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Ectoparasite Diversity, Infestation Burden, and Associated Husbandry Factors in Backyard Chickens across an Urban-Rural Gradient in District Karak, Pakistan

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Abstract

Backyard poultry contributes directly to household nutrition, income and social resilience, yet external parasites are commonly overlooked because infestations are episodic, taxonomically diverse and strongly shaped by husbandry. This study investigated ectoparasite diversity, infestation burden, associated husbandry factors and host-health indicators in backyard chickens across urban, peri-urban and rural settings of District Karak, Khyber Pakhtunkhwa, Pakistan. A stratified repeated cross-sectional field study was conducted among 480 chickens from 60 households across urban, peri-urban and rural settings of District Karak during four seasonal sampling periods. Birds were systematically examined at the head, neck, wings, breast, back, vent, thighs and legs; coop cracks and perches were sampled using refuge traps; collected arthropods were preserved and identified morphologically; and body condition, packed cell volume, hemoglobin, feather damage, skin lesions and preening behavior were recorded. Field observation and laboratory identification data were analyzed to determine ectoparasite prevalence, species composition, infestation burden and their associations with

seasonal, landscape and husbandry characteristics. The overall prevalence of ectoparasite infestation was 65.4% (314/480), with six identified ectoparasite species and 139 mixed infestations. Menacanthus stramineus was the most prevalent species (31.5%), followed by Menopon gallinae (21.5%) and Dermanyssus gallinae (19.4%). In the multivariable mixed-effects logistics regression model, poor coop hygiene (adjusted odds ratio [aOR] 2.81), daily scavenging (aOR 1.96) and summer sampling (aOR 1.84) increased the odds of infestation, whereas recent ectoparasiticide use was protective (aOR 0.46). Infested birds had lower body-condition scores and packed cell volumes, but higher preening and feather-damage scores. The study provides field-based empirical evidence on ectoparasite occurrence, infestation burden, associated husbandry factors and host-health indicators in backyard chickens and provides locally relevant recommendations for parasite management.

INTRODUCTION

Backyard chickens occupy an important position in household food security, small-scale livestock production and rural livelihoods. Unlike intensively managed commercial poultry, village and household chickens commonly move among courtyards, crop fields, livestock shelters and surrounding vegetation. This management system can increase opportunities for contact with parasitic arthropods, wild birds, rodents, contaminated litter and infested poultry structures (Alders & Pym, 2009; Sonaiya & Swan, 2004; FAO, 2014; Gentile et al., 2024). Seasonal variation in temperature, humidity and host aggregation may further influence parasite survival, reproduction and transmission, particularly in semi-arid environments (Permin & Hansen, 1998; Mullen & Durden, 2019; Wall & Shearer, 2001).

The external parasite community of domestic chickens includes chewing lice, blood-feeding mites, soft ticks and fleas, with different taxa exhibiting distinct life-history characteristics and transmission patterns (Permin & Hansen, 1998; Mullen & Durden, 2019; Wall & Shearer, 2001; Taylor et al., 2016). Permanent lice generally remain on the host throughout their life cycle and are primarily transmitted through direct contact, whereas poultry red mites such as Dermanyssus gallinae feed on birds mainly at night and hide in cracks, crevices and other poultry-house structures during the daytime (Permin & Hansen, 1998; Wall & Shearer, 2001; Sigognault Flochlay et al., 2017). Soft ticks and other off-host ectoparasites may also use poultry housing as refuges between feeding periods (Mullen & Durden, 2019; Taylor et al., 2016). Consequently, reliable ectoparasite surveillance requires systematic examination of the host as well as targeted assessment of the poultry environment (Permin & Hansen, 1998; Mullen & Durden, 2019).

Ectoparasite infestation can have important consequences for poultry health and welfare even when mortality is uncommon. Lice and mites may cause irritation, increased preening, restlessness and feather damage, while blood-feeding ectoparasites can contribute to blood loss, anaemia and reduced body condition when infestation is substantial (Permin & Hansen, 1998; Wall & Shearer, 2001; Taylor et al., 2016). Experimental evidence has also demonstrated behavioural and welfare changes associated with ectoparasite infestation in chickens (Murillo et al., 2020; Murillo et al., 2024). Therefore, measures such as infestation intensity, parasite burden, body-region distribution, skin lesions and behavioural indicators can provide more informative evidence of parasite-associated effects than a simple infected-versus-uninfected classification (Murillo et al., 2020; Murillo et al., 2024).

