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Original Research Article

EVALUATION OF RAPID ANTIGEN TEST FOR DETECTING PESTE DES PETITS RUMINANTS VIRUS IN SHEEP AND GOATS IN RURAL SMALL-HOLDER FARMS IN NORTHERN NIGERIA

[1, 2a]CHABIRI, L.A., [1, 2b]WEKA, R.P., [2c]BAMAIYI, P.H., [1c]ADEDEJI, A., [1e]WOMA, T., [1f]ATUMAN, Y. J. & [1g]LUKA, P.D.

[1]National Veterinary Research Institute, Vom, Plateau State, Nigeria; [2]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Jos, Nigeria

ORCID: [a]https://orcid.org/0009-0003-9536-0243; [b]https://orcid.org/0000-0001-6371-9416; [c]https://orcid.org/0000-0002-4031-9599; [d]https://orcid.org/0000-0001-5736-6466; [e]https://orcid.org/0000-0002-7547-7976; [f]https://orcid.org/0000-0002-5864-6790; [g]https://orcid.org/0000-0002-6170-4242

**Correspondence: pamluka08@gmail.com*

ABSTRACT

Peste des petits ruminants (PPR) remains a major constraint to small-ruminant production in Northern Nigeria, where delayed access to laboratory diagnosis can impede outbreak response. This study evaluated the field diagnostic performance of the ID Rapid® PPR Antigen test against real-time reverse-transcription polymerase chain reaction (RT-PCR) among clinically suspected sheep and goats. During outbreak investigations in 13 rural communities across Plateau, Bauchi and Kano States, ocular or nasal swabs were collected from 79 animals. Samples were tested onsite using the antigen-capture lateral-flow assay and subsequently examined by real-time RT-PCR targeting the PPRV nucleoprotein gene. PPRV RNA was detected in 35 samples (44.3%). The rapid test yielded 36 positive, 12 weak-positive, 27 negative and 4 invalid results. When positive and weak-positive outcomes were classified as reactive and invalid results were excluded, analysis of 75 samples yielded sensitivity, specificity and accuracy of 87.9%, 54.8% and 69.3%, respectively. In a secondary analysis restricted to 63 clear positive and negative outcomes, sensitivity was 86.2%, specificity 67.6%, positive predictive value 69.4%, negative predictive value 85.2% and accuracy 76.2%; Cohen's kappa was 0.53, indicating moderate agreement. Concordant positive samples generally had lower cycle-threshold values than discordant samples, consistent with greater detection at higher viral loads. The rapid antigen test offers useful preliminary screening during suspected PPR outbreaks where laboratory access is limited. However, weak-positive, invalid, discordant and clinically suspicious negative results require RT-PCR confirmation. Reliable field use under operational conditions also depends on correct sampling, timely testing, cold-chain maintenance and careful interpretation of faint test lines.

Keywords: Diagnostic accuracy, outbreak investigation, PPRV, rapid antigen test, real-time PCR, small ruminants

INTRODUCTION

Small ruminants are very important to rural households in sub-Saharan Africa, particularly in smallholder communities where families depend on low-input livestock for their livelihoods. They provide meat, milk, manure, income, savings, social security, and quick cash for emergencies such as illness, school fee payments, food shortages, economic shocks, and seasonal market needs (Randolph *et al*., 2007; FAO, 2010; Herrero *et al.,* 2013). Their ability to survive

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harsh climatic conditions, rapid reproduction, and low investment needs make them valuable to resource-poor farmers, women, and young people in rural communities (Njuki & Sanginga, 2013; FAOSTAT, 2021). In Northern Nigeria, sheep and goats are not merely production animals. They are living assets that support nutrition, income generation, social obligations and resilience during periods of uncertainty (FAO, 2013). Despite their value, small ruminant production in Nigeria is limited by infectious diseases, particularly *Peste des petits ruminants* (PPR), which causes high morbidity, mortality, rapid spread, and major socioeconomic losses among sheep and goats (Banyard *et al*., 2010; WOAH, 2021).

PPR is a highly contagious transboundary animal disease caused by *Peste des petits ruminants virus* (PPRV), now classified as small ruminant *Morbillivirus*, an envelope, negative-sense, single stranded RNA virus belonging to the genus *Morbillivirus* in the family *Paramyxoviridae* (ICTV, 2020; Balamurugan *et al.,* 2021). The disease primarily affects goats and sheep but has also been diagnosed in some wildlife and other domestic species (Bello *et al.,* 2018; WOAH, 2021). Clinically, PPR is characterized by fever, depression, ocular and nasal discharges, erosive and ulcerative oral lesions, respiratory distress, coughing, diarrhoea, dehydration, weakness and death in severe cases (Balamurugan *et al.,* 2014; Parida *et al.,* 2015; WOAH, 2021). These clinical signs are important for field recognition, especially in rural areas where laboratory infrastructure is limited. However, clinical diagnosis alone is not sufficient for definitive confirmation because PPR may resemble other small ruminant diseases such as contagious caprine pleuropneumonia, bluetongue, contagious ecthyma, pasteurellosis, and other causes of respiratory or enteric disease (Balamurugan *et al.,* 2014; Santhamani *et al.,* 2016). This is a major challenge during outbreaks because quick decisions are needed for animal movement control, vaccination, farmer awareness, and official disease reporting (Balamurugan *et al.,* 2014; Santhamani *et al.,* 2016). Accurate and timely diagnosis is therefore central to PPR surveillance, outbreak confirmation, and eradication planning (WOAH, 2021).

