

Assessing The Impact of Cattle-Mediated Zooprophylaxis and Mosquito Species Composition on Malaria Endemicity in Ada West District, Ghana: A Quasi-Experimental Study

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Abstract

Background

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, with Ghana bearing a disproportionate burden of the disease. Zooprophylaxis, the deliberate or incidental diversion of blood-feeding mosquitoes from humans to livestock, has been proposed as a cost-effective, community-based vector control strategy. However, empirical evidence of its impact on mosquito species composition and malaria endemicity in Ghana is limited. This study assessed the effects of cattle-mediated zooprophylaxis on mosquito species composition and malaria endemicity in Ghana's Ada West District.

Methods

A quasi-experimental design with a pre-post intervention control structure was employed. The study was conducted in communities within the Ada West District, comparing cattle-keeping (intervention) and non-cattle-keeping (control) communities from July 2025 to March 2026. A multistage sampling approach yielded 412 households and 1,648 participants in the study. Entomological data were collected using CDC light traps and pyrethrum spray catches, and mosquitoes were identified morphologically and by polymerase chain reaction (PCR). Malaria prevalence was determined using rapid diagnostic tests (RDT). Cattle density and management practices were assessed using structured household surveys. The Driver-Pressure-State-Impact-Response (DPSIR) framework guided conceptual integration. Data were analysed using multilevel logistic regression, chi-square tests, and a difference-in-differences (DiD) approach in Stata 17.

Results

A total of 5,842 mosquitoes were collected from traps. *Anopheles gambiae* sensu lato was the predominant species (67.3%), followed by *An. funestus* (21.4%), and *Culex quinquefasciatus* (11.3%). Intervention communities recorded significantly lower *Anopheles* density (2.1 vs. 4.8 per trap-night; $p < 0.001$) and malaria prevalence (11.2% vs. 24.7%; aOR: 0.38, 95% CI: 0.27–0.54; $p < 0.001$) than control communities. The DiD analysis revealed a net reduction in malaria prevalence of $\text{DiD} = (11.2 - 22.8) - (24.7 - 23.9) = -11.6 - 0.8 = -12.4$ percentage points (pp) attributable to zooprophylaxis ($p = 0.003$). Species diversity was significantly higher in the intervention communities (Shannon index $H' = 2.14$ vs. 1.63; $p = 0.021$).

Conclusions

Cattle-mediated zooprophylaxis was associated with significant reductions in *Anopheles* density and malaria endemicity in the study area. These findings support the integration of strategic livestock

management as a complementary approach within Ghana's National Integrated Vector Management Program, particularly in the agro-pastoral communities of the Northern Region.

Background

Malaria continues to represent one of the most significant infectious disease challenges in sub-Saharan Africa. The World Health Organisation World Malaria Report 2024 estimated that Africa accounts for approximately 94% of all global malaria cases and 95% of malaria-related deaths (1). In Ghana, malaria remains hyperendemic and is a leading cause of outpatient visits, hospital admissions, and under-five mortality (2). The principal vectors, *Anopheles gambiae* sensu lato and *Anopheles funestus*, maintain year-round transmission in many ecological zones, including the coastal and riparian communities of the Greater Accra and Volta Regions (3, 4).

Conventional malaria vector control strategies, including indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs), have achieved measurable reductions in transmission intensity in several endemic settings (5–7). However, growing concerns regarding insecticide resistance in *Anopheles* populations, coupled with the limitations of single-strategy approaches, underscore the urgent need for complementary and ecologically grounded interventions (6, 8, 9). Zooprophylaxis, defined as the incidental or deliberate use of animals to divert blood-feeding vectors away from humans, has emerged as a promising low-cost strategy embedded in agricultural and pastoral livelihoods (10).

The theoretical basis for zooprophylaxis is the host preference of malaria vectors (11–13). *Anopheles arabiensis* is a major member of the *An. gambiae* complex and demonstrates notable zoophily compared with the more anthropophilic *An. gambiae* sensu stricto (14, 15). When cattle are present at significant densities, a proportion of mosquito blood meals is redirected from humans to bovine hosts, potentially reducing the entomological inoculation rate (EIR) and, consequently, the risk of malaria in adjacent human populations (16, 17).

However, empirical evidence regarding the effectiveness of zooprophylaxis has been inconsistent. Several studies conducted in East Africa and South Asia have reported significant reductions in malaria incidence associated with livestock presence (18–20), whereas others have raised concerns about zoopotential, the enhancement of vector breeding near livestock due to increased organic matter and standing water (21, 22). This equivocality highlights the importance of investigating this phenomenon in specific ecological and epidemiological contexts.

In Ghana, cattle herding is practiced extensively in communities along the Volta River Basin and coastal lowlands, including the Ada West District, an area characterised by high malaria transmission, extensive water bodies, and agro-pastoral livelihoods (23–25). Despite the ecological plausibility of zooprophylaxis in these settings, no rigorous study has examined its relationship with mosquito species composition and malaria endemicity in this geographical region to date. Additionally, the application of an integrated analytical framework, such as the DPSIR model, to elucidate the causal pathways linking livestock density to malaria outcomes has not been previously attempted in Ghana (23, 26, 27).

Despite the high malaria burden in the Ada West District, a setting characterised by perennial transmission, extensive wetland breeding habitats, and widespread agro-pastoral livelihoods, there is a critical gap in the evidence regarding whether the coexistence of humans and cattle confers a measurable protective effect against malaria. Conventional vector control tools, including LLINs and IRS, have demonstrated limited and inconsistent impact in comparable coastal riparian settings, partly because of insecticide resistance and variable community adherence (28). Zooprophylaxis has been proposed as a complementary approach; however, its effectiveness in West African settings has not yet been rigorously evaluated. Specifically, no study has examined the differential effect of cattle presence on (i) the species composition and abundance of local *Anopheles* populations, (ii) the relative proportions of zoophilic *An. arabiensis* and the anthropophilic *Anopheles gambiae sensu stricto*; and (iii) community-level malaria endemicity in the Ada West District.

Epidemiologically, the Ada West District reports persistently high malaria prevalence exceeding 20% in some communities, even after the sustained deployment of LLINs, indicating that additional or alternative strategies are warranted (29). Ecologically, the district's riparian and wetland landscapes create favourable breeding habitats for *Anopheles gambiae* s.l. and *An. funestus*, and the co-existence of large cattle herds in the same communities presents a natural quasi-experimental opportunity to assess the zooprophylactic effect under real-world conditions in the Ada West District of Ghana. From a public health policy perspective, zooprophylaxis is intrinsically aligned with existing livelihoods and requires no additional costs for communities that own cattle. If proven effective, strategic guidance on cattle shelter placement relative to human dwellings could be incorporated into community health education at minimal cost. Furthermore, generating context-specific evidence from Ghana is necessary because findings from East Africa may not be directly transferable, given the differences in vector species composition, climate, housing types, and husbandry practices (30–32).

This study was designed to assess (i) the impact of cattle-mediated zooprophylaxis on mosquito species composition and abundance, (ii) the relationship between zooprophylaxis exposure and malaria endemicity, and (iii) the determinants of malaria prevalence in communities with different livestock densities.

