately 27,000 newborns globally in 2024.[5] The burden of both diseases is highest among children under five years of age and in low- and middle-income countries, particularly in Africa.[6–8]

As part of global and regional measles and rubella elimination efforts,[9] countries are recommended to implement case-based surveillance systems that ensure prompt epidemiological investigation and laboratory testing of all suspected cases.[10] Because measles and rubella share similar clinical presentations — most notably fever and a generalised maculopapular rash — many African countries have adopted a broad case definition for suspected cases: any person presenting with fever and generalised rash.[11]

A global network of measles and rubella laboratories supports the testing of suspected cases using standardised assays, reagents, and equipment to ensure accurate and timely diagnosis.[12] Serological confirmation typically involves detecting virus-specific IgM antibodies in serum specimens collected in the first 28 days after rash onset.[13] A negative IgM result may be obtained from a proportion of measles cases if serum was collected ≤ 3 days after rash onset (≤ 5 days for rubella cases).[14] Additionally, measles vaccination within 8–56 days prior to blood specimen collection can result in positive IgM results in individuals with measles vaccine-associated reaction.[15]

Mozambique adopted case-based surveillance for measles and rubella in 2016 as part of its national elimination strategy. All suspected cases are notified to the district health department and have blood samples collected for laboratory confirmation, which are sent to the National Reference Laboratory for Measles and Rubella at the National Institute of Health, located in Maputo Province.[16,17] However, the

quality and completeness of epidemiological investigation and laboratory confirmation within Mozambique's surveillance system have not been systematically evaluated at the national level, limiting confidence in the accuracy of reported case counts and incidence estimates.

This study aimed to characterise clinically suspected cases of measles and rubella reported at the national level with valid laboratory test results and assess serological and epidemiological data quality for the period 2016–2021, a period marked by several outbreaks, including a large outbreak in 2020–2021 affecting central and northern provinces.

## Methods

# Study design and population

We conducted a cross-sectional study using data from the measles and rubella surveillance electronic system of the National Institute of Health (*Instituto Nacional de Saude* – INS), maintained by the National Reference Laboratory for Measles and Rubella Serology. The study covered the period 2016–2021 and included suspected measles and rubella cases from all provinces of Mozambique recorded in the INS Measles and Rubella Surveillance Database.

## Epidemiological data

Suspected measles and rubella cases, defined as fever and generalised rash in a person, are reported by districts through the weekly epidemiological bulletin or via rapid communication channels. Healthcare workers complete a case investigation form and collect a blood specimen. Per national surveillance protocol, specimens are collected from all clinically suspected cases during both outbreak and inter-outbreak periods. Measles and rubella testing is not conducted at the health facility level. Serum specimens are prepared and sent in cold chain together with the case investigation forms to the District Health Directorate which then sends these to the Provincial Health Directorate for quality verification before being sent to the National Reference Laboratory.

## Laboratory data

At the National Reference Laboratory, sample quality is assessed before IgM ELISA serological testing. Testing was performed using commercial diagnostic kits Enzygnost® Anti-Measles Virus IgM (Siemens) and Enzygnost® Anti-Rubella Virus IgM ELISA until 2018, and Euroimmun Anti-Measles IgM ELISA and Euroimmun Anti-Rubella Glycoprotein ELISA (IgM) from 2019 onwards. The laboratory is accredited under ISO 15189:2014 by the Portuguese Accreditation Institute and is WHO-certified for measles diagnosis.

## Data management

We included all cases with blood samples collected for serological testing from 2016–2021, excluding those with inadequate or untested samples. We analysed the completeness of data on age, sex, province, year, date of rash onset, date of vaccination, and date of sample collection. The surveillance form did not include a separate field for vaccination status; a missing vaccination date cannot therefore distinguish between an unvaccinated individual and one who was vaccinated but whose date was unknown or not recorded. Individuals with missing vaccination dates were not assumed to be unvaccinated and were retained as a distinct category in all analyses.

Three derived variables were calculated: time from rash onset to sample collection; time from vaccination to sample collection; and time from vaccination to rash onset.

# Data analysis

Data analysis was performed using R v4.5.0. Descriptive statistics were presented as absolute and relative frequencies. Annual incidence rates for IgM-positive and suspected cases were calculated by sex, age, year, province, and district, using age- and year-specific national census projections from the *Instituto Nacional de Estatística* (INE), expressed as cases per 1,000,000 person-years.

Separate multivariable logistic regression models were used to estimate crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for measles IgM positivity and rubella IgM positivity. The binary outcome was defined as IgM positive versus negative or indeterminate; indeterminate results were grouped with negatives as they do not meet the threshold for confirmed positivity. Covariates included sex (reference: female), age group (reference: <1 year), geographic region (reference: southern region; provinces were aggregated into three regions: southern [Maputo City, Maputo Province, Gaza, Inhambane], central [Sofala, Manica, Tete, Zambezia], and northern [Nampula, Niassa, Cabo Delgado], consistent with Mozambique's administrative health classification), interval from vaccination to sample collection (reference: >28 days), and interval from rash onset to sample collection (reference: ≤3 days). Missing values for all covariates were coded as a separate indicator category and included in the model, so that no records were excluded due to missing covariate values. Because the objective was to characterise factors associated with IgM positivity, including potential sources of misclassification, rather than to estimate causal disease risk, all covariates were retained in the multivariable model regardless of statistical significance. As region and province are hierarchically nested, province could not be simultaneously included as a random intercept and region as a fixed effect without inducing collinearity; geographic variation was therefore accounted for by including region as a fixed effect in the multivariable model. All p-values were two-sided, with a significance threshold of $p < 0.05$.

