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Frontline recognition and specimen referral readiness for selected priority zoonoses among health workers in southwestern Uganda

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Abstract

Background Southwestern Uganda is a livestock-intensive region vulnerable to recurrent zoonotic disease outbreaks. Because confirmation of several priority zoonoses depends on higher-level or reference laboratories, preparedness requires both frontline recognition and effective specimen referral. We assessed frontline recognition, specimen-referral readiness, and selected indicators of One Health coordination and cross-sectoral information exchange among health workers.

Methods We conducted a cross-sectional survey of 345 health workers in Mbarara, Kazo, Kiruhura, and Ibanda districts. Participants were purposively recruited from 54 facilities based on roles in recognition, specimen handling, referral, or reporting of suspected zoonoses. Recognition was summarized using an 11-item recognition index covering causative agents and transmission/exposure pathways. The primary outcome was self-reported absence of specimen-referral linkage, defined as a respondent answering 'No' to the question, 'Do you refer samples elsewhere for testing?' We estimated prevalence ratios (PRs) using modified Poisson regression with facility-clustered, small-sample-corrected robust standard errors (CR2), with generalized estimating equations used to assess robustness.

Results Almost all respondents had heard of the selected zoonoses (99.7%), and the median recognition index was 9/11 (interquartile range [IQR] 8–10). Overall, 48/345 (13.9%) respondents reported no specimen-referral linkage. This was more common among those working at peripheral facilities than at HCIVs/hospitals (20.1% vs. 6.8%; aPR = 2.74, 95% CI 1.24–6.04; $p = 0.016$). Among respondents reporting referral, 294/296 (99.3%) with feedback data reported receiving results. Among 280 with referral-to-result interval data, the median interval was 10 days (IQR 7–14). Common barriers were inadequate diagnostic resources (312/345; 90.4%), unreliable transport (259/345; 75.1%), and inadequate intersectoral collaboration (256/345; 74.2%).

Conclusions Frontline recognition was generally strong, but self-reported specimen-referral linkage was less consistently reported at peripheral facilities. Strengthening specimen transport, referral tracking, information exchange, and subnational human-veterinary-public health coordination may improve connectivity with existing diagnostic networks.

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Keywords One Health · Zoonotic diseases · Health workers · Specimen referral · Uganda

Abbreviations

aPR	Adjusted prevalence ratio
CCHF	Crimean-Congo haemorrhagic fever
CI	Confidence interval
CME	Continuing medical education
HC	Health centre
HCII	Health centre II
HCIII	Health centre III
HCIV	Health centre IV
IQR	Interquartile range
KAP	Knowledge, attitudes, and practices
KOFIH	Korea Foundation for International Healthcare
PR	Prevalence ratio
RVF	Rift Valley fever
SMS	Short message service

1 Introduction

Uganda continues to experience outbreaks of priority zoonoses, including anthrax, rabies, Rift Valley fever (RVF), Crimean-Congo haemorrhagic fever (CCHF), and Ebola [1]. Southwestern Uganda has documented recurrent zoonotic events in livestock-keeping settings, including RVF in the Greater Mbarara area [2], CCHF in the cattle corridor [3], and livestock-associated anthrax [4, 5]. These events underscore the importance of timely recognition, reporting, specimen referral, and response at the frontline of the health system.

Frontline detection of priority zoonoses depends on recognition of relevant clinical and exposure features and timely linkage of suspected cases and specimens to surveillance and laboratory systems [6]. This is particularly important for viral haemorrhagic fevers, which may present nonspecifically and often require reference-level confirmation [7]. Uganda's National Technical Guidelines for Integrated Disease Surveillance and Response position frontline facilities within a wider detection, notification, specimen-referral, and response pathway [8]. Evidence from East Africa and Uganda, including community and frontline-worker studies, indicates that high general awareness can coexist with disease-specific recognition and reporting gaps [9–13]; preparedness therefore depends on both health-worker recognition and functional referral, transport, information, and One Health coordination systems [14].

Despite Uganda's investments in One Health coordination, surveillance, and laboratory-referral systems, limited evidence describes whether frontline recognition is accompanied by reported access to specimen-referral pathways across facility levels. We therefore aimed to assess frontline recognition and specimen-referral readiness for RVF, CCHF, Ebola, anthrax, and rabies among health workers in four districts of southwestern Uganda. Specifically, we described disease-specific recognition and selected One Health indicators, characterized reported specimen-referral and result-return pathways, and identified factors associated with self-reported absence of specimen-referral linkage.

