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Original article

Performance of diagnostic procedures for bubonic plague in endemic settings in Madagascar: a prospective test accuracy sub-study within the IMASOY trial

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ABSTRACT

Objectives: We aimed to assess the diagnostic value of a battery of confirmatory tests included in the WHO guidelines for bubonic plague, combined and individually, including culture, PCR and serology, and the on-site and laboratory performance of the F1 antigen-based lateral flow rapid diagnostic test (F1RDT).

Methods: Bubo aspirates from patients (all ages) with suspected bubonic plague enrolled into the IMASOY trial (NCT04110340) underwent routine laboratory diagnosis complemented with serology to measure IgG F1 antibodies from blood samples taken on days 1, 11, and 21. The performance of the F1RDT done on site (on-site F1RDT) and at the Central Laboratory for Plague (reference laboratory F1RDT) was compared against two reference standards: RS1 (culture or PCR-positive), which is used in routine practice, and RS2 (RS1 and serology-positive), including all available confirmatory tests for plague.

Results: Of 438 suspected cases, 184 (42%) were confirmed by culture or PCR (RS1) and 211 (48%) by culture, PCR, or serology (RS2). PCR identified 179 cases (85%), culture 137 (65%), and serology 197 (93%). The combination of PCR and culture identified 87% of confirmed cases while the remaining 13% were identified by serology only. The sensitivity and specificity of on-site F1RDT were 94% (95% CI, 89.6–97.0) and 74% (95% CI, 68.2–79.3) against RS1 and 89.1% (95% CI, 84.1–93) and 77.5% (95% CI, 71.5–82.8) against RS2. The sensitivity and specificity of reference laboratory F1RDT were 91.8% (95% CI, 86.9–95.4), 97.6% (95% CI, 94.9–99.1) against RS1, and 82.0% (95% CI, 76.1–86.9) and 99.1% (95% CI, 96.9–99.9) against RS2.

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Conclusions: PCR outperforms culture, the previous reference standard. While serology adds diagnostic value, it is impractical for routine use. The F1RDT done on site cannot be relied upon for clinical case management decisions. F1RDT performs better under laboratory conditions and could be implemented in peripheral laboratories for surveillance purposes in resource-constraint settings. **Mihaja Raberahona, Clin Microbiol Infect 2026;32:638**

Introduction

Plague is a high-consequence infectious disease caused by *Yersinia pestis*. While the number of reported cases has been steadily decreasing during the 20th century, plague still has epidemic potential due to its widespread reservoir in wild and peridomestic rodents [1] and the possibility of deliberate release [2,3].

The most common clinical manifestation is bubonic plague, characterized by swollen, painful lymph node(s)—the 'bubo (es)'—, fever and other nonspecific symptoms, and a case fatality rate of up to 70% if untreated [4]. Other forms are pneumonic and septicaemic plague [2].

Plague is endemic to Madagascar, which contributes approximately 80% of cases reported worldwide, with transmission occurring mostly during September-March in remote places in the highlands [4,5].

According to Madagascar guidelines, when bubonic plague is suspected based on epidemiology and clinical presentation, a bubo aspirate is taken; part of the sample is tested on site with a F1 antigen-based lateral-flow rapid diagnostic test (F1RDT), and part is sent to the Central Laboratory for Plague, hosted at Institut Pasteur of Madagascar (IPM)—referred to as reference laboratory (RL)—where it is further tested with the F1RDT, PCR, and culture for confirmation.

Prompt diagnosis and treatment are essential to a favourable clinical outcome [6], but this is challenging in practice, as plague tends to occur in remote villages [4]. Therefore, when plague is suspected on clinical grounds, whatever the result of the on-site F1RDT, physicians tend to start treatment; confirmation would normally arrive after treatment is completed.

The F1RDT used in Madagascar was developed by IPM and has been used routinely since 2002 at primary health care centres in plague endemic areas [7]. The same F1RDT is also provided to other countries and is currently recommended in various scenarios by the WHO [8,9]. However, the performance of this F1RDT has not yet been prospectively assessed against all current available confirmatory tests (culture, PCR, and serology) either under field conditions or in the laboratory. Furthermore, the diagnostic value of culture, PCR, and serology in bubonic plague is not completely clarified, particularly because serology is not routinely used due to the difficulty of obtaining day 21 samples. The latter requires an additional follow-up visit, which is difficult to organize in practice, as patients often live several hours' walking distance from health centres. It also involves on-site sample pre-processing and specific transportation conditions, which are not feasible in routine settings.