Methods

Study design

A quasi-experimental design with pre-intervention and control group structures was employed to assess the impact of cattle-mediated zooprophylaxis on mosquito species composition and malaria endemicity. This design was adopted because full randomisation at the community level was neither ethically nor logistically feasible in the study area. Two categories of communities were defined: (i) intervention communities, cattle-keeping communities within the Ada West District (zooprophylaxis-exposed group), and (ii) control communities, non-cattle-keeping communities within the same district. Measurements

were conducted at baseline (July 2025) and endline (January–March 2026), spanning both the peak and off-peak malaria transmission seasons.

Study area

This study was conducted in selected communities in the Ada West District of the Greater Accra Region, Ghana. The communities were stratified based on cattle ownership: those with a high cattle density constituted the intervention group, whereas those with negligible or no cattle presence constituted the control group. Both groups shared the same ecological zone, climate, health system, and socioeconomic environment, which substantially reduced the risk of confounding due to differences at the district level. The district is characterised by riparian and wetland ecosystems that create extensive mosquito breeding habitats and is classified as a malaria-endemic area with perennial transmission (Fig. 1).

Study population and eligibility criteria

The study population comprised three groups: (i) human residents of selected households, (ii) adult mosquito populations collected from households and peridomestic environments, and (iii) household-level cattle as the primary exposure variables. Human participants were eligible if they had resided in the study community for at least six months prior to baseline, provided informed consent, and belonged to households with a clearly defined cattle ownership status. Participants were excluded if they were part of transient or seasonal migrant populations, refused consent, or lived in communities that had undergone vector control interventions in the preceding six months.

Description of Cattle Communities in Ada West District

Table 1 presents the distribution of communities in the Ada West District where cattle are present in the study area. The communities are organised according to their respective areas and sub-districts, including Caesarkope, Amuyaakope Community-based Health Planning and Services (CHPS), Madavunu, Afiadenyigba, Koluedor, Koni Community-based Health Planning and Services (CHPS), Sege, Bornikope Sub-district, and Anyamam areas. Within each area, multiple communities were identified as locations where cattle were maintained. These communities span both community-based health planning and service (CHPS) zones and non-CHPS areas, reflecting the geographic spread of cattle across the districts. The table classifies the study locations based on the presence of cattle and provides a structured overview of the study settings. This categorisation was used to guide data collection and support the spatial organisation of the variables within the study.

Table 1
Summary of Cattle Communities in Ada West District

Area / Sub-district	Communities
Caesarkope Area	Caesarkope; Caesarkope Panya; Mangoase; Odorkope; Tugahkope; Agbenyegahkope; Addodoadjikope
Amuyaokope CHPS	Amuyaokope; Badjorhey; JJ Farm; Ada Luta
Madvunu Area	Madavunu; Wonyi; Ayissah; Dogokope; Kuatsekope
Afiadenyigba Area	Afiadenyigba; Adictrekope; Kponya; English Kenya; Dorgobom
Koluedor	Koluedor; Koluedor Zongo
Koni CHPS	Narkietsekope; Koni–Alavanyo; Amartey Koni; Doratsekope
Sege Area	Sege; Sorkope; Nakomkope
Bornikope Sub-district	Bornikope; Toflokpo; Salom; Osoyaa; Anukpenya; Matsekope; Kpotsum
Anyamam Area	Anyamam; Okorhuesisi
<i>CHPS: Community-based Health Planning and Services.</i>	

Sample size determination

The sample size was calculated using the formula for comparing two independent proportions, assuming a baseline malaria prevalence of 25% in the control group and a 10 percentage-point reduction in the intervention group (15%), with a significance level of 5% (two-tailed) and 80% statistical power. A minimum of 178 households per group was required. After adjusting for a design effect of 1.5 due to cluster sampling and a 10% non-response rate, the final target was 206 households per arm (total: 412). With an average household size of four participants, the total sample was 1,648.

Sampling technique

A multistage sampling approach was used. In the first stage, the Ada West District was purposively selected based on the documented co-existence of cattle-keeping and non-cattle-keeping communities within the same ecological zone. In the second stage, communities were stratified by cattle ownership status (≥ 5 cattle per 10 households versus < 5 cattle per 10 households) using pre-existing agricultural census data [10] and randomly selected within strata. In the third stage, households were sampled using systematic random sampling methods. In the fourth stage, all eligible individuals from the sampled households were enrolled in the study.

Data collection

Entomological data collection

Adult mosquitoes were collected using CDC miniature light traps (John W. Hock, Gainesville, FL, USA) and pyrethrum spray catches (PSC) were conducted in the sleeping rooms of the consenting households. Light traps were operated from 18:00 to 06:00 hours on three consecutive nights during each sampling visit. Larval sampling was conducted at identified breeding sites using the standard dipping technique (10 dips per breeding site). All mosquitoes were morphologically identified at the species level using an established dichotomous key. A random 20% subsample of *Anopheles* specimens was subjected to PCR-based molecular identification as described by Mulamba *et al.* (33). Mosquito density was expressed as the mean number of individuals per trap night (TN). Species diversity was quantified using the Shannon–Wiener (H') and Pielou's evenness (J') indices.

Malaria data collection

Malaria prevalence was determined at baseline (July 2025) and endline (January–March 2026) using WHO-validated malaria rapid diagnostic tests (SD BIOLINE Malaria Ag P.f/Pan) (34). Thick and thin blood smears were prepared from all RDT-positive cases and a random 20% of RDT-negative cases and examined by two independent microscopists, with discordant results adjudicated by a third reviewer. The fever history in the preceding 48 hours and axillary temperature were recorded for each participant.

Cattle exposure assessment

Cattle density and management practices were assessed using structured household questionnaires administered by trained enumerators. The collected data included the number of cattle per household, distance between cattle shelters and the nearest human sleeping room, nocturnal cattle management practices, and the duration of cattle ownership. Community-level cattle density was computed as the number of cattle per 100 people using the 2021 census data.

Environmental and sociodemographic data

Environmental mapping was conducted using handheld GPS devices to record the coordinates of breeding sites, water bodies, vegetation and household locations. Sociodemographic data, including age, sex, household size, housing conditions, socioeconomic status (asset-based index), and ITN use, were collected using interviewer-administered questionnaires.

Conceptual framework

This study employed the Driver-Pressure-State-Impact-Response (DPSIR) framework to examine the ecological pathway through which cattle ownership influences malaria transmission in the Ada West District of Ghana, with malaria endemicity as the terminal outcome variable (35).

Cattle ownership (Driver), characterised by herd size and proximity of livestock to human dwellings, introduces an alternative vertebrate host into the peri-domestic environment, thereby diverting mosquito blood-feeding activity away from humans (Pressure), as measured by shifts in the human blood index

and indoor/outdoor biting ratios. Sustained over time, this behavioural diversion selectively favours zoophilic vector species, such as *Anopheles arabiensis*, over more anthropophilic counterparts, such as *An. gambiae* s.s., reshaping the local vector community composition and abundance

(state). These entomological changes reduce key transmission metrics, including the entomological inoculation rate, sporozoite rate, and vectorial capacity, thereby diminishing human–vector contact (Impact). The cumulative protective effect of this causal chain constitutes zooprophylaxis (response), an emergent ecological outcome rather than a deliberate intervention, quantified by comparing the malaria burden between cattle-owning and non-owning communities while controlling for relevant confounders.