2 Methods

2.1 Study design and setting

We conducted a cross-sectional survey of frontline health workers from 4 December 2024 to 20 January 2025 in Mbarara, Kazo, Kiruhura, and Ibanda districts in southwestern Uganda. The districts were purposively selected to represent agriculturally intensive settings with substantial livestock production, close human-animal interaction, documented recent zoonotic events in the wider region, and a mix of peripheral and higher-level health facilities [2, 4, 5].

Uganda operates a tiered laboratory system in which facilities without specialized testing capacity refer specimens through hub-and-spoke arrangements to higher-level and national reference laboratories and subsequently receive results [15]. Consistent with the national Integrated Disease Surveillance and Response framework, peripheral facilities are expected to recognize and notify suspected priority conditions, collect or facilitate appropriate specimens, and link them to laboratories with confirmatory capacity [8]. For several priority zoonoses, definitive testing is therefore appropriately centralized rather than routinely available at the facility of first presentation.

2.2 Study population and participant selection

We included health workers whose routine responsibilities placed them within the frontline recognition-to-referral pathway for suspected zoonotic diseases, including clinical assessment, diagnostic testing or specimen handling, referral, and reporting. Recruitment was role-based and purposive rather than probability-based. Participants came from 54 facilities: 7 private clinics/medical centres, 8 health centre IIs, 26 health centre IIIs, 5 health centre IVs, and 8 hospitals. The number recruited from each facility varied with facility size, staffing structure, and availability of eligible health workers. The parent survey targeted 406 health workers using a Kish–Leslie sample-size calculation with a 10% allowance for non-response [9]. The present analysis included 345 respondents with complete data on the primary specimen-referral outcome.

2.3 Data collection

Data were collected using a structured, English-language, study-specific questionnaire developed for the parent Zoonotic One Health Engagement and Communication survey from the study objectives and selected priority zoonoses. It was not adopted verbatim from a standardized instrument and was not subjected to formal psychometric content validation. Health workers completed printed hard-copy questionnaires, after which trained research staff entered responses into KoboCollect using tablet devices. The relevant analytic items, response options, coding rules, and referral skip logic are reproduced in Additional file 1.

The questionnaire was pretested among 10 health workers at Mbarara Regional Referral Hospital and the Mbarara Regional Emergency Operations Centre to assess clarity, comprehensibility, sequencing, and feasibility of administration and subsequent data entry. Feedback from the pretest was used to refine wording and flow before the main survey.

The instrument captured sociodemographic and professional characteristics; awareness and recognition of RVF, CCHF, Ebola, anthrax, and rabies; information sources; testing advice; specimen referral and destinations; receipt and communication of referral results; One Health concepts and cross-sectoral information exchange; and perceived barriers to diagnosis and management. Detailed item-level recognition, referral-pathway, barrier, and sensitivity results are provided in the supplementary material.

2.4 Recognition readiness

Recognition was summarized using an 11-item disease-specific recognition index defined a priori from five causative-agent items and six transmission/exposure items. The causative-agent domain covered RVF, CCHF, Ebola, anthrax, and rabies. The transmission/exposure domain covered contact with aborted fetuses or placentas and mosquito transmission for RVF; tick transmission for CCHF; inadequately cooked meat for anthrax; bite exposure from an unvaccinated pet for rabies; and rejection of the misconception that rabies is transmitted through menstrual blood. The combined index was intended as a formative summary of prespecified recognition cues relevant to early suspicion, not as a unidimensional psychometric scale.

Each correct response contributed one point, producing an index from 0 to 11; complete responses to all 11 items were required. For the rabies menstrual-blood misconception, "disagree" or "strongly disagree" was coded as correct, while neutral, agree, or strongly agree responses were coded as incorrect. We did not define good/moderate/poor knowledge thresholds or create a global KAP score. Internal consistency was assessed descriptively using the Kuder–Richardson 20 coefficient (KR-20), which is equivalent to raw Cronbach's alpha for binary items, for the overall index and the two component domains.

2.5 Referral readiness and primary outcome

Referral readiness was characterized using a sequence of respondent-reported indicators following suspicion of a priority zoonosis: advice to test, specimen referral elsewhere for testing, referral destination, receipt of results, usual time to receive results, and communication of results to patients. These indicators describe reported connectivity to the specimen-referral pathway rather than a validated composite readiness scale.