In this paper, we assess the individual and combined diagnostic value of culture, PCR, and serology for bubonic plague and determine the performance of the F1RDT, both on-site and under laboratory conditions.

Materials and method

Study design and settings

This study was embedded in the IMASOY randomized controlled trial (NCT04110340), conducted from 2020 to 2024 in 47 primary health care centres and hospitals spread across 12 districts in plague-endemic area located in the central highlands of Madagascar over five plague transmission seasons [10–12].

Participants

Patients of all ages presenting to the trial sites with a recent onset of fever (less than 10 days) or history of fever, with at least one localised tender lymph node swelling with no clear focus of infection in its drainage area, with residence in or travel to a plague-endemic or outbreak area within 14 days of the onset of symptoms, and with a clinical suspicion of bubonic plague were eligible. Patients with suspected pneumonic plague were excluded.

Test procedures

At inclusion, a bubo aspirate was taken from each participant and tested on site using the F1RDT as per national guidelines for plague management. The remaining sample was placed in Cary-Blair transport medium and sent to the RL in Antananarivo at room temperature according to a standardized procedure.

A two-step procedure is used at the RL for PCR, involving first the detection of *caf1* and *pla* genes using a quantitative PCR (qPCR), followed, if the results are inconclusive, by a conventional PCR targeting three genes: *caf1*, *pla*, and *inv* [13–15].

Detailed test procedures for culture, PCR, and serology are provided in the supplementary materials and Fig. S1 and S2.

The results of all diagnostic tests were then used to classify confirmed cases as per WHO plague case definition [16] (see supplementary materials).

The median transport time of the collected samples from the field to the RL was 2 days (interquartile range: 2–3) in trial conditions.

Index tests and reference standards

Two index tests were assessed: the F1RDT carried out by on-site medical staff, referred to on-site F1RDT and the F1RDT repeated on the sample shipped to the RL, referred to RL F1RDT.

Two reference standards were considered: (a) RS1, mimicking routine practice, defined as positive culture or PCR on Day 1 (D1) for confirmed cases, and negative culture and PCR for non-cases; (b) RS2, representing ideal conditions, which included positive culture, or positive PCR, or seroconversion, or a four-fold increase in anti-F1 IgG antibodies (between D1 and D11 or D21). Confirmatory tests were done at the RL and took several days, so their results were unavailable when the on-site and RL F1RDTs were performed. On-site staff had only clinical and epidemiological data, while RL staff had access to these data plus the on-site F1RDT results.

Statistical methods

Two-by-two tables are presented for the number of positive and negative test results versus each reference standard diagnosis for i) on-site F1RDT and ii) the RL F1RDT performed at the RL.

The performance of the on-site and RL F1RDT against RS1 and RS2 was expressed by calculating sensitivity, specificity, false detection rate (FDR = 1 − PPV [positive predictive value]) and false omission rate (FOR = 1- NPV [negative predictive value]), and F1-score (the harmonic means of PPV and sensitivity). 95% CIs were calculated as exact binomial CIs. Cohen's κ was used to calculate agreement between on-site F1RDT and RL F1RDT.

We also calculated FDR and FOR of the RL F1RDT for various prevalence levels (pre-test probabilities) to simulate hypothetical field situations.

Ethics consideration

The study was approved by the Oxford Tropical Research Ethics Committee (45-18), Comité d'Éthique et de Recherche Biomédicale de Madagascar (Authorisation N°116-MSANP/CERBM dated September 10, 2018), and the London School of Hygiene and Tropical Medicine (17911).

Role of the funding source

The funder had no role in the design of the study, data collection, data analysis, interpretation of the results, or the decision to publish the manuscript.

Results

Of 450 suspected bubonic plague patients enrolled and randomised into the IMASOY trial, 438 had available data for the analyses (see detailed flow diagram in Fig. S3).