# Ethical considerations

A confidentiality agreement was signed and authorised by the case-based measles surveillance coordinator, and data were anonymised to ensure confidentiality. This activity was reviewed by CDC, deemed not research, and was conducted consistent with applicable U.S. law and CDC policy and strictly complied with Decree Nº. 53/2024 (Regulation of the Law on Human Health Research of Mozambique).

Consent to Participate declaration: not applicable. The protocol was approved by the Institutional Bioethics Committee in Health of the Faculty of Medicine/Central Hospital of Maputo (reference CIBSFM&HCM/50/2025).

# Results

A total of 15,782 suspected cases were initially identified. Of these, 2,908 (18%) cases were excluded due to missing both measles and rubella IgM test results (Fig. 1). An additional 116 lacked the date of sample collection and 50 had a missing age, resulting in 12,708 cases (81%) with at least one valid measles or rubella IgM result. Within this group, 34 cases (0.3%) were missing measles IgM results and 531 (4.2%) were missing rubella IgM results. Consequently, 12,674 cases (80% of all suspected) with a valid measles IgM result were included in the measles analysis, 12,177 (77%) with a valid rubella IgM result in the rubella analysis, and 12,143 (77%) with valid results for both tests in the joint analysis. Among the 12,143 suspected cases with valid results for both tests, 1,581 (13%) were positive for measles, 993 (8.2%) for rubella, and 424 (3.5%) for both (Table 1).

Table 1

**Cross-tabulation of measles and rubella IgM test results among suspected cases with valid results for both tests (n = 12,143), Mozambique, 2016–2021**

| | | **Overall** | **Measles IgM** | | |
|---|---|---|---|---|---|
| | | ***n*** **(%[a])** | | | |
| | | | **Negative** | **Positive** | **Indeterminate** |
| | | | **n (%[a])** | **n (%[a])** | **n (%[a])** |
| Overall | | 12,143 (100) | 10,381 (85) | **1,581 (13)** | 181 (1.5) |
| Rubella IgM | Negative | 10,531 (87) | 9,348 (77) | 1,030 (8.5) | 153 (1.3) |
| | **Positive** | **993 (8.2)** | 554 (4.6) | **424 (3.5)** | 15 (0.1) |
| | Indeterminate | 619 (5.1) | 479 (3.9) | 127 (1.0) | 13 (0.1) |

[a] All percentages are column percentages calculated from n = 12,143 (cases with valid IgM results for both tests).

**Bold values** denote cells where both measles and rubella IgM are positive, representing potential co-positivity of interest.

IgM, immunoglobulin M.

# Description of cases

Among all suspected cases with a valid measles IgM result (n = 12,674), 1,697 (13%) tested IgM positive for measles; among those with a valid rubella IgM result (n = 12,177), 996 (8.2%) were positive for rubella (Table 2). The majority of suspected cases with at least one valid measles or rubella IgM result were in children under five years of age (43% aged 1–4 years; 22% under 1 year) and 89% were in children under 10 years of age. Most suspect cases were reported in 2021 (28%), with the highest proportions from the central (43%) and southern (24%) regions.

All provinces consistently reported suspected cases and submitted samples each quarter (Supplementary Fig. 1), though notable differences in case counts and IgM positivity were observed across provinces and years. Maputo Province reported the highest number of suspected cases but low positivity for both diseases. Suspected cases increased in provinces located in the central and northern regions from mid-2020 through 2021, most pronounced in Zambezia Province, which also had the highest IgM positivity rate during this period. Confirmed measles cases were relatively rare from 2016 through 2019, with a marked increase from mid-2020, consistent with outbreaks occurring in the central and northern regions.

Regarding completeness of investigation forms, 3.5% were missing sex, 60% lacked vaccination dates, and 0.8% were missing symptom onset dates (Table 2). Among the 12,708 tested cases, 7,923 (62%) had samples collected within three days of symptom onset; 1.0% had been vaccinated within 14 days of symptom onset; and 4.7% had received a measles and rubella-containing vaccine within 28 days of sample collection.

Among IgM-positive measles cases (n = 1,697), 25% were also rubella IgM-positive. The majority were from samples collected in 2021 (57%) and from provinces in the central and northern regions (58% and 34%, respectively). Additionally, 2.7% had developed symptoms > 28 days before sample collection, 75% were missing vaccination status or onset dates, and 4.1% had been vaccinated within 28 days before sample collection. All variables significantly differed by measles IgM test result status.

Among IgM-positive rubella cases (n = 996), 43% were also measles IgM-positive. The majority (38%) were from 2021 and from the Central and Southern regions (46% and 27%, respectively). Measles IgM result, sex, age, year, and interval from vaccination to sample collection differed significantly by rubella IgM test result.