Importantly, the framework incorporates a feedback loop whereby demonstrated zooprophylactic protection may inform livestock management policies and vector control strategies, thereby modifying the original driver. Malaria endemicity, assessed through parasite prevalence, incidence rates, and transmission intensity classification, serves as the ultimate dependent variable, capturing the net downstream effect of all upstream components in the causal chain (26, 36) (Fig. 2).

Summary of DPSIR Components and Indicators

The Table 2 below summarises the mapping of each DPSIR component to its corresponding study variable and key measurement indicators:

Table 2
DPSIR Components and Indicators

DPSIR Component	Study Variable	Key Indicators
Driver	Cattle ownership	Ownership status, herd size, proximity of cattle to dwellings
Pressure	Altered mosquito host-seeking behaviour	Human blood index, bovine blood index, indoor/outdoor biting ratios
State	Mosquito species composition and abundance	<i>An. gambiae</i> s.l. sibling species ratios, <i>An. funestus</i> density, vector density per trap night
Impact	Malaria transmission dynamics	Entomological inoculation rate, sporozoite rate, human biting rate, vectorial capacity
Response	Zooprophylaxis	Comparative malaria risk between cattle-owning and non-owning communities, blood-meal diversion rates
Outcome	Malaria endemicity	Parasite prevalence, malaria incidence rates, endemicity classification

Data analysis

All data were entered into REDCap and exported to Stata version 17.0 for further analysis. Descriptive statistics were computed for all the variables. Chi-square tests were used to assess the bivariate

associations. Multilevel logistic regression with households nested within communities was used to estimate adjusted odds ratios (aOR) for malaria positivity, accounting for clustering effects. Variables with $p < 0.20$ in the bivariate analyses were included in the multivariable model; the final model retained variables with $p < 0.05$. PAST (PAleontological Statistics Software Package for Education and Data Analysis) was used to analyse and input the Shannon–Wiener (H') and Pielou's evenness (J') indices. The difference-in-differences (DiD) approach was used to estimate the net causal impact of zooprophylaxis on malaria prevalence.

Ethical considerations

Ethical approval was obtained from the Ghana Health Service Ethics Review Committee and the University of Port Harcourt Ethics Review Board. Written informed consent was obtained

Informed consent was obtained from all adult participants, and assent was obtained from children aged 8–17 years with parental consent. All RDT-positive malaria cases were referred for standard care at no cost to the study participants. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Study population and baseline characteristics

A total of 412 households were enrolled, with 206 households in each group. Of the 1,648 eligible participants, 1,591 (96.5%) completed both assessments. The mean age of participants was 28.4 years (SD 17.6) in the intervention arm and 27.9 years (SD 16.8) in the control arm. Females constituted 53.2% and 52.8% of participants in the intervention and control arms, respectively. ITN use was comparable at baseline (64.3% vs. 66.1%; $p = 0.67$) between the two groups. The mean number of cattle per household was 7.4 (SD = 3.1). The baseline malaria prevalence was 22.8% in cattle-keeping communities and 23.9% in non-cattle-keeping communities ($p = 0.74$), confirming group comparability at baseline (Table 3).

Table 3

Baseline sociodemographic and household characteristics of study participants, Ada West District (July 2025)

Characteristic	Cattle-keeping communities (n = 206)	Non-cattle-keeping communities (n = 206)	p-value
Mean age, years (SD)	28.4 (17.6)	27.9 (16.8)	0.71
Female sex, n (%)	437 (53.2%)	422 (52.8%)	0.89
ITN use, n (%)	264 (64.3%)	272 (66.1%)	0.67
Mean cattle per household (SD)	7.4 (3.1)	-	-
Improved housing, n (%)	148 (71.8%)	152 (73.8%)	0.62
Baseline malaria prevalence (%)	22.8%	23.9%	0.74

SD standard deviation, ITN insecticide-treated nets. *p*-values derived from independent-samples *t*-test (continuous variables) and chi-square test (categorical variables); Control communities were selected on the basis of no documented cattle-keeping per agricultural census records

Mosquito species composition and abundance

A total of 5,842 adult mosquitoes were collected across both sites during the six-month observation period: 2,614 from cattle-keeping communities and 3,228 from non-cattle-keeping communities. *Anopheles gambiae* s.l. was the predominant species (67.3%), followed by *An. funestus* (21.4%), and *Culex quinquefasciatus* (11.3%). Molecular analysis of *Anopheles gambiae* s.l. subsample confirmed that *An. arabiensis* constituted 43.7% of the mosquitoes in the intervention arm compared to 18.2% in the control arm ($\chi^2 = 47.3$; $P < 0.001$). *An. gambiae* s.s. constituted 56.3% and 81.8% of the samples, respectively. *Anopheles* density was significantly lower in the intervention communities (2.1 vs. 4.8 per trap night; $p < 0.001$). Species diversity was significantly higher in cattle-keeping communities (Shannon $H' = 2.14$ vs. 1.63; $p = 0.021$) than in other communities (Table 4).

Table 4

Mosquito species composition and diversity indices by study arm, Ada West District (July 2025–March 2026)

Parameter	Cattle-keeping communities	Non-cattle-keeping communities	p-value
Total mosquitoes collected, n	2,614	3,228	—
<i>An. gambiae</i> s.l., %	64.1%	70.2%	0.03
<i>An. arabiensis</i> , %	43.7%	18.2%	< 0.001
<i>An. gambiae</i> s.s., %	56.3%	81.8%	< 0.001
<i>An. funestus</i> , %	24.8%	18.4%	0.08
<i>Culex quinquefasciatus</i> , %	11.1%	11.4%	0.91
Mean <i>An.</i> density/trap-night (95% CI)	2.1 (1.7–2.5)	4.8 (4.2–5.4)	< 0.001
Shannon–Wiener index, H'	2.14	1.63	0.021
Pielou's evenness, J'	0.79	0.61	0.018

An. Anopheles s.l. sensu lato; s.s. sensu stricto; CI, confidence interval. Proportions for An. arabiensis and An. gambiae s.s. are expressed as percentages of molecularly identified An. gambiae s.l. subsample

Mosquito species diversity in cattle-keeping and non-cattle-keeping communities enhanced

Four mosquito species were identified in the study communities: *Anopheles gambiae* sensu stricto, *An. funestus*, *An. arabiensis*, and *Culex quinquefasciatus*. The species composition differed markedly between the study groups (Fig. 1A). In cattle-keeping communities, mosquito species were more evenly distributed, with *Anopheles gambiae* s.s. (36.1%), followed by *Cx. quinquefasciatus* (29.0%), *An. arabiensis* (24.8%), and *An. funestus* (11.1%). In contrast, *Anopheles* mosquitoes dominated the non-cattle-keeping communities. *gambiae* s.s. (42.4%) and *Cx. quinquefasciatus* (31.5%), with smaller proportions of *An. funestus* (12.8%), and *An. arabiensis* (13.4%).