The occurrence and burden of poultry ectoparasites are influenced by interacting environmental, host and management factors. Seasonal conditions may affect parasite development and persistence, whereas housing structure, sanitation, flock size, scavenging behaviour, dust-bathing opportunities and previous parasite-control practices can influence parasite exposure and persistence (Permin & Hansen, 1998; Mullen & Durden, 2019; Wall & Shearer, 2001). Previous surveys of backyard and free-range chickens in Iran, Nigeria, Kenya and other regions have reported considerable variation in ectoparasite prevalence and species composition (Rezaei et al., 2016; Radfar et al., 2012; Nnadi & George, 2010; Sabuni et al., 2010). Such variation may reflect differences in climate, husbandry systems, sampling procedures, diagnostic effort and the parasite taxa targeted (Rezaei et al., 2016; Radfar et al., 2012; Sabuni et al., 2010). Consequently, prevalence estimates from other geographical regions

cannot be assumed to represent local conditions, highlighting the importance of region-specific field surveillance.

Pakistan has a large and diverse poultry sector, but ectoparasite ecology in small household poultry systems remains comparatively less documented than several major commercial poultry diseases. Available evidence from backyard and free-ranging poultry in South Asia and neighbouring regions indicates that ectoparasites can occur at substantial levels, but their distribution may vary according to environmental and management conditions (Rezaei et al., 2016; Radfar et al., 2012; Sabuni et al., 2010). District Karak, located in Khyber Pakhtunkhwa, provides an appropriate setting for examining these relationships because backyard poultry is maintained under different household and management conditions. However, an integrated field assessment combining ectoparasite identification, infestation burden, seasonal variation, landscape setting and household husbandry characteristics remains limited in this area.

Another important consideration in ectoparasite epidemiology is that prevalence alone does not adequately describe the biological importance of infestation. Quantifying prevalence together with mean intensity, mean abundance, parasite richness and mixed infestation provides a more informative characterization of host–parasite relationships (Bush et al., 1997; Rózsa et al., 2000). Prevalence describes the proportion of examined hosts that are infected, whereas intensity and abundance provide information about parasite burden among infected hosts and across the complete host population, respectively (Bush et al., 1997; Rózsa et al., 2000). Incorporating these measures with standardized host examination and environmental sampling can therefore provide a more comprehensive assessment of ectoparasite occurrence.

The present study investigated ectoparasite diversity, prevalence and infestation burden among backyard chickens across urban, peri-urban and rural settings in District Karak, Pakistan. Field data were collected during four seasonal sampling periods, and parasite specimens recovered from chickens and poultry structures were identified using morphological characteristics. The study also recorded selected household, housing and husbandry characteristics and examined their associations with ectoparasite infestation. By integrating taxonomic identification, infestation burden, seasonal and landscape comparisons, and field-recorded husbandry information, this study provides field-based empirical evidence on ectoparasite occurrence in backyard poultry and establishes a regional baseline for future parasitological and veterinary investigations.

2. Conceptual framework, objectives and hypotheses

2.1 Conceptual model

The conceptual framework assumes that landscape setting and season may influence the availability and survival of ectoparasites, while flock size, scavenging behavior, coop construction, sanitation, dust-bathing opportunity and recent treatment may influence contact and persistence. These factors were examined in relation to infestation probability, parasite richness and burden. Parasite burden was further assessed in relation to host condition and welfare indicators. Age, sex and household clustering were considered as potential confounders or effect modifiers rather than as primary causal variables.