The global control and eradication strategy for PPR, jointly promoted by the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, emphasizes improved surveillance, rapid detection, laboratory confirmation, vaccination, and strengthened veterinary services as critical pillars for achieving global eradication by 2030 (FAO & WOAH, 2015; WOAH & FAO, 2016). In endemic regions like Nigeria, PPR control depends not only on vaccine availability but also on early outbreak detection, laboratory confirmation, and reliable diagnostic evidence to guide targeted response (Balamurugan *et al.*, 2014; Santhamani *et al.,* 2016). This is particularly important in rural smallholder communities, where disease reporting is often delayed, samples may be poorly preserved, and farmers may first consult informal animal health providers before official veterinary services are involved (FAO & WOAH, 2015; Woma *et al.*, 2016a).

Several diagnostic methods are available for PPRV detection. Conventional methods such as virus isolation, agar gel immunodiffusion, counter immunoelectrophoresis, virus neutralization, and antigencapture ELISA have historically contributed to PPR diagnosis, but many are time-consuming, technically demanding, or dependent on well-equipped laboratories (Luka *et al.,* 2011; Santhamani *et al.,* 2016). Molecular assays, particularly reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR, have become preferred methods for confirmatory diagnosis because they directly detect viral RNA and offer high sensitivity and specificity (Couacy-Hymann *et al.,* 2002; Luka *et al.,* 2011; Balamurugan *et al.,* 2014). Real-time RT-PCR is useful for rapid and sensitive detection of PPRV RNA and can estimate viral genome load through Ct values. However, its use depends on laboratory facilities, trained personnel, quality reagents, and proper sample handling, which may be limited in remote rural settings (Santhamani *et al.*, 2016; WOAH, 2021).

Field-based rapid antigen tests are useful alternatives to laboratory molecular tests, but they are usually less sensitive because they need enough viral antigens to produce visible test-lines (Baron *et al.,* 2014). These tests are designed to detect PPRV antigen in clinical samples such as ocular, nasal, or oral swabs and can provide results within minutes, making them useful for early outbreak screening and decision-making at farm or community level (Baron *et al.,* 2014; Halecker *et al.,* 2020). The ID Rapid® PPR Antigen test, for example, is an immunochromatographic assay developed for rapid detection of PPRV antigen and has been reported by the manufacturer and comparative evaluations to be simple, field-adaptable, and capable of detecting PPRV across lineages (IDvet, 2019). False-negative results may occur when viral loads are low, samples are collected too early or too late during infection, or sample quality is poor. Similarly, false-positive or discordant results may arise from field-handling errors, difficulties in reading test lines, or differences between antigen-based detection and viral RNA detection methods (Couacy-Hymann *et al.,* 2007; Baron *et al.,* 2014; Halecker *et al.,* 2020).

Evaluating rapid antigen tests under real field conditions is important, as their performance may vary with sample type, infection stage, sample handling, field conditions and disease prevalence. Measures such as sensitivity, specificity, predictive values, agreement, and Cohen's kappa help determine their usefulness for field screening and timely

diagnosis. Comparing rapid test results with real-time RT-PCR and Ct values also helps explain discordant results, especially where viral load is low or antigen detection is limited. PPR remains endemic in Nigeria and continues to cause substantial losses among smallholder sheep and goat farmers.

Previous Nigerian studies have documented PPR occurrence, seroprevalence, molecular detection, and circulation of different viral lineages in several parts of the country (Luka *et al.,* 2011; Woma *et al.,* 2016b; Mantip *et al*., 2021; Esonu *et al.,* 2022). However, there remains limited field-based evidence on how rapid antigen tests perform during suspected PPR outbreaks in rural smallholder settings, particularly in Northern Nigeria, where veterinary access, reporting systems, and laboratory confirmation may be delayed. This evidence-gap has practical implications for surveillance and control because rapid tests are often most valuable in precisely those areas where confirmatory laboratory testing is least immediately accessible. Without local performance data, animal health workers may either over-rely on rapid tests without confirmatory testing or underutilize them despite their potential value for early outbreak response.