The Shannon diversity index (H') was significantly higher in cattle-keeping communities (H' = 2.14) than in non-cattle-keeping communities (H' = 1.63; $p = 0.021$; Fig. 1B). Similarly, Pielou's evenness index (J') was significantly greater in cattle-keeping communities (J' = 0.79) than in non-cattle-keeping communities (J' = 0.61; $p = 0.018$; Fig. 1C), indicating a more equitable distribution of individuals among species in communities with cattle than in those without cattle.

Despite the greater species diversity observed in cattle-keeping communities, the mean *Anopheles* density per trap night was significantly lower in these communities (2.1 mosquitoes per trap night) than

in non-cattle-keeping communities (4.8 mosquitoes per trap night; $p = 0.003$; Fig. 1D). This finding suggests that the presence of cattle may be associated with reduced *Anopheles* abundance, potentially through zooprophylactic effects, whereby cattle serve as alternative blood meal hosts, diverting mosquitoes away from human dwellings (Fig. 3).

Malaria prevalence and zooprophylaxis effect

At the endline, malaria prevalence was significantly lower in cattle-keeping communities (11.2%) than in non-cattle-keeping communities (24.7%) ($\chi^2 = 52.3$; $p < 0.001$), representing an absolute reduction of 13.5 percentage points. In the multivariable analysis, residence in a high-cattle-density community was independently associated with significantly reduced malaria odds (aOR: 0.38, 95% CI: 0.27–0.54; $p < 0.001$), after controlling for ITN use, age, sex, housing type, and socioeconomic status. The DiD analysis yielded a net treatment effect of – 13.5 percentage points (95% CI: –17.8 to – 9.2; $p = 0.003$). Other significant predictors of malaria positivity included nonuse of ITNs (aOR: 2.71), children aged 2–5 years (aOR: 3.14), and poor housing conditions (aOR = 1.89) (Table 5). Cattle shelters located > 50 m from human sleeping quarters were also independently protective (aOR: 0.62, 95% CI: 0.44–0.87; $p = 0.006$). (Table 5).

Table 5
Multilevel logistic regression of predictors of malaria prevalence at endline, Ada West District (January–March 2026)

Variable	Malaria prevalence (%)	aOR (95% CI)	p-value
High cattle density (vs. low)	11.2% vs. 24.7%	0.38 (0.27–0.54)	< 0.001
No ITN use (vs. ITN use)	29.8% vs. 14.1%	2.71 (1.98–3.71)	< 0.001
Age 2–5 years (vs. adults)	38.4% vs. 14.7%	3.14 (2.09–4.72)	< 0.001
Poor housing (vs. improved)	27.3% vs. 14.2%	1.89 (1.32–2.70)	< 0.001
Cattle shelter > 50 m from house	10.4% vs. 18.7%	0.62 (0.44–0.87)	0.006
Low SES (vs. medium/high)	24.6% vs. 15.1%	1.54 (1.11–2.14)	0.009
Female sex (vs. male)	18.2% vs. 17.4%	0.97 (0.74–1.27)	0.83

aOR, adjusted odds ratio; CI, confidence interval; ITN, insecticide-treated net; SES, socioeconomic status. Multilevel logistic regression with households nested within communities. Reference categories shown in parentheses

Difference-in-differences analysis

The DiD analysis compared changes in malaria prevalence between cattle-keeping (intervention) and non-cattle-keeping (control) communities during the study period. At baseline, the prevalence was similar in both groups (22.8% vs. 23.9%), confirming comparability prior to the observation period. At the

endline, malaria prevalence in cattle-keeping communities declined markedly from 22.8% to 11.2% (– 11.6 percentage points), whereas non-cattle-keeping communities experienced a marginal increase from 23.9% to 24.7% (+ 0.8 percentage points). The DiD estimate, representing the net effect after accounting for secular trends in the control group, was – 13.5 pp (95% CI: – 17.8 to – 9.2; p = 0.003). The 95% confidence interval excludes zero, indicating that the observed difference is unlikely to be attributable to chance (37). These findings suggest that cattle-mediated zooprophylaxis played an important role in reducing malaria prevalence, most likely by diverting mosquito blood feeding from humans to bovine hosts (Table 6).

Table 6 Difference-in-differences (DiD) analysis of the effect of cattle-mediated zooprophylaxis on malaria prevalence, Ada West District (July 2025–March 2026)			
Group	Baseline prevalence (%)	Endline prevalence (%)	Change (percentage points)
Cattle-keeping communities (Intervention)	22.8%	11.2%	– 11.6
Non-cattle-keeping communities (Control)	23.9%	24.7%	+ 0.8
Difference-in-differences (DiD)	–	–	– 13.5 (95% CI: – 17.8 to – 9.2; p = 0.003)

DiD, difference-in-differences; CI, confidence interval. The net treatment effect is calculated as (Intervention endline – Intervention baseline) – (Control endline – Control baseline)

Larval survey results

A total of 72 potential breeding sites were identified and sampled across both study arms: 34 in cattle-keeping and 38 in non-cattle-keeping communities. The breeding site types included temporary puddles and hoof prints, irrigation ditches, semi-permanent ponds and marshes, and drainage channels. Cattle-keeping communities had a higher proportion of temporary puddles and hoof-print pools (70.6%, n = 24) than non-cattle-keeping communities (31.6%, n = 12), reflecting the physical disturbance of the soil surface by the livestock. Conversely, semi-permanent ponds and marshes were more prevalent in the control communities (28.9% vs. 8.8%; p = 0.03).

From 720 dip samples collected across the 72 sites (10 dip samples/site), 3,454 mosquito larvae were collected: 1,326 from cattle-keeping communities and 2,128 from non-cattle-keeping communities. The mean larval density was significantly lower in the intervention arm (3.9 per dip; 95% CI: 3.2–4.6) than in the control arm (5.6 per dip; 95% CI: 4.9–6.3; p < 0.001). The mean *Anopheles* larval density was 2.3 per dip (95% CI: 1.8–2.8) in cattle-keeping communities versus 4.0 per dip (95% CI: 3.4–4.6) in non-cattle-keeping communities (p < 0.001), representing a 42.5% reduction, which was consistent with the data from adult mosquito traps. Notably, *Culex quinquefasciatus* larvae were proportionally more abundant in the intervention arm (23.6% vs. 17.4%; p = 0.008), likely attributable to the increased organic matter from

cattle dung in peridomestic breeding habitats, suggesting a degree of species-specific zoopotentialiation (Table 7).