The primary outcome was self-reported absence of specimen-referral linkage, defined as a "No" response to the questionnaire item, "Do you refer samples elsewhere for testing?" A "Yes" response indicated reported specimen-referral linkage. The outcome therefore reflects the individual respondent's reported practice, awareness, or access and must not be interpreted as an audit showing that a facility objectively lacked a functioning referral pathway. To characterize the reliability of this respondent-level measure, we quantified facilities with mixed Yes/No responses among facilities with at least two respondents, overall within-facility pairwise agreement, and a random-intercept logistic intraclass correlation coefficient (ICC).

For the primary comparison, facilities were grouped as peripheral facilities (private clinics/medical centres, HCIIs, and HCIIIs) and HCIVs/hospitals, reflecting their relative position within Uganda's health and laboratory-referral system [16]. Among respondents reporting referral, free-text destinations were classified as national/reference laboratory, regional referral hospital, district/HCIV/hub pathway, private laboratory, or other/multiple destinations. These categories describe reported pathways rather than an audit of the laboratory network.

The referral-to-result interval was the respondent-reported usual number of days between referral and receipt of a result. The questionnaire asked about referral generally rather than for a specific disease; disease-specific interval analyses were therefore not possible. We did not describe this measure as laboratory turnaround time because it could include specimen preparation and transport, laboratory processing, result transmission, and receipt by the originating facility [15]. Intervals were summarized overall and descriptively by facility group.

2.6 One Health and health-system indicators

Selected One Health indicators were analysed as separate item-level measures of multidisciplinary partnership and human–animal–environment linkage. Veterinary officials as an information source and inadequate intersectoral collaboration as a barrier were analysed separately. These measures reflected knowledge and reported context, not verified One Health practice; training/CME was likewise treated as an information source rather than a standardized intervention.

2.7 Statistical analysis

Participant, recognition, referral, and One Health characteristics were summarized using frequencies and percentages for categorical variables and medians with interquartile ranges (IQRs) for continuous variables. Disease-specific recognition items were described individually alongside the 11-item recognition index. KR-20 was calculated for the overall index and for the causative-agent and exposure/transmission domains. Within-facility referral-reporting discordance and pairwise agreement were summarized descriptively, and a random-intercept logistic model was used to estimate a facility-level ICC. The referral-to-result interval was summarized overall and by facility group.

We estimated prevalence ratios (PRs) and 95% confidence intervals (CIs) for self-reported absence of specimen-referral linkage using modified Poisson regression with a log link [17]. The primary adjusted model included facility group, district, professional cadre, and training/CME reported as an information source, selected a priori as contextual and professional characteristics potentially related to referral awareness or access. Because the adjusted analysis included 47 outcome events across 54 facility clusters, we retained this parsimonious prespecified covariate set and used facility-clustered CR2 small-sample-corrected standard errors with Satterthwaite degrees of freedom for primary inference. Generalized estimating equation (GEE) Poisson models with exchangeable and independence working correlations were fitted as sensitivity analyses. Additional sensitivity models incorporated the overall recognition index and the causative-agent and exposure/transmission domains separately and jointly. Additional participant characteristics were retained as crude analyses only and were not selected for adjustment based on statistical significance.

Analyses used complete observations for variables included in each model; missing values were not imputed. All tests were two-sided, and $p < 0.05$ was considered statistically significant. Analyses were conducted using R version 4.6.1 (R Core Team, Vienna, Austria) [18].

3 Results

3.1 Participant and health-facility characteristics

A total of 345 health workers from 54 facilities were included; facilities contributed a median of 4 respondents (range 1–32). The facilities comprised 7 private clinics/medical centres, 8 HCIIs, 26 HCIIIs, 5 HCIVs, and 8 hospitals. The median participant age was 30 years (IQR 26–36), 201 (58.3%) were female, and participants had spent a median of 5 years (IQR 2–10) in their current position and 2 years (IQR 1–4) at their current workstation. Nurses comprised 192/345 (55.7%) of respondents; among them, 129 (67.2%) were certificate-trained, 53 (27.6%) diploma-trained, and 10 (5.2%) degree-trained. Respondents from HCIIIs comprised the largest participant group (39.1%). Training programme and/or CME was reported as an information source by 258/335 (77.0%); 10 respondents had missing data for the parent information-source item. Smartphone ownership was reported by 312/345 (90.4%) (Table 1).