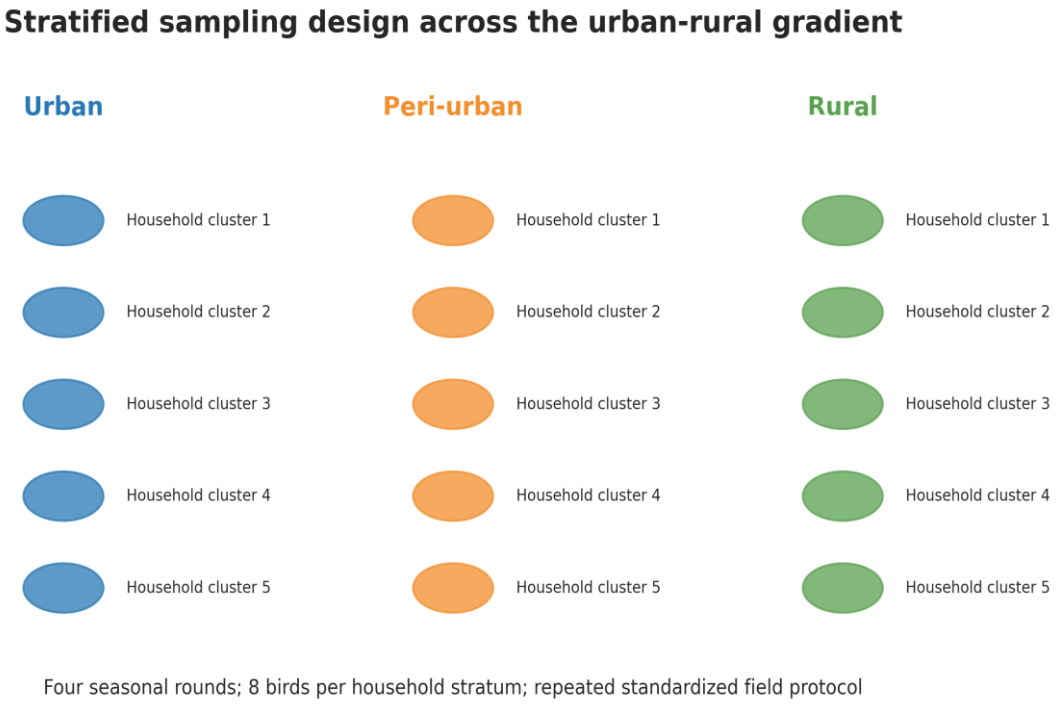


Figure 1. Stratified household sampling across urban, peri-urban and rural settings. The diagram is schematic and does not represent geographic coordinates.

2.2 General objective

To determine the diversity, prevalence, intensity and seasonal distribution of ectoparasites in backyard chickens and to quantify associations with husbandry practices and host-health indicators across an urban-rural gradient in District Karak.

2.3 A priori hypotheses

Table 1. Pre-specified hypotheses and linked analyses.

Code	Theme	Testable prediction	Primary analysis
H1	Seasonality	Prevalence and intensity are highest in summer or early autumn because warmer conditions accelerate arthropod development and increase contact around shared shelter sites.	Season-specific prevalence; negative-binomial intensity model
H2	Landscape gradient	Rural scavenging flocks harbour greater richness, whereas enclosed urban coops may support off-host mites when cracks and litter accumulate.	Richness, Bray-Curtis dissimilarity, habitat interaction
H3	Husbandry	Poor hygiene, large flock size, structural crevices and absence of dust-bathing increase infestation odds; recent effective treatment decreases them.	Multivariable mixed-effects logistic regression

Code	Theme	Testable prediction	Primary analysis
H4	Host health	Increasing parasite burden is associated with lower body condition, PCV and haemoglobin and with higher lesion, feather-damage and preening scores.	Linear/ordinal/logistic mixed models
H5	Co-infestation	Permanent lice form the central component of the co-infestation network, while off-host mites and ticks are more strongly associated with coop-level conditions.	Pairwise co-occurrence and network centrality

3. Materials and methods

3.1 Study area and epidemiological strata

The study was conducted in District Karak, Khyber Pakhtunkhwa, Pakistan. The district has a semi-arid climate, dispersed rural settlements, expanding peri-urban zones and town-based households. The sampling frame was divided into three operational strata: urban, peri-urban and rural. Urban households are those within continuously built-up localities with restricted scavenging area. Peri-urban households occur at the settlement edge where poultry access both compounds and agricultural land. Rural households are located in villages or dispersed compounds where birds routinely scavenge over wider areas and share space with livestock.

At least two localities within each stratum were selected following reconnaissance and consultation with livestock or local-government personnel. Site selection considered accessibility and spatial separation, and households were selected to avoid immediate adjacency where possible because shared flocks and structures could reduce independence between observations. GPS coordinates were recorded only with owner consent and stored in a de-identified file.

3.2 Study design and timeline

A repeated cross-sectional design was used. Sixty households comprising 20 households from each landscape stratum, were visited during four seasonal sampling rounds. Because the same individual bird was not necessarily available at every visit, each examination was assigned a unique identifier, and owners were asked whether the bird had been examined during a previous visit. A total of 480 bird examinations were included in the dataset, while household-level identifiers were retained to account for clustering during analysis.