Table 7
Larval survey findings by study arm, Ada West District (July 2025–March 2026)

Parameter	Cattle-keeping communities	Non-cattle-keeping communities	p-value
Breeding sites sampled, n	34	38	—
Total larvae collected, n	1,326	2,128	—
Mean larval density/dip (95% CI)	3.9 (3.2–4.6)	5.6 (4.9–6.3)	< 0.001
Mean <i>Anopheles</i> density/dip (95% CI)	2.3 (1.8–2.8)	4.0 (3.4–4.6)	< 0.001
<i>An. gambiae</i> s.l. larvae, %	57.8%	66.4%	0.014
<i>An. funestus</i> larvae, %	18.6%	16.2%	0.24
<i>Culex quinquefasciatus</i> larvae, %	23.6%	17.4%	0.008
Temporary puddles/hoof prints, n (%)	24 (70.6%)	12 (31.6%)	0.001
Semi-permanent ponds/marshes, n (%)	3 (8.8%)	11 (28.9%)	0.03
Irrigation ditches/drainage, n (%)	7 (20.6%)	15 (39.5%)	0.09

An. Anopheles s.l. sensu lato; CI, confidence interval. Ten dips were taken at each breeding site using a standard dipping technique to collect larvae. Breeding site types were classified by field enumerators during sampling

Discussion

This study provides empirical evidence that cattle-mediated zooprophyllaxis is associated with statistically significant reductions in both *Anopheles* mosquito density and malaria prevalence in Ghana’s Ada West District. The findings are broadly consistent with those reported from comparable agro-pastoral settings in Ethiopia, Kenya, and Tanzania (7), while contributing novel data specific to the West African epidemiological and ecological contexts of this study population.

The most notable entomological finding was the differential composition of *the Anopheles gambiae* complex between the intervention and control communities. A higher proportion of *An. arabiensis*, the more zoophilic sibling species, in cattle-keeping communities *in this region. gambiae* s.s. suggests that cattle act as a selective host-diversion force favouring more zoophilic individuals (15, 22). This was

further supported by the elevated species diversity index in cattle-dense communities ($H' = 2.14$ vs. 1.63), indicating that a more heterogeneous host environment sustains a more diverse and less anthropophilic mosquito assemblage (17).

The 56.3% reduction in *Anopheles* trap density in the intervention arm was consistent with the meta-analytic estimate of a 35–65% reduction in human-biting rates in livestock-exposed communities reported by Pagès et al. (17). The DiD estimate of a 13.5 percentage-point (pp) net reduction in malaria prevalence attributable to zooprophylaxis was robust to covariate adjustment, suggesting that the observed effect was not merely a product of residual confounding by ITN use, socioeconomic status, or housing quality.

The protective effect associated with a greater distance between cattle shelters and human sleeping quarters (aOR: 0.62) warrants careful interpretation of the results. Although physical separation may reduce the probability of mosquitoes transitioning between bovine and human hosts, it may also reflect broader and deliberate vector risk management behaviours. This finding aligns with the literature on optimal cattle placement as a zooprophylactic design parameter (8) and identifies an actionable spatial threshold in public health guidelines.

The age gradient in malaria risk, with children aged 2–5 years having over three times the odds of malaria positivity compared with adults, was consistent with the established pattern of age-dependent immunity in endemic settings (23) and reinforced the importance of targeting interventions in this vulnerable sub-group. The significant association between poor housing conditions and malaria risk adds to the growing body of evidence linking structural housing improvements to reduced malaria transmission (5).

The application of the DPSIR framework facilitated a system-level interpretation that went beyond conventional effect estimation. By conceptualising cattle ownership (driver), altered host-seeking pressure (pressure), changes in mosquito assemblage composition (state), and malaria burden (impact), this framework illuminates how livestock management practices can be leveraged as a public health response (4, 7). This conceptualisation may be particularly useful for policymakers seeking to frame zooprophylaxis within the Integrated Vector Management (IVM) agenda.

The limitations of this study include the quasi-experimental design, which cannot fully exclude unmeasured confounding; the single six-month study period, which precludes the assessment of longer-term mosquito species dynamics; and the inability to compute full entomological inoculation rates owing to logistical constraints on sporozoite rate determination. Future longitudinal studies with randomised community allocation and molecular xenomonitoring are recommended to confirm and extend these findings.

Conclusions

This study demonstrated that cattle-mediated zooprophylaxis was associated with significant reductions in both *Anopheles* mosquito density and malaria endemicity in communities in Ghana's Ada West District, Ghana. The 13.5 pp reduction in malaria prevalence was accompanied by a demonstrable shift in the vector species composition toward the less anthropophilic *Anopheles arabiensis* and greater overall vector diversity, consistent with the theoretical basis for zooprophylaxis. These findings support the potential inclusion of strategic livestock management as a complementary vector control tool in Ghana's integrated vector management program, particularly in rural agro-pastoral communities. Future research should determine the optimal cattle-to-human density ratios, evaluate synergies with insecticide-based interventions, and assess community acceptability and cost-effectiveness for the large-scale implementation of deliberate zooprophylaxis.

Abbreviations

aOR

Adjusted odds ratio

CDC

Centers for Disease Control and Prevention

CI

Confidence interval

DiD

Difference-in-differences

DPSIR

Driver-Pressure-State-Impact-Response

EIR

Entomological inoculation rate

GPS

Global positioning system

IRS

Indoor residual spraying

ITN

Insecticide-treated net

IVM

Integrated Vector Management

LLIN

Long-lasting insecticidal net

PAST

PAleontological Statistics Software Package for Education and Data Analysis, PCR:Polymerase chain reaction

PSC

Pyrethrum spray catch

RDT
Rapid diagnostic test
SD
Standard deviation
SES
Socioeconomic status

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the Ghana Health Service Ethics Review Committee (GHS-ERC/026/825). Written informed consent was obtained from all adult participants, and assent was obtained from children aged 8–17 years with parental consent. All RDT-positive malaria cases were referred for standard care at no cost to study participants. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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Author Contribution

VO conceived and designed the study, supervised data collection, conducted data analysis, and drafted the manuscript. AB and MOY contributed to the data collection and critically reviewed the manuscript. JBEN contributed to community engagement, participant recruitment and manuscript review. MAAM contributed to the data management, health information analysis, and manuscript review. MB, EK, RD, CBN, and RH served as data officers and analysts, contributing to field data collection, entry, and quality verification. AC and MOF provided field and institutional supervision and reviewed the manuscript. CTW provided academic supervision, contributed to the conceptual framework and reviewed the manuscript. All authors have read and approved the final manuscript

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Availability of data and materials

The de-identified datasets used and analysed in the current study are available from the corresponding author upon reasonable request.