3.2 Frontline recognition and One Health linkage

General awareness was high: 344/345 (99.7%) respondents had heard of the selected priority zoonoses, and the median 11-item recognition index was 9 (IQR 8–10; $n = 344$). Recognition was highest for the causative agent of Ebola (96.8%), rabies exposure through a bite from an unvaccinated pet (93.0%), and anthrax exposure through inadequately cooked meat (92.5%). Lower proportions recognized RVF exposure through contact with an aborted fetus or placenta (58.8%), rejected the rabies menstrual-blood misconception (60.9%), recognized mosquito transmission of RVF (69.9%), and recognized tick transmission of CCHF (75.7%). KR-20 was 0.50

Table 1 Participant and health-facility characteristics

Characteristic	Overall ($N=345$), n (%)
Age, years, median (IQR)	30 (26–36)
Sex	
Female	201 (58.3)
Male	144 (41.7)
Years in current position, median (IQR)	5 (2–10)
Years at workstation, median (IQR)	2 (1–4)
District	
Ibanda	69 (20.0)
Kazo	91 (26.4)
Kiruhura	83 (24.1)
Mbarara	102 (29.6)
Health-facility type	
Private clinic/medical centre	35 (10.1)
Health centre II	14 (4.1)
Health centre III	135 (39.1)
Health centre IV	76 (22.0)
Hospital	85 (24.6)
Professional cadre	
Medical/clinical	54 (15.7)
Nursing	192 (55.7)
Nursing qualification ($n=192$)	
Certificate	129 (67.2)
Diploma	53 (27.6)
Degree	10 (5.2)
Laboratory	52 (15.1)
Other	47 (13.6)
Training programme and/or CME reported as an information source*	258/335 (77.0)
Owns smartphone	312 (90.4)

IQR interquartile range, *CME* continuing medical education

**The training/CME denominator was 335 because 10 respondents did not answer the parent information-source item*

for the overall 11-item index, 0.60 for the five causative-agent items, and 0.36 for the six exposure/transmission items. Full item-level results are shown in Supplementary Figure S1.

For the selected One Health indicators, 205/338 (60.7%) recognized linkage of human, animal, and environmental health, 254/338 (75.1%) recognized medical-veterinary-public-health partnership, and 94/335 (28.1%) reported veterinary officials as a source of information on the selected zoonoses (Table 2).

3.3 Referral readiness and completion of the referral pathway

Nearly all respondents (338/345; 98.0%) reported advising testing when they suspected one of the selected zoonoses. Overall, 297/345 (86.1%) reported referring specimens elsewhere for testing, whereas 48/345 (13.9%) reported no specimen-referral linkage. Of the 297 respondents reporting referral, 296 answered the feedback item and 294/296 (99.3%) reported receiving results. Referral-to-result interval data were available for 280/297 (94.3%) referrers; the median reported interval was 10 days (IQR 7–14), and 134/280 (47.9%) reported intervals of at least 14 days. Descriptively, the median interval was 14 days (IQR 7–14; $n=136$) among respondents at peripheral facilities and 7 days (IQR 5.75–14; $n=144$) among those at HCIVs/hospitals. Results were reported as communicated to patients by 294/297 (99.0%) respondents who referred specimens (Fig. 1).

3.4 Self-reported specimen-referral linkage across facility groups

Self-reported absence of specimen-referral linkage was reported by 37/184 (20.1%) respondents at peripheral facilities compared with 11/161 (6.8%) at HCIVs/hospitals. By facility type, the prevalence was 42.9% at HCIIs,

Table 2 Selected recognition, referral-readiness, and One Health indicators

Indicator	Result
Recognition readiness	
Heard of selected priority zoonoses	344/345 (99.7%)
11-item recognition index, median (IQR), range 0–11	9 (8–10); $n=344$
RVF: recognises aborted fetus/placenta exposure	203/345 (58.8%)
RVF: recognises mosquito transmission	241/345 (69.9%)
CCHF: recognises tick transmission	261/345 (75.7%)
Rabies: rejects menstrual-blood misconception	210/345 (60.9%)
Referral readiness	
Advises testing when zoonosis is suspected	338/345 (98.0%)
Reports specimen referral elsewhere for testing	297/345 (86.1%)
Self-reported absence of specimen-referral linkage	48/345 (13.9%)
Receives referral feedback, among referrers with feedback data	294/296 (99.3%)
Referral-to-result interval, median (IQR), days	10 (7–14); $n=280$
Referral-to-result interval ≥ 14 days	134/280 (47.9%)
Communicates results to patients, among respondents reporting referral	294/297 (99.0%)
One Health and system context	
Recognises human-animal-environment linkage	205/338 (60.7%)
Recognises medical-veterinary-public-health partnership	254/338 (75.1%)
Veterinary officials reported as an information source	94/335 (28.1%)
Inadequate/lack of diagnostic resources reported as a barrier	312/345 (90.4%)
Unreliable transport reported as a barrier	259/345 (75.1%)
Inadequate collaboration with other relevant sectors reported as a barrier	256/345 (74.2%)
Paper-based system reported as a barrier	230/345 (66.7%)