Amongst these 438 suspected cases, the plague-positive rates were 42% without serology (n = 184) and 48% including serology-positive-only cases (n = 211). Baseline characteristics of enrolled participants and comparison between non-case, culture, or PCR confirmed cases and serology-only confirmed cases are detailed in Table 1.

The patterns emerging when considering the confirmatory tests—PCR, culture, and serology—are presented in Fig. 1.

Prior treatment with antibiotics recommended for plague did not differ between confirmed cases with positive (12/137, 8.8%) and negative baseline culture (i.e. confirmed by PCR and/or serology) (8/74, 0.8%) (p = 0.637).

There was no evidence of difference by age or sex between those confirmed by serology only or by PCR and/or culture (p > 0.5). Patients who were confirmed based only on serology could have had a shorter duration of fever (mean 1.1 days vs. 1.5 days, ANOVA p = 0.058). None of the serology-only confirmed cases experienced any of the treatment failure outcomes of death, fever at D11, development of secondary pneumonic plague or requirement for extra anti-plague antibiotics. Power was too low for a formal comparison of outcomes between PCR and/or culture diagnosed and serology only diagnosed cases.

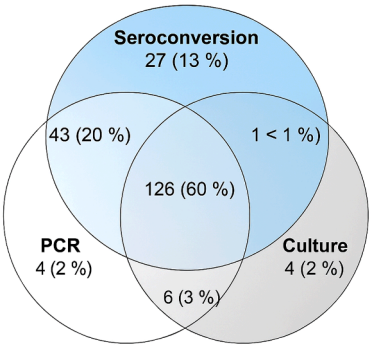

Fig. 1. Venn diagram amongst 211 confirmed cases.

Raw data for the comparisons between on-site and RL F1RDT against both reference standards and between the two RDTs are presented in Table 2. The performance of on-site and RL F1RDT against both reference standards, overall and by age group, is shown in Table 3.

The between-F1RDT agreement was 83.1%, and Cohen's κ was 0.67, expressing moderate agreement. The main difference between the two tests resides in a higher number of positive results returned by the on-site test.

Both incidence and test performance varied with patients' age (Table 3). The accuracy of clinical diagnosis—i.e. the proportion of patients who eventually had bubonic plague confirmed amongst those clinically suspected—was lower in children under 10 and higher in older patients (Table 4).

The projected FDR and FOR results with the two F1RDTs in different prevalence scenarios are presented in Fig. 2. Fig. 3 compares the FDR and FOR of RL F1RDT done once or twice. The FOR of single F1RDT performed in the laboratory is 1% and 2% when incidence is 5% and 10%, respectively, but would be 0% for incidence ranging 0–30% if the test is done twice. The respective FDR would be 19% and 10% for the single and 4% and 1% for the repeat test.

Discussion

This is the first study to assess prospectively the performance of the WHO-recommended set of diagnostic tests for bubonic plague. The study was part of a clinical trial, which strengthens the validity of the findings through the implementation of standardized procedures, training, and monitoring. Nevertheless, efforts were made to ensure that these procedures reflected real-world operational conditions, thereby enhancing the relevance of the results to routine clinical practice. This approach adds value to the study but

Table 1
Baseline characteristics of participants

		Total	Non case	PCR/culture	Serology only	p across 3 groups[a]	p across 2 groups[b]
Enrolled	N	438	227 (51.8)	184 (42.0)	27 (6.2)		
Age (y)	Mean ± SD	15.2 ± 12.8	12.0 ± 10.4	18.8 ± 14.4	17.9 ± 12.8	<0.001	0.746
	Median (range)	12.0 (0–72)	10.0 (0–64)	13.5 (2–72)	15.0 (2–45)	<0.001	0.784
Sex	Male	262	150 (57.3)	97 (37.0)	15 (5.7)	0.021	0.838
	Female	176	77 (43.8)	87 (49.4)	12 (6.8)		

Cases confirmed by PCR or Culture may have also seroconverted (all but 16/184).
[a] p across 3 groups: ANOVA by diagnosis group for means, Kruskall-Wallis for medians, and Fisher's exact test for proportions.
[b] p across 2 case groups (PCR/culture/seroconversion vs. serology only): ANOVA by diagnosis group for means, rank sum for medians, and Fisher's test for proportions.