References

1. Gething PW, Battle KE, Bhatt S, Smith DL, Eisele TP, Cibulskis RE, et al. Declining malaria in Africa: Improving the measurement of progress. *Malar J.* 2014;13(1):39. 10.1186/1475-2875-13-39.
2. Gafaru Mohammed A, Sunkwa Tamal C, Akos Odikro M, Lwanga Noora C, Romeo Quarshie P, Andrew Afari E, et al. Epidemiology of Malaria Cases in Sunyani Municipality, Ghana, 2020. *JIEPH.*

- 2024;7(3):31. 10.37432/jieph.2024.7.3.122.
3. Peprah MS. Land Use Land Cover Classification for Africa: A Case Study of The Republic of Ghana Using Systematic Review and Meta-Analysis. *Int J Earth Sci Knowl Appl*. 2025;7(2):164–92.
 4. Beltrame A. Evaluating How Malaria Transmission Season, Residency, and Travel History Modify the Association Between Vector Control Method Use and *P. falciparum* Infection in Accra, Ghana. 2024.
 5. Zhou Y, Zhang WX, Tembo E, Xie MZ, Zhang SS, Wang XR, et al. Effectiveness of indoor residual spraying on malaria control: a systematic review and meta-analysis. *Infect Dis Poverty*. 2022;11(04):29–42.
 6. Nalinya S, Musoke D, Deane K. Malaria prevention interventions beyond long-lasting insecticidal nets and indoor residual spraying in low-and middle-income countries: a scoping review. *Malar J*. 2022;21(1):31.
 7. Mapua SA, Samb B, Nambunga IH, Mkandawile G, Bwanaly H, Kaindoa EW, et al. Entomological survey of sibling species in the *Anopheles funestus* group in Tanzania confirms the role of *Anopheles parensis* as a secondary malaria vector. *Parasites vectors*. 2024;17(1):261.
 8. Namountougou M, Soma DD, Kientega M, Balboné M, Kaboré DPA, Drabo SF, et al. Insecticide resistance mechanisms in *Anopheles gambiae* complex populations from Burkina Faso, West Africa. *Acta Trop*. 2019;197:105054.
 9. Dusfour I, Vontas J, David JP, Weetman D, Fonseca DM, Corbel V, et al. Management of insecticide resistance in major *Aedes* vectors of arboviruses: Advances and challenges. *PLoS Negl Trop Dis*. 2019;13(10):e0007615.
 10. Kassiri H, Dehghani R, Khodkar I, Ahaki A. Role of ecological values, natural ecosystems, and wildlife in natural zooprophylaxis and prevention of vector-borne diseases: A review. *J Entomol Res*. 2022;46(1):137–44.
 11. Lyons N. Disease investigation and initial response. *Transboundary Diseases of Cattle and Bison, An Issue of Veterinary Clinics of North America: Food Animal Practice: Transboundary Diseases of Cattle and Bison, An Issue of Veterinary Clinics of North America: Food Animal Practice*. E-Book. 2024;40(2):205.
 12. Kassiri H, Dehghani R, Khodkar I, Ahaki A. Role of ecological values, natural ecosystems and wildlife in the natural zooprophylaxis and prevention of vector borne diseases: A review. *J Entomol Res*. 2022;46(1):137–44.
 13. Mondal R, Azmi SA, Sinha S, Bose C, Ghosh T, Bhattacharya S, et al. Ecology and behavior. *Mosquitoes of India*. CRC; 2025. pp. 239–76.
 14. Ajayi F, Ibrahim K, Oguayo V, Anumudu C, Noutcha A. Host preferences, bloodmeal sources, and gonotrophic cycles of *Anopheles gambiae* complex mosquitoes in rural South West Nigeria. *J Vector Borne Dis*. 2026;63(1):131–7.
 15. Hosack GR, El-Hachem M, Ickowicz A, Beeton NJ, Wilkins A, Hayes KR, et al. Prediction of mosquito vector abundance for three species in the *Anopheles gambiae* complex. *Parasites Vectors*. 2025;18(1):497.

16. Heinrich AP, Pooda SH, Porciani A, Zéla L, Schinzel A, Moiroux N, et al. An ecotoxicological view of malaria vector control with ivermectin-treated cattle. *Nat Sustain.* 2024;7(6):724–36. 10.1038/s41893-024-01332-8.
17. Pagès N, Cohnstaedt LW. 8. Mosquito-borne diseases in the livestock industry. In: Garros C, Bouyer J, Takken W, Smallegange RC, editors. *Pests and vector-borne diseases in the livestock industry* [Internet]. Brill | Wageningen Academic; 2018 [cited 2026 Apr 1]. pp. 195–219. Available from: <https://brill.com/view/book/9789086868636/BP000011.xml> 10.3920/978-90-8686-863-6_8
18. Pagès N, Cohnstaedt LW. 8. Mosquito-borne diseases in the livestock industry. In: Garros C, Bouyer J, Takken W, Smallegange RC, editors. *Pests and vector-borne diseases in the livestock industry* [Internet]. Brill | Wageningen Academic; 2018 [cited 2026 Apr 1]. pp. 195–219. Available from: <https://brill.com/view/book/9789086868636/BP000011.xml> 10.3920/978-90-8686-863-6_8
19. Loha E. Association between Livestock Ownership and Malaria Incidence in South-Central Ethiopia: A Cohort Study. *Am J Trop Med Hyg.* 2023;108(6):1145–50. 10.4269/ajtmh.22-0719.
20. Mwalugelo YA, Mponzi WP, Muyaga LL, Mahenge HH, Katusi GC, Muhonja F, et al. Livestock keeping, mosquitoes, and community viewpoints: a mixed methods assessment of relationships between livestock management, malaria vector biting risk, and community perspectives in rural Tanzania. *Malar J.* 2024;23(1):213. 10.1186/s12936-024-05039-1.
21. Hasyim H, Dhimal M, Bauer J, Montag D, Groneberg DA, Kuch U, et al. Does livestock protect against malaria or facilitate malaria prevalence? A cross-sectional study was conducted in endemic rural areas of Indonesia. *Malar J.* 2018;17(1):302. 10.1186/s12936-018-2447-6.
22. Anderson L, Reynolds T, Lipson J. Environmental Implications of Livestock Series: Chickens [Internet]. 2019 23 Apr [cited 2026 1 Apr]. Available from: <https://gatesopenresearch.org/documents/3-1316> 10.21955/gatesopenres.1116221.1
23. Nuvey FS, Mensah GI, Zinsstag J, Hattendorf J, Fink G, Bonfoh B, et al. Management of diseases in a ruminant livestock production system: a participatory appraisal of the performance of veterinary services delivery and utilisation in Ghana. *BMC Vet Res.* 2023;19(1):237. 10.1186/s12917-023-03793-z.
24. Ahmed A, Kuusaana ED. Cattle Ranching and Farmer-Herder Conflicts in Sub-Saharan Africa: Exploring the Conditions for Successes and Failures in Northern Ghana. *Afr Secur.* 2021;14(2):132–55. 10.1080/19392206.2021.1955496.
25. Etwire ES, Onyam I, Otabil MA, Kwansa-Aidoo K, Adadey SM, Ekloh W. Prevalence and Impact of Cattle Infections in Ghana: Challenges in Livestock Health and Disease Management. *Adv J Grad Res.* 2024;15(1):22–33. 10.21467/ajgr.15.1.22-33.
26. Chan K, Cano J, Massebo F, Messenger LA. Cattle-related risk factors for malaria in southwest Ethiopia: A cross-sectional study. *Malar J.* 2022;21(1):179. 10.1186/s12936-022-04202-w.
27. Mwalugelo YA, Mponzi WP, Muyaga LL, Mahenge HH, Katusi GC, Muhonja F, et al. Livestock keeping, mosquitoes and community viewpoints: a mixed methods assessment of relationships between