The 11-item recognition index is a formative summary of prespecified disease-specific items and is not a validated unidimensional scale; One Health indicators are item-level responses and do not directly measure operational practice The referral-to-result interval is a generic health-worker-reported interval to receipt of results and should not be interpreted as analytic laboratory turnaround time

25.7% at private clinics/medical centres, 16.3% at HCIIIs, 6.6% at HCIVs, and 7.1% at hospitals (Fig. 2). Among the 44 facilities with at least two respondents, 16 (36.4%) had mixed Yes/No reports about specimen referral; overall within-facility pairwise agreement was 85.5%, and the random-intercept logistic ICC was 0.58.

In crude analysis, respondents at peripheral facilities were 2.94 times as likely to report no specimen-referral linkage as those at HCIVs/hospitals (PR 2.94, 95% CI 1.43–6.07; $p=0.003$). In the prespecified adjusted model using CR2 small-sample-corrected facility-clustered inference, the association persisted (aPR 2.74, 95% CI 1.24–6.04; $p=0.016$). The adjusted analysis included 335 respondents, 47 outcome events, and 54 facility clusters. There was no clear evidence of association between district, professional cadre, or training/CME and self-reported absence of specimen-referral linkage (Table 3).

Sensitivity analyses yielded similar facility-group estimates. GEE models produced estimates for peripheral facilities using exchangeable (aPR 2.63, 95% CI 1.30–5.32; $p=0.007$) and independence (aPR 2.74, 95% CI 1.41–5.32; $p=0.003$) working correlations. Adding the total 11-item recognition index to the CR2 model yielded an aPR of 2.73 (95% CI 1.25–5.93; $p=0.015$); the recognition index itself showed no clear evidence of association (PR 1.08 per point, 95% CI 0.82–1.42; $p=0.564$). Models using the causative-agent and exposure/transmission domains separately or jointly produced facility-group aPRs of 2.72–2.75. These analyses are summarized in Supplementary Table S2.

3.5 Reported specimen-referral destinations

Among 273 respondents who named a referral destination, 121 (44.3%) described a national/reference laboratory pathway, 70 (25.6%) a regional referral hospital, and 58 (21.2%) a district/HCIV/hub pathway. Private laboratories accounted for 20 (7.3%); three respondents reported multiple referral levels and one response was other/unclear. Additional referral-pathway details, including facility-type/group prevalence and result-return modes, are provided in Supplementary Table S1.

