

Reviewer 1

Dear Reviewer,

Thank you for your valuable suggestions and for the time you dedicated to evaluating our manuscript. Your comments have been extremely helpful in improving the quality and clarity of our work.

Below we provide a point-by-point response to the comments raised.

R1: The most substantive methodological concern relates to the construction of age-specific incidence rates. Throughout the manuscript, incidence is calculated using the total resident population as denominator without adjustment for vaccination status or underlying susceptibility. This approach implicitly treats the entire population, including individuals who are vaccinated and therefore largely protected against infection, as uniformly at risk. In the context of pertussis, where primary vaccination coverage in infants is relatively high and booster coverage among adolescents is substantially lower (reported as approximately 75–79% for 16-year-olds in Tuscany), this assumption is not epidemiologically neutral. It means that the denominators for different age strata contain markedly different proportions of susceptible individuals, rendering direct comparisons of age-specific incidence rates methodologically problematic.

The consequence is non-trivial. The 10–14 year age group, identified in this manuscript as carrying the highest cumulative incidence (217.61 per 100,000), has suboptimal booster coverage and therefore a larger proportion of genuinely susceptible individuals in its denominator than, say, the 0–4 year group, where primary vaccination rates are higher. Cross-age comparisons of population-based incidence rates under these conditions do not isolate differential susceptibility from differential exposure or case ascertainment; they conflate all three. The authors therefore are encouraged to calculate or discuss incidence rates restricted to the unvaccinated or incompletely vaccinated stratum within each age group, where vaccination records permit, or at minimum to explicitly acknowledge this limitation and its direction of bias in the text. The recently published analysis by Moa and colleagues on the burden of pertussis in Australian adults, which similarly grapples with the intersection of population-based incidence estimates and vaccination status, may provide a useful methodological reference (<https://doi.org/10.3390/vaccines13101029>)

Answer R1:

We sincerely thank the reviewer for this valuable suggestion. We had already considered this aspect during the analytical phase; however, the specific information is not available within the surveillance system used, nor can it be retrieved from the currently available vaccination registries. In light of this limitation, we have explicitly acknowledged this issue among the study limitations in the Discussion section. Additionally, we have expanded the description of the PREMAL surveillance system in the Materials and Methods section, providing a clearer explanation of both its potential and its limitations with respect to the availability of the variables of interest.

"The PREMAL system is a national passive surveillance platform for notifiable diseases in Italy. It relies on routine reporting by healthcare providers and local health authorities (ASLs), without active case finding. Standardized forms collect demographic and clinical information, including, since 2024, the degree of severity of cases. Laboratory confirmation and vaccination status are not consistently available. Incidence estimates are calculated using the total resident population as the denominator, without adjustment for vaccination coverage or underlying susceptibility. Strengths of PREMAL include nationwide coverage, longitudinal data collection, and the ability to monitor trends over time and across age groups. Limitations are inherent to passive surveillance: case ascertainment depends on clinical recognition and reporting practices, which may vary over time and by region; mild or subclinical cases are likely underreported. Consequently, passive surveillance cannot directly capture the true disease burden. Despite these limitations, PREMAL provides a cost-effective and sustainable tool for population-level monitoring and public health decision-making. PREMAL provides a cost-effective and sustainable framework for monitoring infectious diseases population-level monitoring and public health decision-making"

We believe that these revisions improve the methodological transparency of the manuscript and help to appropriately contextualize the interpretation of the results within the constraints of the surveillance system used.