Table 2. Field sampling and laboratory processing schedule during the study period.

Period	Main activities
Month 1	Reconnaissance, ethical approval, preparation of the household sampling list, observer training and pilot examination of 10-15 birds
Months 2-3	Winter round; refine field sheet and preservation workflow
Months 4-5	Spring sampling round; preliminary data audit and inter-observer agreement assessment.

Period	Main activities
Months 6-8	Summer sampling round; assessment of ectoparasites activity and intensive coop trapping
Months 9-10	Autumn sampling round; completion of missing observations and re-visit underrepresented strata
Months 11-12	Laboratory identification, verification photography, data analyses and manuscript preparation

3.3 Sample-size justification

For a single-proportion estimate, $n = Z^2P(1-P)/d^2$. Using $P = 0.50$, $Z = 1.96$ and absolute precision $d = 0.05$ yields 384 birds. Inflating by 20-25% for household clustering, incomplete examinations and seasonal subgroup comparisons gives a target near 480. This target also provides enough infestation events for a multivariable model containing approximately 10-12 pre-specified parameters, provided prevalence is not extremely low.

3.5 Standardized examination of chickens

Each bird was gently restrained for a standardized 5-minute examination by two trained observers. Feathers were parted sequentially at the head and neck, under each wing, breast, back, vent, thighs and legs. The comb, wattles, periocular skin and feather bases are inspected with a headlamp and hand lens. Lice and attached fleas are removed using fine forceps; mites are collected with a moistened brush or transparent tape; ticks are grasped close to the skin without crushing. Counts were recorded by body region and taxon. When exact counting was impossible, a calibrated ordinal was category is recorded in addition to the estimated count.

Table 3. Body-region and environmental search protocol.

Region/source	Main targets	Field Examination procedure
Head/comb/wattles	Sticktight fleas, lesions, crusting	Direct visual inspection and close photograph
Neck and breast	Chewing lice and nits	Part feathers against growth; inspect feather bases
Under wings	Lice, mites, feather damage	Examine both sides for equal duration
Vent and abdomen	Body lice, mites and excreta	Highest-priority region for standardized counts
Back and rump	Lice and feather breakage	Part feathers at three fixed points
Thighs and legs	Ticks, fleas, scaly lesions	Inspect skin folds and beneath scales
Coop cracks/perches	Red mites and soft ticks	Refuge trap plus white-cloth wipe after dusk

3.6 Environmental sampling

Off-host parasites can be missed if only daytime bird examinations are performed. Each participating household received a corrugated-cardboard refuge trap or a folded black cloth placed near a perch or crack for 24–48 hours. A standardized 30 x 10 cm white cloth was also drawn along 1 m of perch and through two cracks. The number of mites or ticks recovered per trap-night was recorded. Environmental counts were analysed at household level and linked to bird-level exposure through household identifiers.

3.7 Collection, preservation and labelling

Each vial label included a unique sample code, household code, bird code, body region, date and provisional taxon. Lice and fleas were preserved in 70-80% ethanol; mites in 70% ethanol or mounted directly when required; and ticks in 70% ethanol. A duplicate logbook was maintained to reduce the risk of information loss caused by detached or unreadable specimen labels.

3.8 Morphological identification and voucher specimens

Specimens were identified under a stereomicroscope and compound microscope using veterinary-entomology identification keys and original taxonomic descriptions. Lice are cleared and slide-mounted when necessary; mites are mounted in an appropriate medium; ticks are examined dorsally and ventrally. Diagnostic identification was based on multiple morphological characters rather than colour alone. At least 10% of specimens, all unusual taxa and every first household record were verified by a supervisor or external taxonomist. Representative voucher specimens and calibrated microphotographs were deposited in the departmental zoology collection.