- livestock management, malaria vector biting risk and community perspectives in rural Tanzania. *Malar J.* 2024;23(1):213. 10.1186/s12936-024-05039-1.
28. Dev V. Long-Lasting Insecticidal Nets: An Evidence-Based Technology for Malaria Vector Control and Future Perspectives. In: Tyagi BK, editor. *Genetically Modified and other Innovative Vector Control Technologies* [Internet]. Singapore: Springer Singapore; 2021 [cited 2026 1 Apr]. pp. 297–309. Available from: https://link.springer.com/10.1007/978-981-16-2964-8_15 doi:10.1007/978-981-16-2964-8_15.
 29. Ghana Health Service G. *Ada West Annual Report [End of Year Report]* [Internet]. 2025. Available from: <https://chimgh.org/>
 30. Wilcox BA, Echaubard P, De Garine-Wichatitsky M, Ramirez B. Vector-borne disease and climate change adaptation in African dryland social-ecological systems. *Infect Dis Poverty.* 2019;8(1):36. 10.1186/s40249-019-0539-3.
 31. Obame-Nkoghe J, Agossou AE, Mboowa G, Kamgang B, Caminade C, Duke DC, et al. Climate-influenced vector-borne diseases in Africa: a call to empower the next generation of African researchers to find sustainable solutions. *Infect Dis Poverty.* 2024;13(1):26. 10.1186/s40249-024-01193-5.
 32. Thomson MC, Muñoz ÁG, Cousin R, Shumake-Guillemot J. Climate drivers of vector-borne diseases in Africa and their relevance to control programs. *Infect Dis Poverty.* 2018;7(1):81. 10.1186/s40249-018-0460-1.
 33. Mulamba C, Riveron JM, Ibrahim SS, Irving H, Barnes KG, Mukwaya LG et al. Widespread Pyrethroid and DDT Resistance in the Major Malaria Vector *Anopheles funestus* in East Africa Is Driven by Metabolic Resistance Mechanisms. Brooke B, editor. *PLoS ONE.* 2014;9(10):e110058. 10.1371/journal.pone.0110058
 34. Ngalamé AL, Watching D, Kibu OZ, Zeukóo EM, Nsagha DS. Assessment of the diagnostic performance of the SD Bioline Malaria antigen test for the diagnosis of malaria in the Tombel health district, Southwest region of Cameroon. Aninagyei E, editor. *PLoS ONE.* 2025;20(3):e0298992. 10.1371/journal.pone.0298992
 35. Singh A, Rathour A, Gupta RK, Kumar DVR, Acharya S, Jamuna DKV. Employing the Drivers-Pressures-State-Impact-Response (DPSIR) Framework to Analyse Water Pollution Trends. *NES.* 2025;10(3):261–74. 10.28978/nesciences.1811122.
 36. Zeru MA, Shibru S, Massebo F. Exploring the impact of cattle on human exposure to malaria mosquitoes in the Arba Minch area district of southwest Ethiopia. *Parasites Vectors.* 2020;13(1):322. 10.1186/s13071-020-04194-z.
 37. Chilton A, Rozema K. *Difference-in-Differences.* In: *Trial by Numbers* [Internet]. 1st edn. Oxford University Press New York; 2024 [cited 2026 Apr 5]. pp. 104–31. Available from: <https://academic.oup.com/book/56321/chapter/445422206> 10.1093/oso/9780197747858.003.0006

Figures

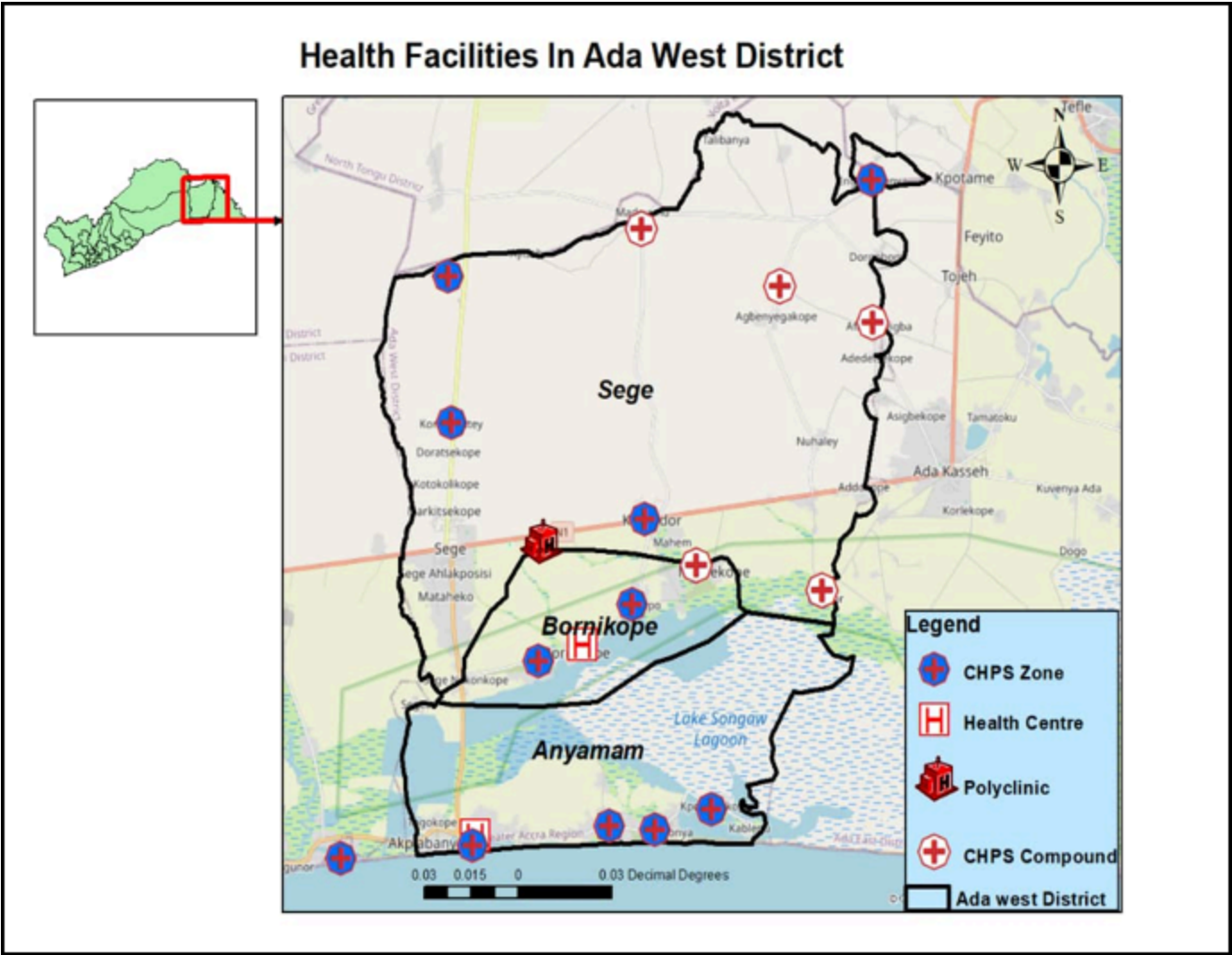


Figure 1

The Profile of Ada West District, 2026 (**Source;** District Health annual report, 2025)