Table 2
Comparison between on-site F1RDT, RL F1RDT, and the two reference standards

Test		Reference standard regardless of serology (RS1)			Reference standard including serology (RS2)			RL F1RDT		
		Positive	Negative	Total	Positive	Negative	Total	Negative	Positive	Total
RL F1RDT	Positive	169	6	175	173	2	175	—	—	—
	Negative	15	248	263	38	225	263	—	—	—
	Total	184	254	438	211	227	438	—	—	—
Site F1RDT	Positive	173	66	239	188	51	239	69	170	239
	Negative	11	188	199	23	176	199	194	5	199
	Total	184	254	438	211	227	438	263	175	438

Reference standard regardless of serology: Positive = PCR positive or culture positive at D1; Negative = PCR negative and culture negative at D1.
Reference standard including serology: Positive = PCR positive at D1, culture positive at D1, or seropositive at D11 or 21; Negative = PCR negative and culture negative at D1, with seronegative results at all timepoints D1, D11 and D21.

Table 3
Diagnostic performance of F1RDT at RL and on-site by age against the two reference standards

Age (y)	Reference standard	Test	N	Sensitivity	Specificity	FDR	FOR	F1
All	RS1 (regardless of serology)	RL	438	91.8 (86.9–95.4)	97.6 (94.9–99.1)	3.4 (1.3–7.3)	5.7 (3.2–9.2)	0.942
		Site	438	94.0 (89.6–97.0)	74.0 (68.2–79.3)	27.6 (22.0–33.7)	5.5 (2.8–9.7)	0.818
	RS2 (with serology)	RL	438	82.0 (76.1–86.9)	99.1 (96.9–99.9)	1.1 (0.1–4.1)	14.4 (10.4–19.3)	0.896
		Site	438	89.1 (84.1–93.0)	77.5 (71.5–82.8)	21.3 (16.3–27.1)	11.6 (7.5–16.8)	0.836
1–9	RS1 (regardless of serology)	RL	158	88.1 (74.4–96.0)	98.3 (93.9–99.8)	5.1 (0.6–17.3)	4.2 (1.4–9.5)	0.914
		Site	158	92.9 (80.5–98.5)	72.4 (63.3–80.3)	45.1 (33.2–57.3)	3.4 (0.7–9.7)	0.690
	RS2 (with serology)	RL	158	79.6 (65.7–89.8)	100 (96.7–100)	0.0 (0.0–9.0)	8.4 (4.1–14.9)	0.886
		Site	158	91.8 (80.4–97.7)	76.1 (67.0–83.8)	36.6 (25.5–48.9)	4.6 (1.3–11.4)	0.750
10–17	RS1 (regardless of serology)	RL	152	93.3 (85.1–97.8)	97.4 (90.9–99.7)	2.8 (0.3–9.7)	6.3 (2.1–14.0)	0.952
		Site	152	94.7 (86.9–98.5)	75.3 (64.2–84.4)	21.1 (13.2–31.0)	6.5 (1.8–15.7)	0.861
	RS2 (with serology)	RL	152	85.5 (76.1–92.3)	98.6 (92.2–100.0)	1.4 (0.0–7.5)	15.0 (8.0–24.7)	0.916
		Site	152	91.6 (83.4–96.5)	79.7 (68.3–88.4)	15.6 (8.8–24.7)	11.3 (4.7–21.9)	0.879
18 and above	RS1 (regardless of serology)	RL	127	92.5 (83.4–97.5)	96.7 (88.5–99.6)	3.1 (0.4–10.8)	7.9 (2.6–17.6)	0.947
		Site	127	94.0 (85.4–98.3)	75.0 (62.1–85.3)	19.2 (11.2–29.7)	8.2 (2.3–19.6)	0.869
	RS2 (with serology)	RL	127	79.7 (69.2–88.0)	97.9 (88.9–99.9)	1.6 (0.0–8.4)	25.4 (15.3–37.9)	0.881
		Site	127	84.8 (75.0–91.9)	77.1 (62.7–88.0)	14.1 (7.3–23.8)	24.5 (13.3–38.9)	0.854

FDR, false detection rate; FOR, false omission rate.