This study evaluated the field performance of a rapid antigen test for detecting *Peste des petits ruminants virus* among sheep and goats during suspected outbreaks in rural smallholder communities across Plateau, Bauchi and Kano states of Nigeria. The study specifically assessed the clinical presentation of suspected PPR cases, confirmed PPRV infection by real-time RT-PCR as the reference diagnostic method, determined the diagnostic sensitivity, specificity, predictive values, accuracy, and agreement of the rapid antigen test, and examined the relationship between RT-PCR Ct values and rapid outcomes of the test. By generating field-based evidence from rural outbreak conditions, the findings provide practical information on the usefulness and limitations of rapid antigen testing as an early screening tool for PPR control and eradication efforts in Nigeria.

MATERIALS AND METHODS

STUDY DESIGN

This study adopted a field-based cross-sectional diagnostic accuracy design to evaluate the performance of a rapid antigen test for detecting *Peste des petits ruminants virus* in clinically suspected sheep and goats during outbreaks in rural smallholder communities of Northern Nigeria. Outbreak investigations were conducted following reports of suspected PPR in sheep and goats. Field screening was performed using a rapid antigen test kit, while real-time RT-PCR was used as the confirmatory reference method for detecting PPRV infection (Couacy-Hymann *et al.,* 2002; Baron *et al*., 2014; Santhamani *et al.,* 2016; WOAH, 2021).

STUDY AREA

The study was conducted in selected rural smallholder communities in Plateau, Bauchi, and Kano States, located in the North-Central, North-Eastern, and North-Western geopolitical zones of Nigeria, respectively.

These states are important small ruminant-producing areas with predominantly agrarian rural economies, where sheep and goats serve as key livelihood assets. Although the states differ in ecology, climate, population, and production systems, previous studies have reported PPR occurrence and exposure among small ruminants in these areas, supporting their suitability for evaluating a fieldbased rapid antigen test during suspected PPR outbreaks (Woma *et al.,* 2016b; Bello *et al.,* 2018; Esonu *et al.,* 2022; Chabiri *et al.,* 2023; Rayyanu *et al*., 2023).

OUTBREAK LOCATIONS

Outbreak investigations were conducted in 13 communities across 11 local government areas (LGAs) where farmers reported clinical disease and mortality among sheep and goats. The communities were purposively selected based on reports of suspected PPR outbreaks and included Bisichi, Buhit, Bwall, Miango, Dakang, and Pasankai in Plateau State; Nabordo, Bakin-Kogi, and Yakubari in Bauchi State; and Ajumawa, Bagwai, Gurjiya, and Panshekara in Kano State.

STUDY POPULATION

The study population comprised sheep and goats from rural communities in Plateau, Bauchi, and Kano States where suspected PPR outbreaks were reported. Animals of different ages and sexes were considered eligible for sampling during outbreak investigations. The focus on sheep and goats was based on their known susceptibility to PPR and their importance in rural livestock production systems in northern Nigeria, where the disease has been previously reported among small ruminants (Woma *et al.,* 2016b; Esonu *et al.,* 2022; Rayyanu *et al.,* 2023).

CASE DEFINITION

A suspected PPR case was defined as any sheep or goat from an affected flock presenting with clinical signs suggestive of PPR, including fever, depression, ocular or nasal discharge, oral erosions or lesions, salivation, cough, dyspnoea, diarrhoea, dehydration, pasted hindquarters, or death, particularly where morbidity or mortality was reported within the flock (FAO, 1999; WOAH, 2024).

MATERIALS

The materials used included sterile nasal and ocular swabs, sample collection tubes, disposable gloves, cool boxes, ice packs, field data collection forms, permanent markers,

personal protective equipment, and the ID Rapid® PPR Antigen test kit. Laboratory materials included RNA extraction materials, QIAGEN One Step RT-PCR Kit, PPRV-specific primers, nuclease-free water, PCR tubes, micropipettes, filter tips, cold storage facilities, and real-time PCR equipment.

SAMPLE COLLECTION

Swabs of ocular or nasal discharge were collected from sheep and/or goats which were manifesting oral lesions, diarrhoea, respiratory signs, depression or weakness, consistent with standard clinical descriptions of PPR in small ruminants (FAO, 1999; WOAH, 2024). After collection, samples were tested immediately in the field using the rapid antigen test and preserved and transported under cold-chain conditions to the laboratory for molecular confirmation.