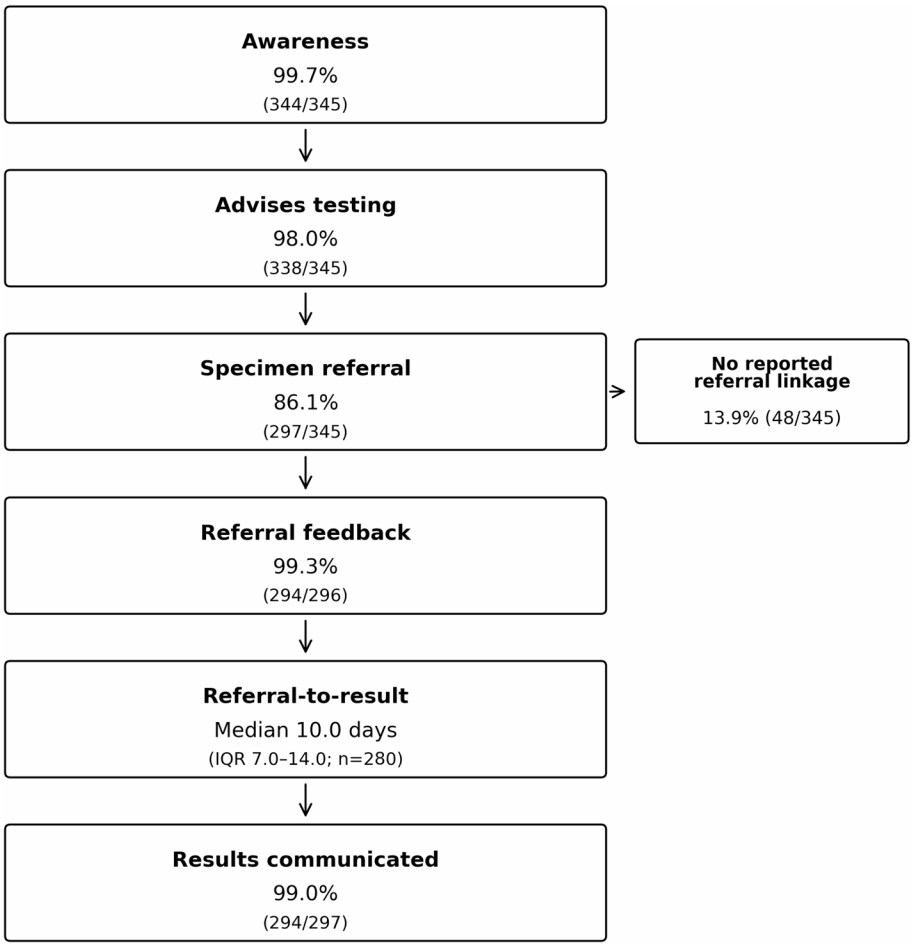


Fig. 1 Frontline recognition and specimen-referral pathway assessed in the study. Arrows depict a conceptual pathway rather than longitudinal attrition; denominators differ by indicator. The referral-to-result interval is respondent-reported and generic and should not be interpreted as analytic laboratory turnaround time

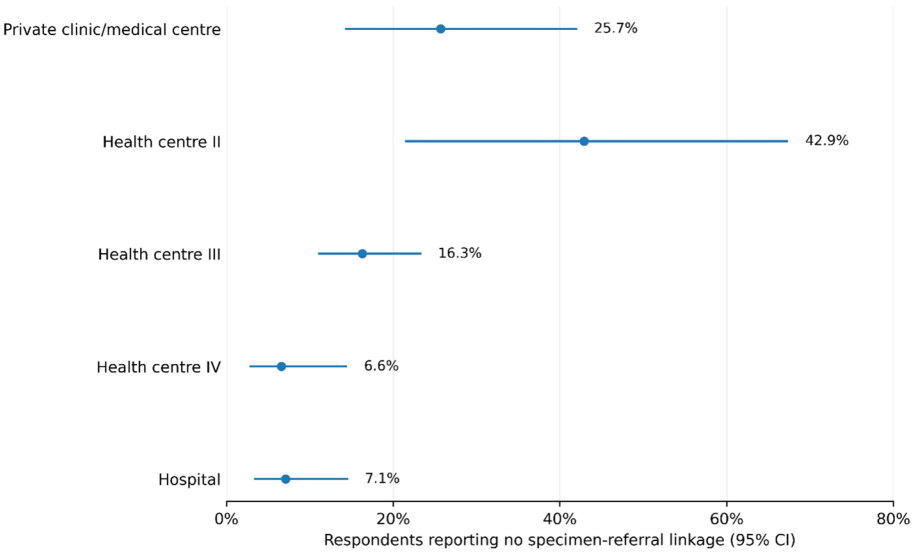


Fig. 2 Self-reported absence of specimen-referral linkage by health-facility type. Points show crude respondent-level prevalences with Wilson 95% confidence intervals. The measure represents health-worker self-report and not an audited facility attribute

Table 3 Factors associated with self-reported absence of specimen-referral linkage