Table 4
Accuracy of clinical diagnostic amongst suspected bubonic plague cases

Age (y)	Suspected cases n (%)	Confirmed cases by RS1 n (%)	Confirmed cases by RS2 n (%)
All	438	184 (42)	211 (48)
Under 10	158	42 (26.6)	49 (31)
10–17	152	75 (49.3)	83 (54.6)
18 and above	127	67 (52.8)	79 (62.2)

RS1, reference standard 1 including PCR and culture; RS2, reference standard 2 including PCR, culture, and serology.

may also introduce certain limitations, e.g.: single-reader interpretation of the F1RDT results, as done routinely, prevents assessing observer bias. RL staff were not blinded to the on-site F1RDT results.

We found that F1RDT performance varies depending on whether it is used in laboratory conditions or at point-of-contact, with the latter performing less well, and that PCR outperforms culture—the previous reference standard [17]—but none of the available confirmatory tests can detect all cases.

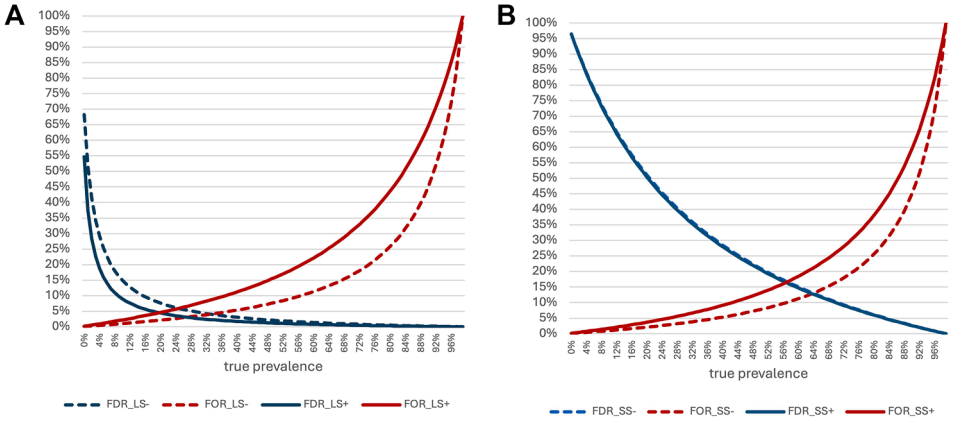

Fig. 2. FDR and FOR of the RL F1RDT and on-site F1RDT at different prevalence scenarios. A, the F1RDT done at the reference laboratory with 92% sensitivity and 98% specificity (against reference standard not including serology: FDR_LS-, FOR_LS-) and 82% sensitivity and 99% specificity (against reference standard including serology: FDR_LS+, FOR_LS+). B, the F1RDT done on site with 94% sensitivity and 74% specificity (against reference standard not including serology: FDR_SS-, FOR_SS-) and 89% sensitivity and 76% specificity (against reference standard including serology: FDR_SS+, FOR_SS+). F1RDT, F1 antigen-based lateral flow rapid diagnostic test; FDR, false detection rate; FOR, false omission rate.

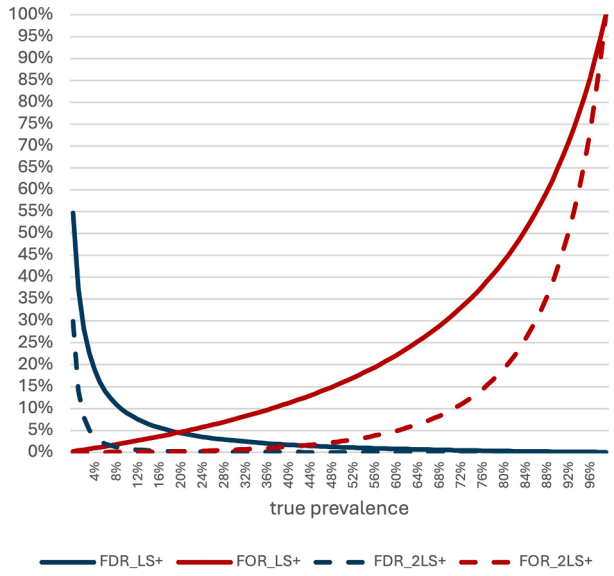

Fig. 3. Comparison FDR and FOR of the F1RDT with 82% sensitivity and 99% specificity against reference standard including serology performed once (FDR_LS+, FOR_LS+) or repeated twice (FDR_2LS+, FOR_2LS+). F1RDT, F1 antigen-based lateral flow rapid diagnostic test; FDR, false detection rate; FOR, false omission rate.