Table 2
Characteristics of suspected cases (n = 12,708), measles IgM-positive cases (n = 1,697), and rubella IgM-positive cases (n = 996), Mozambique, 2016–2021

| **Characteristic** | **Suspected cases tested (*n* = 12,708) *n* (%[a])** | **Measles IgM positive (*n* = 1,697 of 12,674 tested) *n* (%[a])** | ***P*** | **Rubella IgM positive (*n* = 996 of 12,177 tested) *n* (%[a])** | ***P*** |
|---|---|---|---|---|---|
| **Measles IgM** | | | | | < 0.01 |
| Negative | 10,784 (85) | — | | 554 (56) | |
| **Positiveb** | **1,697 (13)** | — | | **424 (43)** | |
| Indeterminate | 193 (1.5) | — | | 15 (1.5) | |
| Result missing | 34 (0.3) | — | | 3 (0.3) | |
| **Rubella IgM** | | | < 0.01 | | |
| Negative | 10,560 (83) | 1,030 (61) | | — | |
| **Positiveb** | **996 (7.8)** | **424 (25)** | | — | |
| Indeterminate | 621 (4.9) | 127 (7.5) | | — | |
| Result missing | 531 (4.2) | 116 (6.8) | | — | |
| **Sex** | | | < 0.01 | | 0.2 |
| Female | 5,738 (45) | 812 (48) | | 457 (46) | |
| Male | 6,531 (51) | 794 (47) | | 495 (50) | |
| Missing | 439 (3.5) | 91 (5.4) | | 44 (4.4) | |
| **Age group, years** | | | < 0.01 | | < 0.01 |
| < 1 | 2,790 (22) | 89 (5.2) | | 49 (4.9) | |
| 1–4 | 5,424 (43) | 674 (40) | | 472 (47) | |
| 5–9 | 3,036 (24) | 534 (31) | | 294 (30) | |
| 10–14 | 931 (7.4) | 196 (12) | | 94 (9.7) | |
| 15–19 | 218 (1.7) | 46 (2.8) | | 22 (2.3) | |
| ≥ 20 | 347 (2.8) | 108 (6.6) | | 38 (3.9) | |

| Characteristic | Suspected cases tested (*n* = 12,708) *n* (%[a]) | Measles IgM positive (*n* = 1,697 of 12,674 tested) *n* (%[a]) | *P* | Rubella IgM positive (*n* = 996 of 12,177 tested) *n* (%[a]) | *P* |
|---|---|---|---|---|---|
| **Year of sample collection** | | | < 0.01 | | < 0.01 |
| 2016 | 1,503 (12) | 22 (1.3) | | 109 (11) | |
| 2017 | 1,559 (12) | 29 (1.7) | | 65 (6.5) | |
| 2018 | 1,769 (14) | 30 (1.8) | | 188 (19) | |
| 2019 | 2,125 (17) | 91 (5.4) | | 105 (11) | |
| 2020 | 2,208 (17) | 565 (33) | | 155 (16) | |
| 2021 | 3,544 (28) | 960 (57) | | 374 (38) | |
| **Region** | | | < 0.01 | | < 0.01 |
| South | 4,194 (33) | 130 (7.7) | | 272 (27) | |
| Centre | 5,470 (43) | 984 (58) | | 462 (46) | |
| North | 3,044 (24) | 583 (34) | | 262 (26) | |
| **Interval from vaccination to symptom onset** | | | < 0.01 | | 0.3 |
| > 14 days before onset | 4,520 (36) | 370 (22) | | 335 (34) | |
| **≤ 14 days before onsetb** | **127 (1.0)** | **16 (0.9)** | | **9 (0.9)** | |
| Vaccination after onset | 464 (3.7) | 42 (2.5) | | 29 (2.9) | |
| Vaccination or onset date missingc | 7,597 (60) | 1,269 (75) | | 623 (63) | |
| **Interval from vaccination to sample collection** | | | < 0.01 | | < 0.01 |
| > 28 days | 4,457 (35) | 356 (21) | | 318 (32) | |
| **8–28 daysb** | **204 (1.6)** | **35 (2.1)** | | **34 (3.4)** | |
| **≤ 7 daysb** | **393 (3.1)** | **34 (2.0)** | | **18 (1.8)** | |
| Vaccination date missingc | 7,654 (60) | 1,272 (75) | | 626 (63) | |
| **Interval from rash onset to sample collection** | | | < 0.01 | | 0.06 |

| Characteristic | Suspected cases tested (*n* = 12,708) *n* (%[a]) | Measles IgM positive (*n* = 1,697 of 12,674 tested) *n* (%[a]) | *P* | Rubella IgM positive (*n* = 996 of 12,177 tested) *n* (%[a]) | *P* |
|---|---|---|---|---|---|
| **≤ 3 days (measles) / ≤5 days (rubella)b,d** | **7,923 (62)** | **953 (56)** | | **786 (79)** | |
| 4–28 days (measles) / 6–28 days (rubella)d | 4,368 (34) | 684 (40) | | 178 (18) | |
| > 28 days | 311 (2.4) | 46 (2.7) | | 18 (1.8) | |
| Onset date missing | 106 (0.8) | 14 (0.8) | | 14 (1.4) | |

[a] Column percentages. Denominators differ between columns due to missing IgM results (34 cases missing measles IgM; 531 missing rubella IgM). For proportions IgM-positive within each characteristic category, see Table 3.