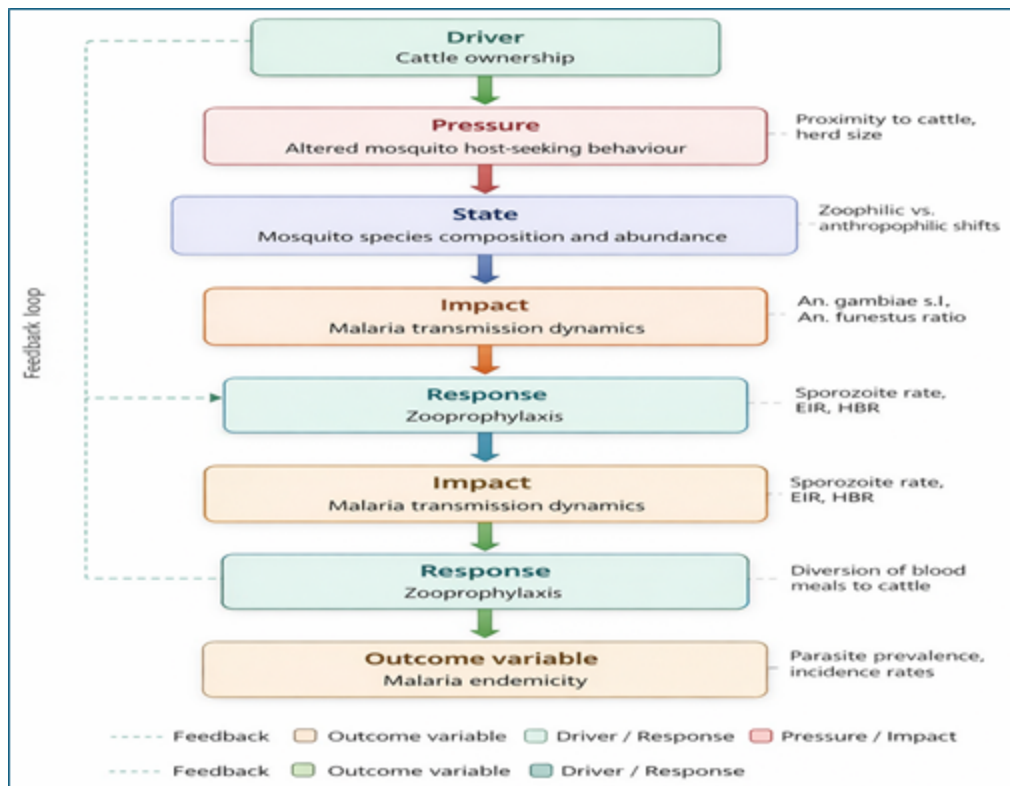


Figure 2

Conceptual framework guided by the Driver-Pressure-State-Impact-Response (DPSIR) model showing pathways linking cattle ownership to malaria transmission outcomes.

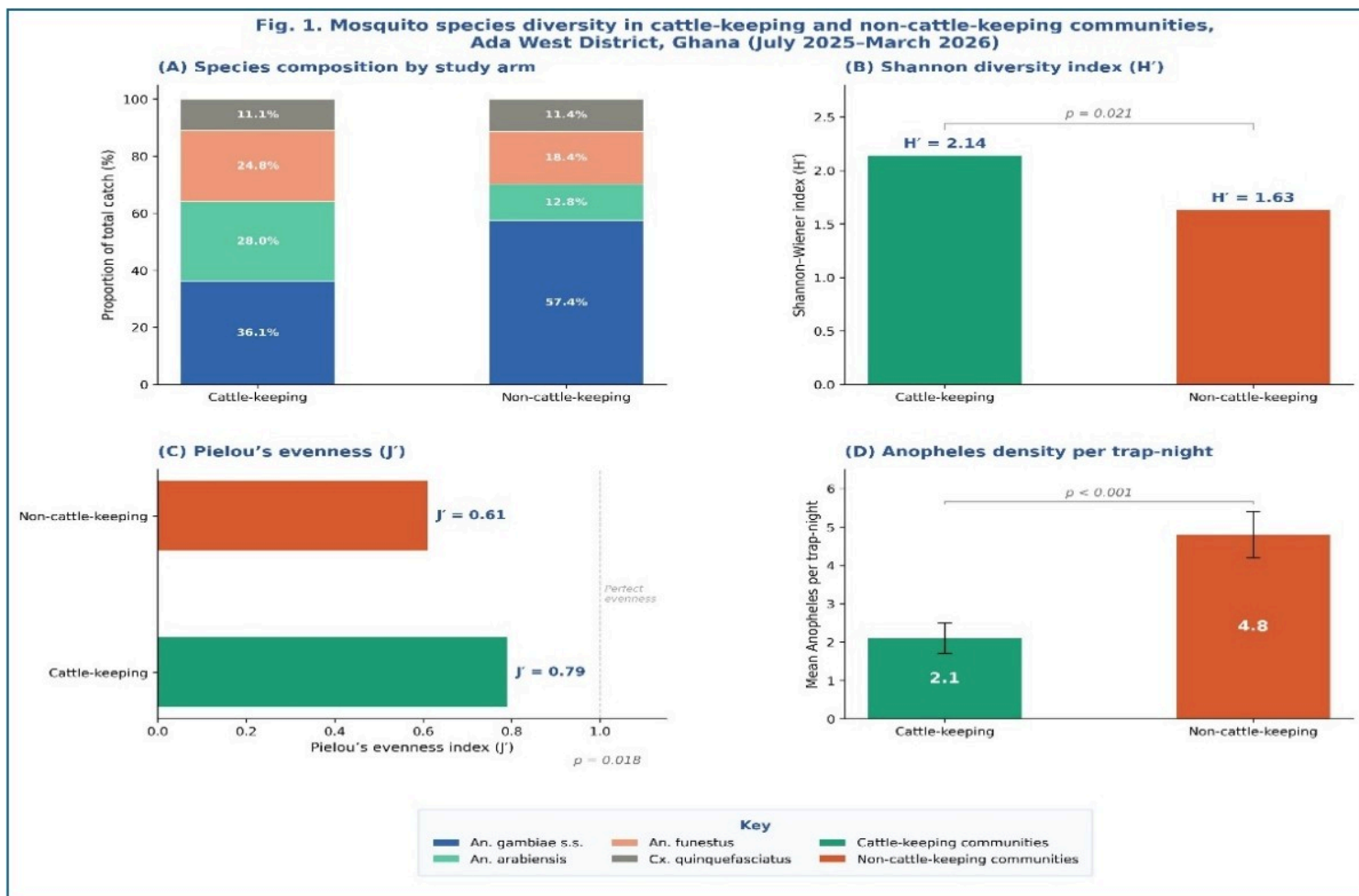


Figure 3

Mosquito species diversity in cattle-keeping and non-cattle-keeping communities in the Ada West District (July 2025–March 2026). (A) Species composition in each study arm. (B) Shannon–Wiener diversity index (H'). (C) Pielou's evenness index, J' . Molecular identification of *Anopheles gambiae* s.l. subsample was by PCR (20%). Pielou's evenness index (J') measures how equally individuals are distributed across species, ranging from 0 (complete dominance by one species) to 1.0 (perfectly equal distribution of individuals across species).