The interpretation and potential applications of the findings of this study depend on the intended use case of the diagnostic test—whether for disease surveillance or case-management. Importantly, these data were generated in a situation where nearly half of the clinically suspected cases are laboratory confirmed, which determines the predictive value of a test result.

Regarding surveillance, we found that, in the system of laboratory confirmation of plague currently used in Madagascar, most cases are detected by PCR. Culture misses about one in four cases identified by PCR, and adds little. There was no obvious explanation for this finding. There was no evidence that culture performance is affected by prior antibiotic intake or age. It is possible that smaller buboes yield smaller volumes of aspirate, which might negatively affect test performance, but this could not be verified, as measurement of bubo size with callipers proved unreliable [18]. Transport conditions might influence results.

Since the 2017 urban outbreak, the PCR previously used [19] was updated to a two-step algorithm [14]. While PCR is now considered a confirmatory test in WHO's updated case definition [16], our study is the first to report the use of this improved PCR protocol as confirmatory tests for plague. A triplex assay has been recently developed using 3 genes—*caf1*, *pla*, and *yopM*—in a single reaction, which yields improved performance and turnaround time but will require further field evaluation [20].

Still, between PCR and culture, 13% of cases are missed and only detected by serology. Serology, however, is not suited for practice, as it requires multiple visits and sample shipment to the RL under particular conditions. While these serology-only positive cases seem to be less severe—shorter duration of fever, all resolved—-they are too few for any conclusive analyses.

Concerning case management, bubonic plague is a serious and potentially life-threatening disease requiring immediate treatment. The ideal point-of-contact diagnostic does not exist at the moment. Physicians cannot confidently rule in or rule out plague based on the available F1RDT performed on sites for case-management decisions, and their clinical judgement is clearly influenced by the patient's age, as they are more likely to suspect, and thus overtreat, plague in young children than adults.

The performance of the F1RDT used in Madagascar for plague was previously assessed in retrospective studies and a systematic review and meta-analysis of retrospective studies [19,21,22]. In this study, the sensitivity of the F1RDT, even when performed at the RL against all available confirmatory tests, is lower than previously reported [19]. The WHO guideline for plague management suggests that a negative F1RDT would help exclude bubonic plague cases in endemic areas [9]. However, in view of the results of this study, the role of the F1RDT should be revisited, taking into account use cases and expected local incidence.

F1RDT performance may be influenced by various factors, such as patient's age and clinical severity—more severe cases may have higher bacterial loads. However, the absence of well-defined and validated baseline criteria for classifying the severity of bubonic plague—particularly across age groups—makes robust analyses challenging. Why F1RDT performed better in the laboratory is not entirely clear. Additional sample processing (dilution and vortex mixing) in the laboratory could facilitate sample migration and improve reading. Faint red lines caused by a viscous bubo aspirate usually mixed with blood may have been misinterpreted as a positive test by the on-site staff but were negative after laboratory processing. Although F1RDT accuracy can be affected by staff proficiency [21], regular training and oversight within the IMASOY trial mitigated this risk.

Decisions on deploying F1RDT depend on the local plague risk. In high-prevalence settings, like in this study, the laboratory-based F1RDT has a low FDR, making it comparable to a confirmatory test in a laboratory lacking PCR and culture—common in endemic areas. However, its FOR is too high to confidently exclude plague. Conversely, in very low-prevalence areas where plague is not endemic, a negative result effectively excludes plague, while a positive result requires confirmatory testing due to the high risk of false positives.

Conclusion

For plague surveillance, we have a set of tests that, together, provide reliable data. However, it may not be operationally and financially sustainable to adopt the F1RDT, PCR, culture, and serology in all settings and incidence rates.