R2: A central analytical challenge for any passive surveillance study of this kind is the assumption that case ascertainment probability remains constant over time. This assumption is implausible here. The dramatic increase from 10 cases in 2023 to 598 cases in 2024 is interpreted primarily as a true resurgence of pertussis, and this may well be correct. However, the ECDC Communicable Disease Threats Report reintroduced pertussis as a monitored threat from 2023 onwards after nearly a decade of absence, and a dedicated Rapid Risk Assessment was published in May 2024. The authors themselves acknowledge that heightened awareness among clinicians may have increased diagnostic sensitivity and reporting completeness in 2024. These institutional and behavioural changes in the surveillance system constitute a time-varying confounder of unknown magnitude that cannot be separated from the true underlying trend using passive surveillance data alone. This limitation is particularly consequential for two specific claims: first, the inference about changes in the age distribution of cases, which could reflect differential improvements in case detection across age groups rather than genuine epidemiological shifts; and second, the interpretation of the declining hospitalization proportion in 2024 (10.4% versus 60% in 2023). The latter is almost certainly influenced by surveillance inflation of the denominator, as milder cases become detectable through heightened diagnostic vigilance, the total number of reported cases increases disproportionately relative to severe cases, mechanically reducing the hospitalization fraction without any necessary change in disease severity. The authors should discuss this numerator-denominator artifact explicitly and avoid presenting the decline in hospitalization proportion as straightforward evidence of attenuated clinical severity. The severity of underreporting in Italian surveillance, estimated at approximately 141-fold among adolescents and over 3,000-fold among adults, provides essential context for interpreting apparent changes in age-specific incidence [please cite <https://doi.org/10.3390/jcm12051732>]. Active surveillance data from comparable settings,

such as the community-based study conducted in Yiwu, China, which estimated pertussis incidence at 108.3 per 100,000 compared to far lower passive estimates, illustrates the systematic gap between passive and active surveillance systems and the magnitude of true burden likely obscured by notification-only approaches [cite <https://www.mdpi.com/2076-2607/12/11/2186>].

Answer R2:

We thank the reviewer for this insightful comment. As correctly noted, our analysis is based on data derived from a passive surveillance system. We acknowledge that such systems inherently rely on the assumption of relatively stable case ascertainment over time and are therefore subject to variations in diagnostic awareness, testing practices, and reporting completeness. We agree that the renewed attention to pertussis at the European level and the increased clinical awareness observed in 2024 may have contributed to improved case detection and reporting.

To address this important point, we have strengthened the Discussion by explicitly acknowledging the potential impact of time-varying changes in surveillance sensitivity and reporting practices on the observed increase in cases. We also clarified that the apparent changes in the age distribution should be interpreted cautiously. In this context, we have included the suggested reference on pertussis underreporting in Italy (Bagordo et al., 2023) to better contextualize the well-documented magnitude of underascertainment in passive notification systems. Regarding hospitalization, we agree that the observed reduction in the proportion of hospitalized cases in 2024 may be influenced by increased detection of milder cases expands the denominator without a proportional increase in severe cases. We have therefore revised the Discussion to explicitly note that this decrease should not be interpreted as straightforward evidence of reduced clinical severity, but rather as a potential consequence of improved surveillance sensitivity. We believe these revisions better clarify the methodological constraints of passive surveillance data and provide a more cautious interpretation of the observed trends.

We also thank the reviewers for the suggested bibliographic references. The reference: “Bagordo, F.; Grassi, T.; Savio, M.; Rota, M.C.; Baldovin, T.; Vicentini, C.; Napolitano, F.; Trombetta, C.M.; Gabutti, G.; Seroepidemiological Study Group. *Assessment of Pertussis Underreporting in Italy*. J. Clin. Med. 2023, 12, 1732. Available online: <https://doi.org/10.3390/jcm12051732>” was already included in our manuscript (Ref. 2). Conversely, we decided not to include the following paper: “Dai, H.; He, H.; Xu, J.; Zhu, Y.; Fu, T.; Chen, B.; Li, J.; Gao, Y.; Qin, A.; Zhang, M.; et al. *Underestimated Incidence Rate of Pertussis in the Community: Results from Active Population-Based Surveillance in Yiwu, China*. Microorganisms 2024, 12, 2186. <https://doi.org/10.3390/microorganisms12112186>”.

Instead, we considered it more appropriate to expand the Materials and Methods section by providing a more detailed description of the PREMAL surveillance system, which is based on passive surveillance, and by highlighting its main limitations and potential sources of underreporting.