[b] **Bold values** denote categories associated with potential IgM misclassification: co-positivity for both diseases (IgM result blocks); vaccine-induced IgM responses (recent vaccination windows); or increased probability of false-negative results due to early sample collection.

[c] Vaccination date missing; vaccination status unknown. Individuals with a missing vaccination date are not assumed to be unvaccinated (see Methods).

[d] Disease-specific early-window thresholds applied in analysis: ≤3 days after rash onset for measles; ≤5 days for rubella. Optimal collection window: 4–28 days for measles; 6–28 days for rubella. Row labels reflect the measles threshold; rubella analysis used disease-specific cutpoints.

IgM, immunoglobulin M. *P*-values from $\chi^2$ test.

# Factors associated with measles IgM positivity

Among participants tested for measles IgM (n = 12,674), positivity was higher among those aged ≥ 10 years compared to those under 1 year (24% vs 15%; 2.2 [1.7–2.9], respectively) and from the northern and central versus southern regions (19% and 18% vs 3.1%; AOR = 8.1 [6.6–9.9] and 7.0 [5.8–8.4], respectively) (Table 3). Positivity was highest among those who had received measles-rubella (MR) vaccine 8–28 days before sample collection and those missing vaccination dates (17% for both groups compared with 8.0% for those vaccinated > 28 days; AOR = 2.2 [1.5–3.3] and 1.6 [1.4–1.9], respectively). Compared with those whose sample was collected ≤ 3 days from rash onset, those with samples collected 4–28 days after rash onset had increased odds of testing positive (16% vs 12%; AOR = 1.2 [1.0–1.3]).

# Factors associated with rubella IgM positivity

Among participants tested for rubella IgM (n = 12,177), positivity was also higher among those aged ≥ 10 years compared with those under 1 year (11% vs 8.5%; AOR = 1.4 [1.0–2.0]) and from the northern and central versus southern regions (9.1% and 8.8% vs 6.7%; AOR = 1.4 [1.2–1.7] and 1.3 [1.1–1.5], respectively). Rubella positivity was highest among those vaccinated 8–28 days before sample collection compared with those vaccinated > 28 days (18% vs 7.5%; AOR = 2.8 [1.9–4.1]). Positivity was 14% among those missing onset date, compared with 8.3% among those whose onset was within 5 days of sample collection (AOR = 1.9 [1.0–3.2]).

Table 3
Factors associated with measles IgM and rubella IgM positivity among suspected cases, Mozambique, 2016–2021

| Characteristic | Measles IgM (*n* = 12,674) | | | | Rubella IgM (*n* = 12,177) | | | |
|---|---|---|---|---|---|---|---|---|
| | n (% positive[a]) | OR (95% CI) | AOR (95% CI) | P | n (% positive[a]) | OR (95% CI) | AOR (95% CI) | P |
| **Sex** | | | | | | | | |
| Female (ref) | 812 (14) | *1.00* | *1.00* | | 457 (8.3) | *1.00* | *1.00* | |
| Male | 794 (12) | 0.8 (0.8–0.9) | 0.9 (0.8–1.0) | 0.05 | 495 (7.9) | 1.0 (0.8–1.1) | 1.0 (0.9–1.1) | 0.7 |
| Missing | 91 (21) | 1.6 (1.2–2.0) | 1.5 (1.1–1.9) | < 0.01 | 44 (10) | 1.3 (0.9–1.7) | 1.3 (0.9–1.7) | 0.2 |
| **Age group, years** | | | | | | | | |
| < 1 (ref) | 89 (15) | *1.00* | *1.00* | | 49 (8.5) | *1.00* | *1.00* | |
| 1–4 | 674 (9.7) | 0.6 (0.5–0.8) | 0.8 (0.7–1.1) | 0.2 | 472 (7.1) | 0.8 (0.6–1.1) | 0.8 (0.6–1.1) | 0.2 |
| 5–9 | 534 (16) | 1.1 (0.8–1.4) | 1.6 (1.3–2.1) | < 0.01 | 294 (8.9) | 1.0 (0.8–1.5) | 1.1 (0.8–1.6) | 0.5 |
| ≥ 10 | 350 (24) | 1.8 (1.4–2.3) | 2.2 (1.7–2.9) | < 0.01 | 181 (11) | 1.4 (1.0–1.9) | 1.4 (1.0–2.0) | 0.03 |
| **Region** | | | | | | | | |
| South (ref) | 130 (3.1) | *1.00* | *1.00* | | 272 (6.7) | *1.00* | *1.00* | |
| Centre | 984 (18) | 6.9 (5.7–8.3) | 7.0 (5.8–8.4) | < 0.01 | 462 (8.8) | 1.3 (1.1–1.6) | 1.4 (1.2–1.6) | < 0.01 |
| North | 583 (19) | 7.5 (6.2–9.1) | 8.1 (6.6–9.9) | < 0.01 | 262 (9.1) | 1.4 (1.2–1.7) | 1.4 (1.2–1.7) | < 0.01 |
| **Interval from vaccination to sample collection** | | | | | | | | |
| > 28 days (ref) | 356 (8.0) | *1.00* | *1.00* | | 318 (7.5) | *1.00* | *1.00* | |