Variable	cPR (95% CI)	*p*-value	aPR (95% CI)	*p*-value
Facility group				
HCIV/hospital	1.00 (Ref)	–	1.00 (Ref)	–
Peripheral facilities (private clinic/medical centre, HCII, HCIII)	2.94 (1.43–6.07)	0.003	2.74 (1.24–6.04)	0.016
District				
Mbarara	1.00 (Ref)	–	1.00 (Ref)	–
Ibanda	1.58 (0.61–4.09)	0.342	1.35 (0.48–3.78)	0.551
Kazo	0.88 (0.27–2.85)	0.832	0.76 (0.23–2.52)	0.636
Kiruhura	0.70 (0.26–1.91)	0.489	0.85 (0.27–2.66)	0.753
Professional cadre				
Medical/clinical	1.00 (Ref)	–	1.00 (Ref)	–
Nursing	1.09 (0.49–2.42)	0.832	0.95 (0.41–2.21)	0.900
Laboratory	0.52 (0.15–1.79)	0.300	0.48 (0.13–1.74)	0.248
Other	0.72 (0.24–2.16)	0.556	0.78 (0.21–2.93)	0.699
Training programme/CME reported as an information source				
No	1.00 (Ref)	–	1.00 (Ref)	–
Yes	0.78 (0.35–1.72)	0.540	0.86 (0.37–2.02)	0.720
Additional participant characteristics (crude analyses only)				
Age, per 1-year increase	1.01 (0.97–1.04)	0.797	–	–
Sex				
Male	1.00 (Ref)	–	–	–
Female	1.19 (0.68–2.10)	0.537	–	–
Education level				
College/university	1.00 (Ref)	–	–	–
Primary/secondary	0.79 (0.25–2.52)	0.696	–	–
Years in current position, per 1-year increase	0.98 (0.93–1.04)	0.496	–	–
Years at workstation, per 1-year increase	0.95 (0.84–1.07)	0.377	–	–

cPR crude prevalence ratio, *aPR* adjusted prevalence ratio, *CI* confidence interval, *Ref* reference category, *CME* continuing medical education, *HCII* health centre II; *HCIII* health centre III; *HCIV* health centre IV. Adjusted estimates use CR2 small-sample-corrected standard errors clustered by facility with Satterthwaite degrees of freedom. The adjusted model included facility group, district, professional cadre, and training/CME reported as an information source ($N=335$; 47 outcome events; 54 facility clusters). Additional participant characteristics are shown as crude analyses only and were not selected for adjustment based on statistical significance

3.6 One Health and health-system constraints

Health workers reported multiple constraints. The most frequent were inadequate or absent diagnostic resources (90.4%), inadequate community awareness (86.4%), and late presentation to health facilities (86.4%). Unreliable transport (75.1%), nonspecific clinical signs and symptoms (74.5%), inadequate collaboration with other relevant sectors (74.2%), inadequate knowledge and skills (73.3%), and paper-based systems (66.7%) were also reported. The full distribution of reported barriers is shown in Supplementary Figure S2.

Among respondents with data on result-return modes, physical collection of results on paper was reported by 52.1%, digital technology by 38.4%, and phone call/SMS by 37.0%; modes were not mutually exclusive. For communication of results to patients, physical paper collection was reported by 64.5%, phone call/SMS by 49.8%, and digital technology by 8.5%.

4 Discussion

This multi-district survey found generally high frontline recognition of selected priority zoonoses but a consistent respondent-level difference in reported specimen-referral linkage across facility groups. Health workers at peripheral facilities more often reported no specimen-referral linkage than those at HCIVs/hospitals, and this association remained after conservative small-sample correction and across GEE and recognition-domain sensitivity analyses. Among respondents reporting referral, receipt and communication of results were highly

complete, although the reported time from referral to result receipt was often prolonged. Together, these findings distinguish individual disease recognition from the system connectivity needed to move specimens and results through the diagnostic network.

The facility-group association should be interpreted as a difference in health-worker reports, not as evidence that peripheral facilities objectively lacked referral pathways. Within-facility responses were mixed in more than one-third of facilities with at least two respondents, underscoring that awareness of, access to, or use of the same referral pathway may differ among workers at a facility. Nevertheless, the stability of the facility-group estimate across CR2 and GEE analyses suggests that the observed respondent-level pattern is not explained by the choice of clustered modelling approach. This distinction is important because peripheral facilities are not expected to perform confirmatory testing for many high-consequence zoonoses; their role is to recognize suspected cases and connect them to Uganda's higher-level and reference diagnostic network [19, 20].

Among respondents who reported referral and answered the feedback item, almost all reported receiving results, indicating high reported completeness of feedback rather than proof of overall referral-system effectiveness. Timeliness remains a concern: the median reported referral-to-result interval was 10 days, almost half of reported intervals were at least 14 days, and the descriptive median was longer among respondents at peripheral facilities. The interval combined transport, processing, result transmission, and receipt and was based on usual recall rather than a disease-specific episode. A 10-day interval would be concerning for time-sensitive outbreak investigation, particularly for suspected viral haemorrhagic fevers, but these data cannot be interpreted as VHF-specific turnaround performance. Prospective linkage of facility, courier, and laboratory timestamps would be needed to identify where delays occur [15, 21].