Culture, which was considered the reference standard test, is outperformed by PCR, which is also easier and cheaper. F1RDT could be implemented in peripheral laboratories in low-resource settings for surveillance purposes, particularly in low-incidence areas. Repeating the F1RDT twice would be valuable, as it would improve its FOR and FDR in high-prevalence settings.

Currently, there is no test that can be done at point-of-care to reliably confirm a plague diagnosis for case management purposes.

CRediT authorship contribution statement

Mihaja Raberahona: Conceptualisation, Methodology, Visualization, Validation, Writing - Original draft, reviewing, and editing. Minoarisoa Rajerison: Data curation, Investigation, Supervision. Beza Ramasindrazana, Salohiniana Manuel Randriamanantena, Voahangy Andrianaivoarimanana: Investigation. Tansy Edwards: Formal analysis, Data curation, Methodology, Visualization. Josephine Bourner, Elise Pesonel, Lisy Hanitra Razananaivo, Gabriella Zadonirina, Theodora Mayouya-Gamana: Project administration. Reziky Tiandraza Mangahasimbola: Data curation. Rindra Vatosoa Randremanana: Investigation, Project administration. Mamy Randria, Rivonirina Andry Rakotoarivelo: Supervision. Peter Horby: Supervision, Funding acquisition. Piero Olliaro: Conceptualisation, Methodology, Visualization, Formal analysis, Data curation, Supervision, Validation, Writing - Original draft, reviewing, and editing.

Transparency declaration

Potential conflict of interest

The authors declare that they have no conflicts of interest.

Financial report

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Data availability

We will share de-identified data used in published analyses. The data will be made available in an existing repository (either Oxford or LSHTM) with access granted requiring specific request. The data will be made available approximately 3 months after publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2025.12.002.