| Characteristic | Measles IgM (*n* = 12,674) | | | | Rubella IgM (*n* = 12,177) | | | |
|---|---|---|---|---|---|---|---|---|
| | n (% positive[a]) | OR (95% CI) | AOR (95% CI) | P | n (% positive[a]) | OR (95% CI) | AOR (95% CI) | P |
| 8–28 days | 35 (17) | 2.4 (1.6–3.4) | 2.2 (1.5–3.3) | < 0.01 | 34 (18) | 2.7 (1.8–4.0) | 2.8 (1.9–4.1) | < 0.01 |
| ≤ 7 days | 34 (8.7) | 1.1 (0.7–1.6) | 1.0 (0.7–1.4) | 0.9 | 18 (4.9) | 0.6 (0.4–1.0) | 0.6 (0.4–1.0) | 0.05 |
| Vaccination date missing[b] | 1,272 (17) | 2.3 (2.0–2.6) | 1.6 (1.4–1.9) | < 0.01 | 626 (8.5) | 1.1 (1.0–1.3) | 1.0 (0.8–1.1) | 0.6 |
| **Interval from rash onset to sample collection** | | | | | | | | |
| ≤ 3 days / ≤5 days (ref)[c] | 953 (12) | *1.00* | *1.00* | | 786 (8.3) | *1.00* | *1.00* | |
| 4–28 days / 6–28 days[c] | 684 (16) | 1.4 (1.2–1.5) | 1.2 (1.0–1.3) | < 0.01 | 178 (7.8) | 0.9 (0.8–1.1) | 0.9 (0.8–1.1) | 0.3 |
| > 28 days | 46 (15) | 1.3 (0.9–1.7) | 1.0 (0.7–1.4) | 0.9 | 18 (5.9) | 0.7 (0.4–1.1) | 0.6 (0.4–1.0) | 0.09 |
| Onset date missing | 14 (13) | 1.1 (0.6–1.9) | 1.0 (0.5–1.7) | > 0.9 | 14 (14) | 1.8 (1.0–3.1) | 1.9 (1.0–3.2) | 0.03 |

[a] % positivity among suspected cases tested within each analysis (n = 12,674 for measles; n = 12,177 for rubella).

[b] Vaccination date missing; vaccination status unknown. Not assumed to be unvaccinated (see Methods).

[c] Disease-specific thresholds: reference category is ≤ 3 days for measles and ≤ 5 days for rubella; optimal window is 4–28 days for measles and 6–28 days for rubella.

AOR, adjusted odds ratio; CI, confidence interval; IgM, immunoglobulin M; OR, odds ratio; ref, reference category.

*P*-values for fixed effects are from two-sided Wald z-tests; Age categories 10–14, 15–19, and ≥ 20 years were collapsed into a single ≥ 10 years category due to sparse counts in older age groups and similar

odds ratios across these categories, which did not warrant separate reporting; missing values are retained as their own category and included in analysis.

# Incidence rates

For IgM-positive measles cases, the highest incidence occurred among children aged 1–4 years (incidence rate [IR] = 27.8; 95% CI: 25.7–29.9 per 1,000,000 person-years), while the lowest was among individuals aged ≥ 10 years (IR = 2.9; 95% CI: 2.6–3.2 ) (Table 4). Regionally, the central region had the highest incidence (IR = 11.6; 95% CI: 10.9–12.3) and the southern region the lowest (IR = 3.2; 95% CI: 2.7–3.8). Zambezia Province reported the highest provincial incidence (IR = 21.2; 95% CI: 19.7–22.7) and Maputo City the lowest (IR = 1.3; 95% CI: 0.6–2.2). The highest annual incidence was observed in 2021 (IR = 29.6; 95% CI: 27.8–31.5) and the lowest in 2016 (IR = 0.7; 95% CI: 0.4–1.0).

For IgM-positive rubella cases, the highest incidence was also among children aged 1–4 years (IR = 19.5; 95% CI: 17.7–21.2) and the lowest among individuals aged ≥ 10 years (IR = 1.3; 95% CI: 1.1–1.5). Regionally, the southern region had the highest incidence (IR = 6.8; 95% CI: 6.0–7.6) and the northern region the lowest (IR = 3.8; 95% CI: 3.3–4.2). Niassa had the highest provincial incidence (IR = 10.6; 95% CI: 8.9–12.4) and Nampula the lowest (IR = 2.0; 95% CI: 1.6–2.5). The highest annual incidence occurred in 2021 (IR = 11.5; 95% CI: 10.4–12.7) and the lowest in 2016 (IR = 2.0; 95% CI: 1.5–2.5).

Nearly all districts reported at least one suspected measles case annually, with samples submitted for laboratory confirmation (Supplementary Fig. 2). The annual incidence of suspected cases was highest in southern provinces, exceeding 301 cases per 1,000,000 population in four southern districts and one northern district. IgM-positive rubella incidence was also highest in the south, though elevated rates appeared across the country without a discernible clustering pattern. IgM-positive measles incidence was highly clustered in central and northern regions, surpassing 30 cases per 1,000,000 population in 10 districts.