FIELD RAPID ANTIGEN TESTING

Field testing was conducted on-site using the ID Rapid® PPR Antigen test according to the manufacturer's instructions (IDvet, 2019). The rapid antigen test was used as a pen-side screening tool because it detects PPRV antigen directly from clinical samples and provides results within 10-15minutes, making it useful during field outbreak investigations where access to laboratory confirmation may be delayed (Baron *et al.,* 2014; Halecker *et al.,* 2020; WOAH, 2024). Swab specimens were placed into extraction buffer supplied with the ID Rapid® PPR Antigen kit and mixed thoroughly to release viral antigen. The test is an antigen-capture lateral-flow immunochromatographic assay. As the extract migrated along the dipstick by capillary action, any PPRV antigen present bound to labelled anti-PPRV antibodies, and the resulting antigen–antibody complexes were captured at the test region to produce a visible coloured band. The control band confirmed adequate sample migration and reagent functionality. The manufacturer-recommended volume of extract was applied to the test strip, and the results were read after 10–15 min (IDvet, 2019). A visible control line with a visible test line was recorded as positive, a visible control line without a test line was recorded as negative, while absence of the control line was recorded as invalid (Baron *et al.,* 2014; IDvet, 2019; Halecker *et al.,* 2020). The four invalid results were excluded from the diagnostic-performance analysis. Corresponding specimens were transported under cold-chain conditions to the Biotechnology Centre, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, for real-time RT-PCR confirmation.

MOLECULAR DETECTION BY REAL-TIME PCR

Viral RNA was extracted from clinical samples and amplified using the QIAGEN OneStep RT-PCR Kit according to the manufacturer's instructions. The assay targeted the PPRV nucleoprotein (N) gene using the PPRV-specific primers NP3:
5′-GTCTCGGAAATCGCCTCACAGACT-3′ and NP4: 5′CCTCCTCCTGGTCCTCCAGAATCT-3′, as described by Couacy-Hymann *et al.* (2002). Amplification was performed on a real-time PCR platform, using one-step RT-PCR cycling conditions that included reverse transcription, initial enzyme activation/denaturation, and repeated amplification cycles with fluorescence detection during amplification. Each run included extracted test RNA, a PPRV-positive control, a negative extraction control, and a non-template control to validate the assay. Results were interpreted using amplification curves and cycle threshold (Ct) values. A sample was considered positive for PPRV RNA when a typical amplification curve was observed with a Ct value of ≤35. Samples with no amplification or Ct values >35 were interpreted as negative or as showing late, non-confirmatory amplification, provided that the positive control amplified appropriately and the negative extraction and no-template controls showed no amplification. Ct values were used as an indirect indicator of viral RNA concentration, with lower Ct values suggesting a higher viral genome load and higher Ct values suggesting a lower viral genome load (Bustin, 2002; Couacy-Hymann *et al.,* 2002; Balamurugan *et al.,* 2014; Santhamani *et al.,* 2016; WOAH, 2023).

DIAGNOSTIC PERFORMANCE ANALYSIS

The field-based rapid antigen test was evaluated as the index test, using real-time RT-PCR as the reference standard for PPRV detection. Diagnostic accuracy was assessed using a 2 × 2 contingency table, as recommended for diagnostic accuracy studies (Bossuyt *et al.*, 2015; Cohen *et al.,* 2016). Samples positive by both the rapid antigen test and RT-PCR were classified as true positives, while samples negative by both tests were classified as true negatives. Samples positive by the rapid antigen test but negative by RT-PCR were classified as false positives, whereas samples negative by the rapid antigen test but positive by RT-PCR were classified as false negatives.

Sensitivity, specificity, positive predictive value, negative predictive value, overall accuracy, and Cohen's kappa were calculated to assess diagnostic performance of the rapid antigen test under field conditions. Sensitivity was calculated as TP / (TP + FN), while specificity was calculated as TN / (TN + FP). Positive predictive value was calculated as TP / (TP + FP), negative predictive value as TN / (TN + FN), and overall diagnostic accuracy as (TP + TN) / total number of samples tested. These indices were used to determine ability of the rapid antigen test to correctly identify PPRV-positive and PPRV negative samples (Shreffler & Huecker, 2023). The overall agreement and Cohen's kappa were used to

assess the level of agreement between the rapid antigen test and real-time RT-PCR beyond chance (Dohoo *et al.*, 2009; Thrusfield, 2018).

DATA ANALYSIS

Data were summarized using frequencies, percentages, and diagnostic performance indices. The association between Ct values and rapid test outcomes was assessed by comparing Ct-value distributions among concordant and discordant samples. Statistical interpretation focused on the ability of the rapid antigen test to support field screening and outbreak investigation under rural smallholder conditions. Diagnostic performance was assessed using sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, with real-time RT-PCR as the reference standard. Cohen's kappa statistic was used to determine the level of agreement between the rapid antigen test and real-time RT-PCR beyond chance. Ct values were further compared between concordant and discordant results to assess the influence of viral load on rapid test outcomes (Bustin *et al*., 2009; Dohoo *et al*., 2009; Santhamani *et al*., 2016; Thrusfield, 2018; Halecker *et al*., 2020).