R3: The manuscript calculates a "cumulative incidence" across the six-year observation period using the average resident population as denominator (reported as 18.19 per 100,000). This is not a standard epidemiological measure. Cumulative incidence, properly defined, estimates the probability of disease occurrence in a defined population over a specific period under the assumption that all individuals are followed from the start of the period and are event-free at baseline. Its calculation requires a closed cohort or a well-specified open cohort with person-time denominators. The approach used here, dividing total cases over six years by an average annual population count, yields a period incidence rate rather than a true cumulative incidence, and conflates the very different epidemiological conditions of the pandemic suppression period (2020–2023, 32 total cases) with the 2024 resurgence (598 cases). The resulting figure (18.19 per 100,000) is dominated by a single calendar year and is unlikely to be informative for surveillance purposes. The authors should either adopt standard incidence rate terminology, present annual rates individually, or, if a multi-year summary measure is desired, calculate an age-standardised average annual incidence rate with appropriate denominators and a confidence interval.

Answer R3:

We thank the reviewer for this valuable suggestion. In response, we considered different approaches for presenting the incidence estimates. Specifically, we calculated incidence rates, presented the annual rates individually, and reported the period incidence. In addition, we included a summary measure in the form of an average annual incidence rate with its corresponding confidence interval. These estimates are presented in the main tables of the manuscript as well as in the supplementary tables.

R4: The authors note that a "high proportion of notifications with incomplete information on hospitalization status ('NA') limited severity analysis, but neither the proportion of missing values nor any assessment of the likely missingness mechanism is provided. If missing hospitalization records are concentrated in particular years, age groups, or local health authorities, their omission could systematically bias estimates of hospitalization rates and their apparent temporal trends. The authors should quantify the extent of missing data for this variable, stratified by year and local health authority if possible, and discuss whether a missing-not-at-random mechanism is plausible given the surveillance context. At minimum, a sensitivity analysis presenting hospitalization rates under complete-case assumptions and under a plausible worst-case imputation should be reported.

Confidence intervals are not reported for any of the incidence estimates, including those presented in Tables 1 and 2 and in the supplementary material. For a surveillance study with small absolute case counts in certain strata (notably 2021–2023, where zero to ten cases per year were recorded), point estimates without uncertainty bounds are unreliable bases for comparison. Poisson-based confidence intervals should be provided for all rates, with exact methods applied where counts are small. The geographical analysis attributes the majority of cases (64%) to the Central Tuscany Local Health Authority and offers population density as one explanatory factor. While this is

plausible, the authors acknowledge the alternative explanation of differential reporting capacity. These two explanations have entirely different public health implications. The manuscript would benefit from a more systematic treatment of this ambiguity, for example by examining whether the proportion of laboratory-confirmed cases (if available) differs across local health authorities, which would serve as a partial proxy for surveillance quality.

Answer R4:

Thank you for your comments. The PREMAL surveillance system is not affected by differences in reporting practices across Local Health Authorities (ASLs), as data collection is standardized within the system. Data collection has been implemented starting from 2024 and now includes the further clinical information. However, information on laboratory confirmation is not available within the system. To improve clarity, we have expanded the description of the PREMAL surveillance tool in the Materials and Methods section, explicitly specifying that it is a passive surveillance system, with the associated strengths and limitations inherent to this type of data source.

Furthermore, we have added confidence intervals to the estimates presented in the tables in the main manuscript as well as in the supplementary tables.

R5: The use of the phrase "significant differences" in relation to the annual variation in incidence (Methods section) is potentially misleading in the absence of formal statistical testing. If no inferential tests were performed, which is methodologically defensible for a descriptive surveillance study, the language should be revised to "substantial" or "notable" differences throughout.

Answer R5:

Thank you for this comment. We confirm that no inferential statistical tests were performed in the analysis. We agree that the previous wording was not appropriate in this context; therefore, we have revised the manuscript by replacing the expression "significant differences" with more appropriate descriptive terms such as "substantial" or "notable" differences throughout the text