Table 4
Incidence of IgM-positive measles and rubella cases per 1,000,000 person-years, Mozambique, 2016–2021

| Variable | Measles IgM positive, *n* | IR | 95% CI | Rubella IgM positive, *n* | IR | 95% CI |
|---|---|---|---|---|---|---|
| Age group, years | | | | | | |
| < 1 | 89 | 13.3 | 10.6–16.1 | 49 | 7.3 | 5.4–9.4 |
| 1–4 | 674 | 27.8 | 25.7–29.9 | 472 | 19.5 | 17.7–21.2 |
| 5–9 | 534 | 19.6 | 18.0–21.3 | 294 | 10.8 | 9.6–12.0 |
| ≥ 10 | 400 | 2.9 | 2.6–3.2 | 181 | 1.3 | 1.1–1.5 |
| Region | | | | | | |
| South | 130 | 3.2 | 2.7–3.8 | 272 | 6.8 | 6.0–7.6 |
| Centre | 984 | 11.6 | 10.9–12.3 | 462 | 5.4 | 4.9–5.9 |
| North | 583 | 8.4 | 7.7–9.1 | 262 | 3.8 | 3.3–4.2 |
| Province | | | | | | |
| Zambezia (C) | 762 | 21.2 | 19.7–22.7 | 258 | 7.2 | 6.3–8.1 |
| Niassa (N) | 252 | 19.1 | 16.7–21.5 | 140 | 10.6 | 8.9–12.4 |
| Cabo Delgado (N) | 155 | 9.4 | 8.0–10.9 | 41 | 2.5 | 1.8–3.3 |
| Manica (C) | 69 | 5.0 | 3.8–6.2 | 71 | 5.1 | 4.0–6.4 |
| Maputo Province (S) | 72 | 4.8 | 3.8–6.0 | 125 | 8.4 | 7.0–9.9 |
| Sofala (C) | 76 | 4.7 | 3.7–5.9 | 75 | 4.7 | 3.7–5.7 |
| Nampula (N) | 176 | 4.4 | 3.8–5.1 | 81 | 2.0 | 1.6–2.5 |
| Tete (C) | 77 | 4.0 | 3.2–5.0 | 58 | 3.0 | 2.3–3.8 |
| Inhambane (C) | 29 | 3.1 | 2.0–4.2 | 68 | 7.2 | 5.5–9.0 |
| Gaza (S) | 20 | 2.3 | 1.4–3.3 | 39 | 4.4 | 3.0–5.9 |
| Maputo City (S) | 9 | 1.3 | 0.6–2.2 | 40 | 5.9 | 4.1–7.8 |

| Variable | Measles IgM positive, *n* | IR | 95% CI | Rubella IgM positive, *n* | IR | 95% CI |
|---|---|---|---|---|---|---|
| Year of sample collection | | | | | | |
| 2021 | 960 | 29.6 | 27.8–31.5 | 374 | 11.5 | 10.4–12.7 |
| 2020 | 565 | 17.4 | 16.0–18.9 | 155 | 4.8 | 4.0–5.6 |
| 2019 | 91 | 2.8 | 2.3–3.4 | 105 | 3.2 | 2.6–3.9 |
| 2018 | 30 | 0.9 | 0.6–1.3 | 188 | 5.8 | 5.0–6.6 |
| 2017 | 29 | 0.9 | 0.6–1.2 | 65 | 2.0 | 1.5–2.5 |
| 2016 | 22 | 0.7 | 0.4–1.0 | 109 | 3.4 | 2.7–4.0 |

C, central region; CI, confidence interval; IgM, immunoglobulin M; IR, incidence rate per 1,000,000 person-years; N, northern region; S, southern region.

## Discussion

Case-based measles and rubella surveillance aims to monitor transmission patterns and rapidly detect outbreaks to support timely prevention and control measures. In this study, we described suspected measles and rubella cases using data reported through Mozambique's national surveillance system. IgM positivity was highest in 2021 and in the central and northern provinces; positivity rates were highest among persons aged ≥ 10 years, whereas the highest incidence of confirmed cases was observed among children aged 1–4 years. All provinces consistently reported suspected cases and submitted samples for laboratory confirmation. However, findings suggest potential misclassification, both underestimation and overestimation, of measles and rubella cases, highlighting opportunities to strengthen case investigation, sample collection, and laboratory practice.

Although the national testing algorithm requires all suspected cases to be tested for both measles and rubella IgM, only three in four suspected cases had results for both tests. Among suspected cases with at least one valid result, three in five lacked information on vaccination history, a key variable for accurate case classification.

IgM testing, while central to measles and rubella surveillance, can be an imperfect indicator of true infection when used without additional epidemiological data. Samples collected ≤ 3 days after rash onset for measles, or ≤ 5 days for rubella, are more likely to produce false-negative results.[13] In our analysis, only one in three samples was collected within the optimal window (4–28 days for measles; 6–28 days for rubella); as expected, these samples had higher odds of testing positive than those collected earlier or later. This pattern aligns with evidence that measles-specific IgM typically rises around day 4 after rash onset, rubella IgM around day 6, and both decline after approximately day 28.[14] These patterns

suggest that incidence estimates may be underestimated due to suboptimal collection timing. Where program capacity allows, obtaining a second sample from suspected cases with a negative result on an initial sample collected within the early window (≤ 3 days for measles; ≤5 days for rubella), or conducting PCR testing on nasopharyngeal or throat swabs for selected early-negative or outbreak-linked cases, may improve the sensitivity of case ascertainment.