ETHICAL CONSIDERATIONS

Field investigations were conducted with the consent and cooperation of livestock owners. Ethical clearance was obtained from the University of Jos before the commencement of the study (REF/UJ/FPS/PCL/AEU/2). Informed consent was obtained from livestock owners before clinical examinations and samples collection. All animals were handled humanely, and samples were collected following standard veterinary diagnostic procedures.

RESULTS

OVERVIEW OF SAMPLE CHARACTERISTICS AND PPRV DETECTION

A total of 79 sheep and goats with clinical suspicion of PPR were sampled during outbreaks in rural communities of Plateau, Bauchi, and Kano States. Forty-three (43) were ovine and 36 were caprine, comprising both young and adult animals of either sex (Table I).

Table I: Species, Age and Sex Distribution of Sheep and Goats (n = 79) sampled during suspected PPR Outbreaks in Plateau, Bauchi and Kano States, Nigeria.

Variable	Category	Frequency (n)	Percentage (%)
Species	Ovine	43	54.4
	Caprine	36	45.6
Age	Young	43	54.4
	Adult	36	45.6
Sex	Female	47	59.5
	Male	32	40.5
Total		**79**	**100.0**

CLINICAL PRESENTATION OF SUSPECTED PPR OUTBREAK CASES

Clinical signs were recorded for the 79 sampled outbreak cases with multiple signs documented in some animals (Table II). The sampled sheep and goats showed clinical features consistent with suspected PPR infection, including diarrhoea, nasal discharges, coughing, ocular discharges, anorexia, weakness, depression, and fever. The most frequently observed clinical sign was diarrhoea (n = 30), followed by nasal discharges (n = 27), coughing (n = 24), ocular discharges (n = 22), and anorexia (n = 21). Other signs included weakness (n = 12), depression (n = 8), fever (n = 6), and oral lesions (n = 4).

Table II: Frequency of clinical signs observed among sheep and goats (n = 79) sampled during suspected PPR outbreaks in Plateau, Bauchi and Kano States, Nigeria.

Clinical sign	Frequency (n)
Diarrhoea	30
Nasal discharge	27
Coughing	24
Ocular discharge	22
Anorexia	21
Weakness	12
Depression	8
Fever	6
Oral lesions	4

Note: Individual animals could present with more than one clinical sign; therefore, the frequencies and percentages do not sum to the total number of sampled animals or 100%, respectively.

Thirty-five (35) of the samples were confirmed positive for PPRV by Real-time RT-PCR, giving an overall molecular

detection rate of 44.3% (35/79). The rapid antigen test yielded 36 positive, 12 weak-positive, 27 negatives, and 4 invalid results (Table III).

Table III: Cross-tabulation of ID Rapid® PPR Antigen test outcomes against real-time RT-PCR Results among sheep and goats (n = 79) sampled during suspected PPR outbreaks in Plateau, Bauchi and Kano States, Nigeria

ID Rapid® PPR Antigen test result	Real-time RT-PCR positive	Real-time RT-PCR negative	Total
Positive	25	11	36
Weak-positive	4	8	12
Negative	4	23	27
Invalid	2	2	4
Total	**35**	**44**	**79**

Note: **Weak-positive and invalid rapid-test outcomes were retained in this descriptive cross-tabulation but were excluded from the clear-result diagnostic-performance analysis.**

DISTRIBUTION OF RAPID ANTIGEN TEST AND RT-PCR RESULTS BY STATE

Rapid test reactivity was highest in Plateau State, where 26 of 38 samples were reactive, followed by Bauchi with 11 of 19 samples and Kano with 11 of 22 samples (Table IV). Using RTPCR as the confirmatory test, 35 samples were positive for PPRV RNA, giving an overall molecular detection rate of 44.3%. Plateau State recorded the highest RT-PCR positivity rate, with 21 of 38 samples positive. This was followed by Bauchi State, with 8 of 19 samples positive, while Kano State had the lowest positivity rate, with 6 of 22 samples positive. Overall, the findings confirmed the presence of PPRV in suspected outbreak cases across all three states, with the highest proportion of confirmed cases observed in Plateau State.

AGREEMENT BETWEEN RAPID ANTIGEN AND RT-PCR

Of the 36 samples that tested positive using the rapid antigen test, 25 were positive and 11 were negative by real-time RT-PCR. Among the 27 rapid-test-negative samples, 23 were negative and 4 were positive by real-time RT-PCR.

The 12 weak-positive rapid-test results comprised 4 real-time RT-PCR-positive and 8 real-time RT-PCR-negative samples. Four rapid-test results were invalid, comprising 2 real-time RT-PCR-positive and 2 real-time RT-PCR-negative samples. For the reactive/non-reactive analysis, positive and weak-positive rapid-test results were classified as reactive, whereas negative results were classified as non-reactive.