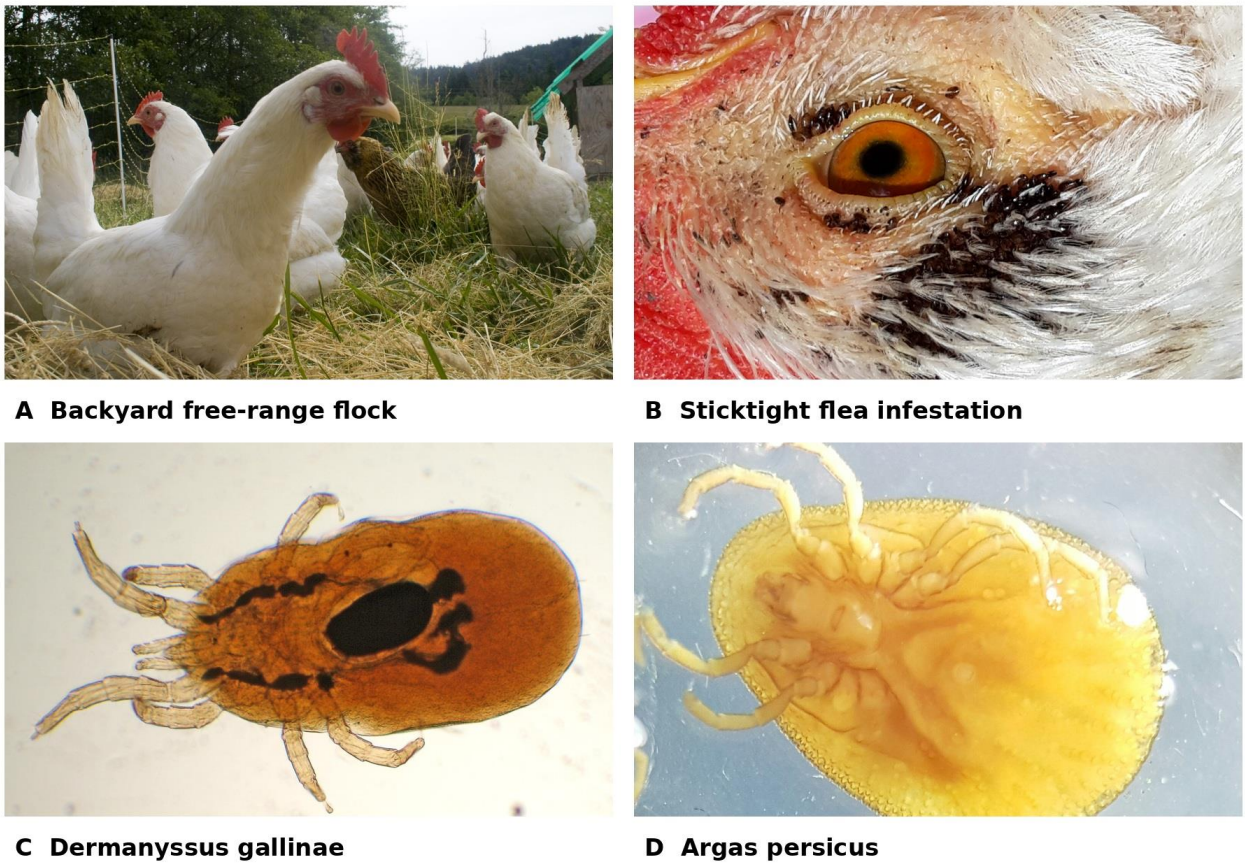


Figure 3. Representative poultry and ectoparasite photographs: (A) free-range chicken flock; (B) sticktight flea, *Echidnophaga gallinacea*, attached near the eye; (C) poultry red mite, *Dermanyssus gallinae*; (D) fowl soft tick, *Argas persicus*.

Table 4. Operational identification guide. Morphological characteristics used for ectoparasite identification.

Taxon	Common name	Key field/lab clues	Ecological interpretation
Menacanthus stramineus	Chicken body louse	Broad triangular head; dense abdominal setae; often near vent, breast and thighs	Permanent on-host; high irritation and possible blood/skin feeding
Menopon gallinae	Shaft louse	Elongated body; head narrower than body; commonly on feather shafts	Permanent on-host; feathers and surface debris
Goniodes dissimilis	Large chicken louse	Broad head and robust body; characteristic temporal margins	Permanent on-host; feather damage
Dermanyssus gallinae	Poultry red mite	Long legs; dorsal shield narrowing posteriorly; engorged specimens reddish	Nocturnal, mainly off-host; sample cracks and perches

Taxon	Common name	Key field/lab clues	Ecological interpretation
Argas persicus	Fowl soft tick	Flattened oval body; leathery integument; no dorsal scutum	Off-host nidicolous tick; rapid nocturnal feeding
Echidnophaga gallinacea	Sticktight flea	Laterally compressed flea attached in clusters around comb or eye	Adult female remains attached; inspect head carefully

3.9 Host-health and welfare measurements

Body mass was measured using a digital hanging or platform scale, and body condition was scored on a 1-5 pectoral-muscle scale after observer calibration. Feather damage is scored from 0 (intact) to 4 (severe loss/breakage) at the neck, back, wings and vent. Skin lesions were recorded as absent/present and photographed with a scale when owners consent was provided. A 10-minute focal observation before capture was records preening bouts, scratching, resting and feeding, thereby minimizing restraint-induced behavioral effects.