Post-vaccination IgM responses also complicate interpretation. Measles IgM positivity following vaccination has been documented to rise from 2% at one week to 62% at two weeks post-vaccination[18], and to decrease from 85.8% at one month to 16.7% at six months post-vaccination.[19] In this dataset, the interval from vaccination to sample collection was used as a proxy for vaccination recency because rash-onset dates were missing for the majority of records with available vaccination dates; this differs from the vaccination-to-rash-onset interval used in WHO case definitions, and estimates of vaccine-induced positivity should be interpreted with this in mind. Nearly 5% of suspected cases had been vaccinated within 28 days before sample collection; these individuals had twice the odds of testing IgM-positive. The highest post-vaccination IgM rates occurred in the 8–28-day window (17% for measles; 18% for rubella), compared with 8.0% and 7.5%, respectively, among those vaccinated > 28 days before sample collection. Additionally, 25% of measles IgM-positive cases were also rubella IgM-positive, and 43% of rubella IgM-positive cases were also measles IgM-positive. Given the low likelihood of concurrent active infections, these findings may reflect vaccine-induced IgM or nonspecific IgM reactivity. The lower adjusted odds of measles IgM positivity among children aged 1–4 years compared with infants under 1 year may reflect higher vaccine coverage in this age group following the first measles dose administered at 9 months in Mozambique.

The diagnostic kit used for IgM testing may also contribute to observed co-positivity. Differences between assay platforms in specificity under tropical conditions have been previously noted, and immune activation during acute measles or rubella infection can trigger non-specific IgM responses detectable by heterologous assays.[20] Molecular confirmation via PCR would optimally distinguish true co-infections from cross-reactive results; however, specimens suitable for PCR testing were not routinely collected from suspected cases during the study period.

Nonspecific IgM reactions represent another interpretive challenge.[21] Conditions such as elevated rheumatoid factor levels can lead to false-positive results.[22] Geospatial patterns in our study — particularly isolated IgM-positive cases in non-outbreak years — are consistent with this interpretation. IgM positivity rates in surveillance data reflect not only true infection but also vaccine-induced IgM responses, non-specific or cross-reactive serology, and the proportion of suspected cases attributable to other rash-fever illnesses. Provinces and time periods with low IgM positivity may reflect non-outbreak conditions or high rates of competing aetiologies rather than true low disease incidence.

Accurate case classification requires integration of laboratory results with epidemiological information. Per WHO guidelines and Mozambique's national surveillance protocol,[13,17] a case may be classified as: laboratory-confirmed (IgM-positive in the absence of recent vaccination or in the presence of a

consistent epidemiological context); epidemiologically linked (meeting the clinical case definition with a documented link to a laboratory-confirmed case); or discarded (IgM-negative with no epidemiological link). Recently vaccinated individuals who test IgM-positive but lack an epidemiological link to a confirmed case benefit from further assessment for vaccine-induced IgM before confirmation. Surveillance systems where case investigation forms more completely capture vaccination dates, symptom onset, and epidemiological linkage are better positioned to apply these classification criteria accurately; facility-level investment in documentation quality is associated with more accurate case ascertainment.

Geographic patterns have implications for surveillance. Southern provinces, particularly Maputo Province, reported high numbers of suspected cases but low measles IgM positivity. This pattern may partly reflect the central laboratory's location in the south of the country; evidence from Mozambique suggests that health facilities farther from a central laboratory tend to face greater financial, transport, and logistical barriers to consistent sample submission,[23] though the specific contribution of distance to the observed north–south differential in positivity rates could not be directly quantified in this study. Facilities that receive targeted logistical and financial support may benefit from improved capacity to submit samples consistently, with downstream gains in surveillance coverage and data quality; operational assessment of specific barriers would support the prioritisation of such investments.

This study has several limitations. Not all individuals with suspected measles seek healthcare, meaning some cases may have been missed — particularly in remote or resource-limited settings, or in highly vaccinated populations where breakthrough infections cause mild symptoms.[24] Surveillance performance varies across provinces, particularly in districts with fewer resources and geopolitical challenges in northern Mozambique. The change in diagnostic assay platform in 2019 (from Siemens to Euroimmun kits) may have introduced temporal variation in sensitivity or specificity that cannot be fully disentangled from true epidemiological trends across the study period. The surveillance database did not include variables for epidemiological linkage to confirmed cases, precluding application of standard WHO case classification criteria. Missing vaccination status for approximately 60% of records may not be random across provinces or facility types, potentially introducing differential bias into regression estimates. The regression models did not account for potential clustering by district, province, or outbreak period; confidence intervals may therefore be underestimated, and associations may partly reflect outbreak dynamics rather than individual-level factors. COVID-19-related disruptions to health services during 2020–2021 may have affected surveillance activities in ways that cannot be quantified from available data.[25] Finally, without PCR confirmation, it was not possible to distinguish true measles or rubella infection from vaccine-induced IgM or cross-reactive serology, particularly for cases co-positive for both diseases.