After excluding the four invalid results, 52 of the 75 results were concordant, comprising 29 reactive/real-time RT-PCR-positive and 23 non-reactive/real-time RT-PCR-negative results. The remaining 23 results were discordant, comprising 19 reactive/real-time RT-PCR-negative and 4 non-reactive/real-time RT-PCR-positive results (Table V).

In the clear-result analysis, weak-positive and invalid rapid-test results were excluded, leaving 63 samples.

Of these, 48 results were concordant, comprising 25 concordant positive and 23 concordant negative results, while 15 were discordant, comprising 11 rapid-test-positive/real-time RT-PCR-negative and 4 rapid-test-negative/real-time RT-PCR-positive results.

Table IV: **State-level distribution of ID Rapid® PPR Antigen test and real-time RT-PCR results among sheep and goats (n = 79) sampled during suspected PPR outbreaks in Plateau, Bauchi and Kano States, Nigeria**

Variable	Plateau n (%)	Bauchi n (%)	Kano n (%)	Total n (%)
Samples tested	38 (48.1)	19 (24.1)	22 (27.8)	79 (100.0)
Rapid test positive	20 (52.6)	8 (42.1)	8 (36.4)	36 (45.6)
Rapid test weak positive	6 (15.8)	3 (15.8)	3 (13.6)	12 (15.2)
Rapid test negative	11 (28.9)	6 (31.6)	10 (45.5)	27 (34.2)
Rapid test invalid	1 (2.6)	2 (10.5)	1 (4.5)	4 (5.1)
Rapid test reactive*	26 (68.4)	11 (57.9)	11 (50.0)	48 (60.8)
RT-PCR positive	21 (55.3)	8 (42.1)	6 (27.3)	35 (44.3)
RT-PCR negative	17 (44.7)	11 (57.9)	16 (72.7)	44 (55.7)

Note: Rapid test reactive = positive + weak positive

Table V: Cross-tabulation of reactive and non-reactive ID Rapid® PPR Antigen test results against real-time RT-PCR results among suspected PPR outbreak samples in Plateau, Bauchi and Kano States, Nigeria (n = 75).

ID Rapid® PPR Antigen test classification	Real-time RT-PCR positive	Real-time RT-PCR negative	Total
Reactive	29	19	48
Non-reactive	4	23	27
Total	33	42	75

Note: Reactive results comprised positive and weak-positive rapid-test result whereas non-reactive results comprised negative rapid-test results. Four invalid results were excluded.

DIAGNOSTIC PERFORMANCE OF THE RAPID ANTIGEN TEST

For the reactive/non-reactive analysis, 75 samples were included after excluding the four-invalid rapid-test results. Positive and weak-positive results were classified as reactive. This classification produced 29 true-positive, 23 true-negative, 19 false-positive and 4 false-negative results. Accordingly, the rapid antigen test had a sensitivity of 87.9%, specificity of 54.8% and overall accuracy of 69.3% relative to real-time RT-PCR.

A secondary analysis was conducted using only clear positive and negative rapid-test results. After excluding 12 weak-positive and 4 invalid results, 63 samples remained. This analysis produced 25 true-positive, 23 true-negative, 11 false-positive and 4 false-negative results.

The resulting sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy were 86.2%, 67.6%, 69.4%, 85.2% and 76.2%, respectively.

Cohen's kappa was 0.53, indicating moderate agreement between the rapid antigen test and real-time RT-PCR (Table VI).

Using the diagnostic table:

True positive: RDT reactive and RT-PCR positive
False positive: RDT reactive but RT-PCR negative
False negative: RDT non-reactive but RT-PCR positive
True negative: RDT non-reactive and RT-PCR negative 23

29
19
4

The sensitivity of the rapid antigen test was:

$$\text{Sensitivity} = 29 / (29 + 4) \times 100 = 87.9\%$$

The specificity was:

$$\text{Specificity} = 23 / (23 + 19) \times 100 = 54.8\%$$

The overall accuracy was:

$$\text{Accuracy} = (29 + 23) / 75 \times 100 = 69.3\%$$

Table VI: Diagnostic performance of the ID Rapid® PPR Antigen test relative to real-time RT-PCR using only clear positive and negative rapid-test results from sheep and goats sampled during suspected PPR outbreaks in Plateau, Bauchi and Kano States, Nigeria (n = 63).

Performance indicator	Value
True positive	25
True negative	23
False positive	11
False negative	4
Sensitivity	86.2%
Specificity	67.6%
Positive predictive value	69.4%
Negative predictive value	85.2%
Overall accuracy	76.2%
Cohen's kappa	0.53

Note: The analysis excluded 12 weak-positive and 4 invalid rapid-test results.

RELATIONSHIP BETWEEN CT VALUES AND RAPID TEST OUTCOMES

Cycle threshold values differed between concordant rapid antigen test-positive/RT-PCR-positive samples and rapid antigen test-positive/RT-PCR-negative discordant samples. Samples that were positive by both rapid antigen test and RT-PCR had lower Ct values, with a median Ct value of 23.1 and a range of 15.8–32.0 (Figure I).