References

[1] Rajerison M, Andrianaivoarimanana V, Olliaro P. Plague. Manson's tropical diseases. 24 ed. Elsevier; 2024.
[2] Nelson CA, Meaney-Delman D, Fleck-Derderian S, Cooley KM, Yu PA, Mead PS, et al. Antimicrobial treatment and prophylaxis of plague: recommendations for naturally acquired infections and bioterrorism response. MMWR Recomm Rep 2021;70:1–27. https://doi.org/10.15585/mmwr.rr7003a1.
[3] Wren BW. The yersiniae–a model genus to study the rapid evolution of bacterial pathogens. Nat Rev Microbiol 2003;1:55–64. https://doi.org/10.1038/nrmicro730.
[4] Andrianaivoarimanana V, Piola P, Wagner DM, Rakotomanana F, Maheriniaina V, Andrianalimanana S, et al. Trends of human plague, Madagascar, 1998-2016. Emerg Infect Dis 2019;25:220–8. https://doi.org/10.3201/eid2502.171974.
[5] World Health Organization = Organisation mondiale de la Santé. Plague around the world in 2019 – La peste dans le monde en 2019. Weekly Epidemiological Rec [Relevé Épidémiologique Hebdomadaire] 2019;94:289–92.
[6] Nelson CA, Fleck-Derderian S, Cooley KM, Meaney-Delman D, Becksted HA, Russell Z, et al. Antimicrobial treatment of human plague: a systematic review of the literature on individual cases, 1937-2019. Clin Infect Dis 2020;70:S3–10. https://doi.org/10.1093/cid/ciz1226.
[7] Chanteau S, Rahalison L, Ralafiarisoa L, Foulon J, Ratsitorahina M, Ratsifasoamanana L, et al. Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. Lancet 2003;361:211–6. https://doi.org/10.1016/S0140-6736(03)12270-2.
[8] World Health Organization. Manual for plague surveillance, diagnosis, prevention and control. Geneva: World Health Organization; 2024.
[9] World Health Organization. WHO guidelines for plague management: revised recommendations for the use of rapid diagnostic tests, fluoroquinolones for case management and personal protective equipment for prevention of post-mortem transmission. Geneva: World Health Organization; 2021.
[10] Randremanana RV, Raberahona M, Randria MJDD, Rajerison M, Andrianaivoarimanana V, Legrand A, et al. An open-label, randomized, non-inferiority trial of the efficacy and safety of ciprofloxacin versus streptomycin + ciprofloxacin in the treatment of bubonic plague (IMASOY): study protocol for a randomized control trial. Trials 2020;21:722. https://doi.org/10.1186/s13063-020-04642-2.
[11] Randremanana RV, Raberahona M, Randria MJ de D, Bourner J, Zadonirina G, Razananaivo H, et al. An open-label, randomized, non-inferiority trial of the efficacy and safety of ciprofloxacin versus an aminoglycoside + ciprofloxacin in the treatment of bubonic plague (IMASOY): study protocol for a randomized control trial-an update to the published protocol. Trials 2024;25:457. https://doi.org/10.1186/s13063-024-08302-7.
[12] Randremanana RV, Raberahona M, Bourner J, Rajerison M, Edwards T, Randriamparany R, et al. Ciprofloxacin versus aminoglycoside-ciprofloxacin for bubonic plague. N Engl J Med 2025;393:544–55. https://doi.org/10.1056/NEJMoa2413772.
[13] D'Ortenzio E, Lemaître N, Brouat C, Loubet P, Sebbane F, Rajerison M, et al. Plague: bridging gaps towards better disease control. Med Mal Infect 2018;48:307–17. https://doi.org/10.1016/j.medmal.2018.04.393.
[14] Randremanana R, Andrianaivoarimanana V, Nikolay B, Ramasindrazana B, Paireau J, Ten Bosch QA, et al. Epidemiological characteristics of an urban plague epidemic in Madagascar, August-November, 2017: an outbreak report. Lancet Infect Dis 2019;19:537–45. https://doi.org/10.1016/S1473-3099(18)30730-8.
[15] Ten Bosch Q, Andrianaivoarimanana V, Ramasindrazana B, Mikaty G, Rakotonanahary RJL, Nikolay B, et al. Analytical framework to evaluate and optimize the use of imperfect diagnostics to inform outbreak response: application to the 2017 plague epidemic in Madagascar. PLoS Biol 2022;20:e3001736. https://doi.org/10.1371/journal.pbio.3001736.
[16] Manual for Plague Surveillance. Diagnosis, prevention and control. 1st ed. World Health Organization; 2024.
[17] Rajerison M, Melocco M, Andrianaivoarimanana V, Rahajandraibe S, Rakotoarimanana F, Spiegel A, et al. Performance of plague rapid diagnostic test compared to bacteriology: a retrospective analysis of the data collected in Madagascar. BMC Infect Dis 2020;20:90. https://doi.org/10.1186/s12879-020-4812-7.
[18] Bourner J, Randriamparany R, Rasoanaivo TF, Denis E, Randremanana RV, Vaillant M, et al. Bubonic plague: can the size of buboes be accurately and consistently measured with a digital calliper? Trials 2023;24:815. https://doi.org/10.1186/s13063-023-07835-7.
[19] Jullien S, Dissanayake HA, Chaplin M. Rapid diagnostic tests for plague. Cochrane Database Syst Rev 2020;6:CD013459. https://doi.org/10.1002/14651858.CD013459.pub2.
[20] Ramasindrazana B, Rahalison ZA, Gauthier P, Mikaty G, Bodoarison ZI, Maminirina LA, et al. Development and evaluation of a triplex real-time PCR assay for enhanced plague diagnostics in Madagascar. PLoS Negl Trop Dis 2025;19:e0013278. https://doi.org/10.1371/journal.pntd.0013278.
[21] Rajerison M, Melocco M, Andrianaivoarimanana V, Rahajandraibe S, Rakotoarimanana F, Spiegel A, et al. Performance of plague rapid diagnostic test compared to bacteriology: a retrospective analysis of the data collected in Madagascar. BMC Infect Dis 2020;20:90. https://doi.org/10.1186/s12879-020-4812-7.
[22] Riehm JM, Rahalison L, Scholz HC, Thoma B, Pfeffer M, Razanakoto LM, et al. Detection of Yersinia pestis using real-time PCR in patients with suspected bubonic plague. Mol Cell Probes 2011;25:8–12. https://doi.org/10.1016/j.mcp.2010.09.002.