# Conclusions

Mozambique's national measles and rubella surveillance system receives reports of suspected cases from all provinces; however, a meaningful proportion of IgM-positive cases may be misclassified. IgM

serology alone, without integration of vaccination history and epidemiological linkage, is insufficient for accurate case classification. Surveillance systems that achieve higher completeness of vaccination and symptom-onset documentation, appropriate sample collection timing, and consistent application of WHO case classification criteria are better positioned to generate accurate incidence estimates and detect outbreaks promptly. Addressing these system-level gaps offers substantial opportunity to improve the quality and utility of measles and rubella surveillance in Mozambique.

# Declarations

### Competing interests

The authors declare no competing interests.

**CDC disclaimer:** *The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the funding agencies.*

### Generative AI Disclosure

The authors used Claude (Anthropic, version Claude Sonnet 4.6) to assist with manuscript editing and revision, including reviewing and rephrasing text for clarity, internal consistency, and alignment with reviewer comments. All substantive intellectual content, analytical decisions, and interpretations are solely the work of the authors. The authors reviewed and take full responsibility for all AI-assisted edits incorporated into the final manuscript.

### Availability of data

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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## Figures

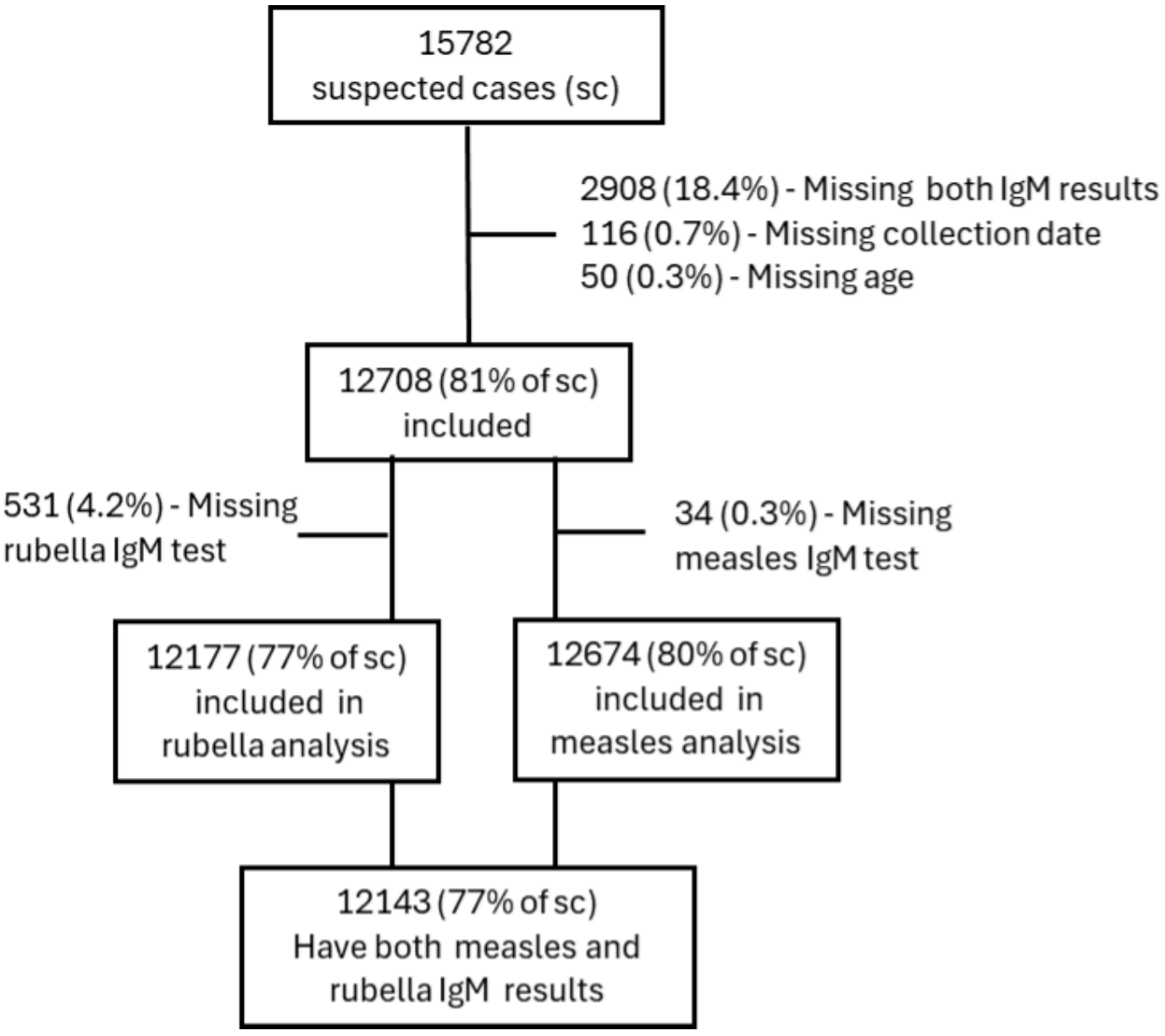


**Figure 1**

**Inclusion of measles and rubella suspected cases, Mozambique, 2016–2021**

# Supplementary Files

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