In contrast, rapid antigen test-positive samples that were negative by RT-PCR but had recorded Ct values showed generally higher Ct values, with a median of 37.0 and a range of 34.0–39.0. Most of these values were close to or above the Ct threshold of 35.

DISCUSSION

This study confirmed the presence of PPRV among clinically suspected sheep and goats sampled during outbreak investigations in Plateau, Bauchi, and Kano States. The detection of PPRV RNA by RT-PCR suggests that continued *PPR virus* is circulating among small ruminant populations in Northern Nigeria. This agrees with previous reports of molecular evidence of PPRV infection in sheep and goats across different parts of Nigeria (Luka *et al.*, 2011; Woma *et al*., 2016a; Mantip *et al.*, 2021; Esonu *et al.*, 2022).

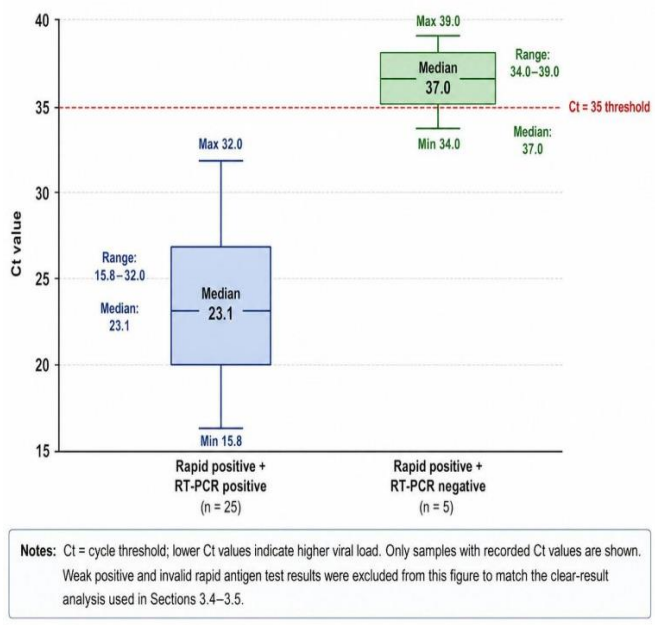

Figure I: Distribution of real-time RT-PCR cycle threshold (Ct) values among samples, stratified by ID Rapid® PPR Antigen test outcome, from sheep and goats sampled during suspected PPR outbreaks in Plateau, Bauchi and Kano States, Nigeria.

The clinical signs observed among the sampled animals were consistent with suspected PPR infection. The signs included diarrhoea, nasal discharges, coughing, ocular discharges, anorexia, weakness, depression, and fever, while oral lesions were less frequently recorded. These findings are consistent with established descriptions of PPR as an acute contagious disease of small ruminants commonly associated with fever, depression, ocular and nasal discharges, respiratory signs, oral erosions, diarrhoea, dehydration, and mortality in severe cases (Balamurugan *et al.*, 2014; Parida *et al.*, 2015; WOAH, 2021). The relatively low occurrence of oral lesions in this study may be related to differences in stage of the disease at the time of sampling, which reinforces the need for laboratory confirmation because clinical diagnosis alone is not sufficient for definitive PPR diagnosis.

The rapid antigen test showed good sensitivity but lower specificity when weak positive results were classified as reactive. When only clear positive and negative rapid test results were considered, the test showed a sensitivity of 86.2%, specificity of 67.6%, and moderate agreement with RT-PCR based on Cohen's kappa value of 0.53. These findings suggest that the rapid antigen test is useful for field level screening of suspected PPR cases, particularly for detecting RT-PCR-positive cases; however, confirmatory RT-PCR remains important for weak, invalid, and discordant results. It showed useful field application for screening suspected PPR cases. Its good sensitivity indicates that it detected many RT-PCR-positive animals during outbreak investigations. However, its lower specificity shows that some reactive rapid test results were not confirmed by RTPCR. This finding supports the role of the rapid antigen test as a field screening tool rather than a standalone confirmatory test. Similar observations have been reported in previous evaluations of immunochromatographic antigen detection tests, which showed that rapid tests can support PPR diagnosis during active outbreaks, particularly when infected animals are shedding detectable levels of viral antigen (Couacy-Hymann *et al.*, 2007; Baron *et al.*, 2014; Halecker *et al.*, 2020).