Adjustment for measured recognition did not materially change the facility-group association. The 11-item recognition index had modest internal consistency, consistent with its use as a formative summary spanning different diseases and both causative-agent and exposure/transmission cues; domain-specific sensitivity analyses produced nearly identical facility-group estimates. These findings support treating disease recognition and referral connectivity as related but distinct components of preparedness [9, 14].

The One Health results should also be interpreted as item-level indicators rather than measures of operational collaboration. About three in five respondents selected the human-animal-environment linkage, three-quarters selected medical-veterinary-public-health partnership, and veterinary officials were infrequently reported as an information source. Inadequate intersectoral collaboration was also commonly reported as a barrier. These responses suggest opportunities to strengthen cross-sector information exchange at subnational level, but they do not establish how frequently or effectively human-health, veterinary, and public-health actors collaborate in practice [14, 20–23].

The practical implication is to strengthen the connection between frontline suspicion and existing diagnostic systems, especially at peripheral facilities. Feasible priorities supported by the reported barriers and pathways include maintaining reliable hub-and-spoke specimen transport, making referral routes and contact points explicit to frontline staff, using simple specimen and result tracking, expanding electronic or phone-based result return within existing surveillance systems, and strengthening routine district-level information exchange between human-health, veterinary, and public-health teams. These system measures should complement, rather than replace, targeted disease-specific education.

4.1 Strengths and limitations

This study has several strengths. It included frontline health workers from four districts, multiple professional cadres, and 54 facilities spanning private clinics/medical centres through hospitals, and the primary facility-group association was robust to alternative clustered modelling approaches. Several limitations should be considered. First, recruitment was role-based and purposive; the parent target was 406 health workers but the analytic dataset did not allow reliable reconstruction of how many individuals were approached or declined. Findings therefore may not be generalized to all health workers or facilities in the four districts. Second, referral measures were

self-reported and may be affected by recall, social-desirability, and reporting differences; they were not verified against facility registers, courier logs, laboratory records, or laboratory confirmation. Within-facility disagreement further emphasizes that the primary outcome reflects individual reported awareness, access, or practice rather than an audited facility characteristic. Third, the referral-to-result interval was recalled, generic rather than disease-specific, and did not distinguish transport, laboratory processing, and result-transmission components. Fourth, the recognition index was a study-specific formative summary rather than a validated psychometric scale, and its overall internal consistency was modest. Fifth, only 47 outcome events were available in the complete-case adjusted model; although CR2 and GEE sensitivity analyses yielded concordant estimates, secondary and null associations remain imprecise and type II error is possible. Residual facility-level confounding also cannot be excluded. Finally, the cross-sectional design does not support causal inference.

5 Conclusion

Frontline recognition of selected priority zoonoses was generally strong, but health workers at peripheral facilities more often reported absence of specimen-referral linkage than those at HCIVs/hospitals. This respondent-level finding was robust across alternative clustered analyses but should not be interpreted as an audited facility-level failure. For the Uganda Ministry of Health and district health teams, priorities include reliable specimen transport for peripheral facilities, clear referral and tracking mechanisms, timely result return integrated with existing surveillance systems, and stronger subnational human-veterinary-public health information exchange. Prospective facility- and laboratory-linked studies are needed to identify where delays or breakdowns occur along the recognition-to-result pathway.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1186/s12982-026-03332-9.

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Author contributions RM, CM, DA, AB, AM, PDK, SA, AWW, and ARA contributed to the conceptualization and/or design of the study. DA and AM contributed to field implementation and data collection. RM led data management and statistical analysis, interpreted the findings, and drafted the initial manuscript. FM contributed to interpretation of the findings from an animal-health and One Health perspective and critically revised the manuscript for important intellectual content. All authors approved the final version for submission. All authors agree to be accountable for the work.

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Data availability The de-identified dataset used and analysed for this study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study was reviewed and approved by the Makerere University School of Public Health Research Ethics Committee (SPH-2024-671) and registered with the Uganda National Council for Science and Technology (HS5420ES). Study procedures were conducted in accordance with the Declaration of Helsinki and the Uganda National Council for Science and Technology National Guidelines for Research Involving Humans as Research Participants (2014), which were applicable when the protocol was approved and data were collected. Written informed consent was obtained from all participants before participation. Participation was voluntary, and respondents could decline to answer

individual questions or withdraw from the study at any time without penalty.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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