The sensitivity and specificity observed in this study were lower than the values stated by the manufacturer of the ID Rapid® PPR Antigen test kit. The manufacturer reports high specificity of > 99% and sensitivity of 100% on ocular swabs, with results available in less than 20 minutes (Innovative Diagnostics/IDvet, n.d.). The lower performance observed in the present study may be due to differences between controlled validation conditions and real field outbreak conditions. In rural field settings, test performance may be affected by sample quality, sample type, timing of collection, storage conditions, transport delay, cold-chain limitations, ambient temperature, and interpretation of weak test lines. Therefore, the lower field specificity observed in this study does not invalidate the test but indicates that its performance under routine outbreak-investigations may be lower than the manufacturer's stated values.

The discordant results observed between the rapid antigen test and RT-PCR may be explained by the different diagnostic targets of the two tests and by field-related sample handling factors. Rapid antigen tests detect viral antigen, while RT-PCR detects viral RNA. Rapid-test-positive but RT-PCR-negative results may occur when viral RNA has degraded due to poor sample preservation, delayed processing, inadequate cold-chain maintenance, high ambient temperature, or power supply interruptions, while viral antigen remains detectable. Such results may also arise from non-specific rapid test reactions, especially where test bands are weak or faint. Weak positive rapid antigen test results require cautious interpretation.

When weak positive results were classified as reactive, the specificity of the rapid test decreased. This suggests that weak test lines may reflect low antigen concentration, borderline reactions, reader subjectivity, poor sample quality, or the effect of testing under field conditions. For this reason, weak positive results should be reported separately and confirmed by RT-PCR where possible, rather than being interpreted as definitive evidence of infection.

Rapid-test-negative but RT-PCR-positive results may occur when viral antigen concentration is below the detection limit of the rapid test, especially during early, late, or low-viral-

load infections. In such cases, RT-PCR may still detect viral RNA because of its higher analytical sensitivity. This supports previous reports that antigen-based tests, although useful for field screening, may miss some infected animals when viral load is low or when clinical samples are suboptimal (Baron *et al.*, 2014; Halecker *et al.*, 2020). Therefore, a negative rapid test result alone should not be used to rule out PPR in animals with strong clinical or epidemiological suspicion.

The Ct value findings provided further insight into rapid test performance. Samples that were positive by both rapid antigen test and RT-PCR had lower Ct values, suggesting higher viral loads. This supports the biological expectation that antigen-based tests perform better when viral antigen concentration is high in the clinical sample (Bustin *et al.*, 2009; Baron *et al.*, 2014; Halecker *et al.*, 2020). In contrast, discordant rapid-test-positive/RT-PCR-negative samples with recorded Ct values generally had high Ct values, close to or above the Ct threshold. These findings may reflect low viral genome concentration, late amplification, borderline molecular signals, or sample degradation, and should therefore be interpreted in relation to the laboratory Ct cut-off, sample history, and field conditions.

When only clear positive and negative rapid antigen test results were considered, the test showed moderate agreement with RT-PCR. This indicates that the rapid test has practical value for early field screening but cannot replace molecular confirmation. This interpretation is consistent with diagnostic guidance that rapid field tests can support early detection and outbreak response, while RT-PCR remains the preferred confirmatory method because of its higher analytical sensitivity and specificity for detecting viral RNA (Santhamani *et al.*, 2016; WOAH, 2021).

Overall, rapid antigen testing can support early PPR outbreak detection in rural smallholder systems, especially where access to laboratory confirmation is delayed by distance, poor roads, insecurity, limited diagnostic infrastructure, or weak cold-chain systems. However, the test should be used within an integrated diagnostic approach that includes clinical assessment, epidemiological information, proper sample collection, field documentation, and confirmatory RT-PCR. Weak positive, invalid, high-Ct, or discordant results should be clearly reported and interpreted cautiously.

STUDY LIMITATIONS

This study was conducted under real field outbreak conditions; therefore, the findings may not fully represent test performance under standardized laboratory conditions. Weak positive rapid antigen test results posed an interpretation challenge. Classifying them as reactive will reduce specificity, while excluding them from the clear-result analysis, may exclude real field cases.

Sample handling may also have influenced the findings. Delays in transport, cold-chain limitations, high ambient temperatures, and fluctuations in power supply during storage may have contributed to RNA degradation and may partly explain some of the discordant rapid test and RT-PCR results.

Despite these limitations, the study provided useful field-based evidence on the performance of the rapid antigen test under real outbreak conditions in rural Northern Nigeria.

CONCLUSION

This study confirmed the circulation of PPRV among clinically suspected sheep and goats in Plateau, Bauchi, and Kano States of Nigeria. The rapid antigen test showed useful field application for early screening of suspected PPR cases, particularly in samples with higher viral loads. Overall, the rapid antigen test can support field-level detection and early outbreak response, especially in rural areas where access to laboratory diagnosis is limited. However, it should not replace confirmatory real-time RT-PCR, particularly for weak positive, invalid, discordant, or clinically suspicious negative results.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial, personal, or professional interests that influenced the conduct, analysis, interpretation, or reporting of this study.

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