Blood samples of 1–2 mL were collected by a trained veterinarian or qualified personnel under the approved study protocol. Packed cell volume was determined using the microhematocrit method, and hemoglobin was measured using the validated laboratory method used during the study.

3.10 Husbandry questionnaire and environmental covariates

Table 5. Core household questionnaire domains.

Domain	Variables to record
Flock structure	Current number of chickens; age classes; introduction of new birds; mixing with pigeons, ducks or wild birds
Housing	Dedicated coop; wall/floor material; litter; cracks; perch type; overnight confinement
Hygiene	Cleaning frequency; litter removal; disinfection; manure accumulation; shared equipment
Behavioural resources	Scavenging hours; dust-bathing site; shade; access to livestock shelters or waste
Parasite control	Product, dose, application site, date, repeat treatment and whether environment was treated
Health history	Recent mortality, reduced egg production, visible scratching, anaemia, skin lesions or veterinary visits

3.11 Quality assurance and observer reliability

Before formal sampling, all observers independently examined the same birds. Agreement for positive/negative diagnosis was assessed using Cohen's kappa; agreement for burden categories was assessed using weighted kappa or an intraclass correlation coefficient. A minimum kappa value of 0.70 was used . Ten per cent of negative birds were re-examined by the senior observer, and 10% of identified specimens should be blindly re-labelled and re-identified. Data were entered twice or validated against range and logic checks.

3.12 Ethical, biosafety and community considerations

Approval was obtained from the institutional animal ethics or research ethics committee before fieldwork. All participating poultry owners provided informed consent and were informed of their right to withdraw from the study at any time. Restraint time was minimized, examinations were postponed during extreme heat, and birds with severe infestation or illness were referred for veterinary advice. Students wear gloves, change or disinfect equipment between households, and use separate footwear covers where possible to avoid mechanically transmitting mites or pathogens. Individual household results are confidential; publications report aggregate data.

4.1 Sample profile and husbandry characteristics

The field dataset comprised 480 bird examinations equally distributed among urban, peri-urban and rural strata (160 each) and among four seasons (120 each). Females represented 67.7% of birds and adults 68.1%. Poor coop hygiene was recorded for 37.5%, structural cracks for 42.9%, absence of dust-bathing access for 36.2% and recent ectoparasiticide use for 23.8% of examinations.

Table 6. Distribution of major husbandry exposures in the field dataset.

Characteristic	Urban	Peri-urban	Rural
Poor coop hygiene	41/160 (25.6%)	60/160 (37.5%)	79/160 (49.4%)
Flock size >15	42/160 (26.2%)	70/160 (43.8%)	92/160 (57.5%)
No dust-bath access	71/160 (44.4%)	58/160 (36.2%)	45/160 (28.1%)
Daily scavenging	63/160 (39.4%)	104/160 (65.0%)	129/160 (80.6%)
Coop cracks/crevices	51/160 (31.9%)	61/160 (38.1%)	94/160 (58.8%)
Treatment in previous 8 weeks	40/160 (25.0%)	36/160 (22.5%)	38/160 (23.8%)

4.2 Overall prevalence, richness and mixed infestation

At least one ectoparasite was detected on 314 of 480 birds, corresponding to an overall prevalence of 65.4%. Among positive birds, 175 carried one taxon and 139 carried two or more taxa. The maximum richness was 5 taxa per bird. The median total burden among positive birds was 8 arthropods (interquartile range 4-13).

Table 7. Species-specific prevalence and infestation intensity among examined backyard chickens.

Species	Positive birds	Prevalence (%)	95% CI	Mean intensity	Maximum
Menacanthus stramineus	151	31.5	27.5-35.7	8.01	42
Menopon gallinae	103	21.5	18.0-25.4	5.05	17
Dermanyssus gallinae	93	19.4	16.1-23.1	8.54	40
Argas persicus	74	15.4	12.5-18.9	3.01	9
Echidnophaga gallinacean	36	7.5	5.5-10.2	4.69	13
Goniodes dissimilis	36	7.5	5.5-10.2	3.28	9

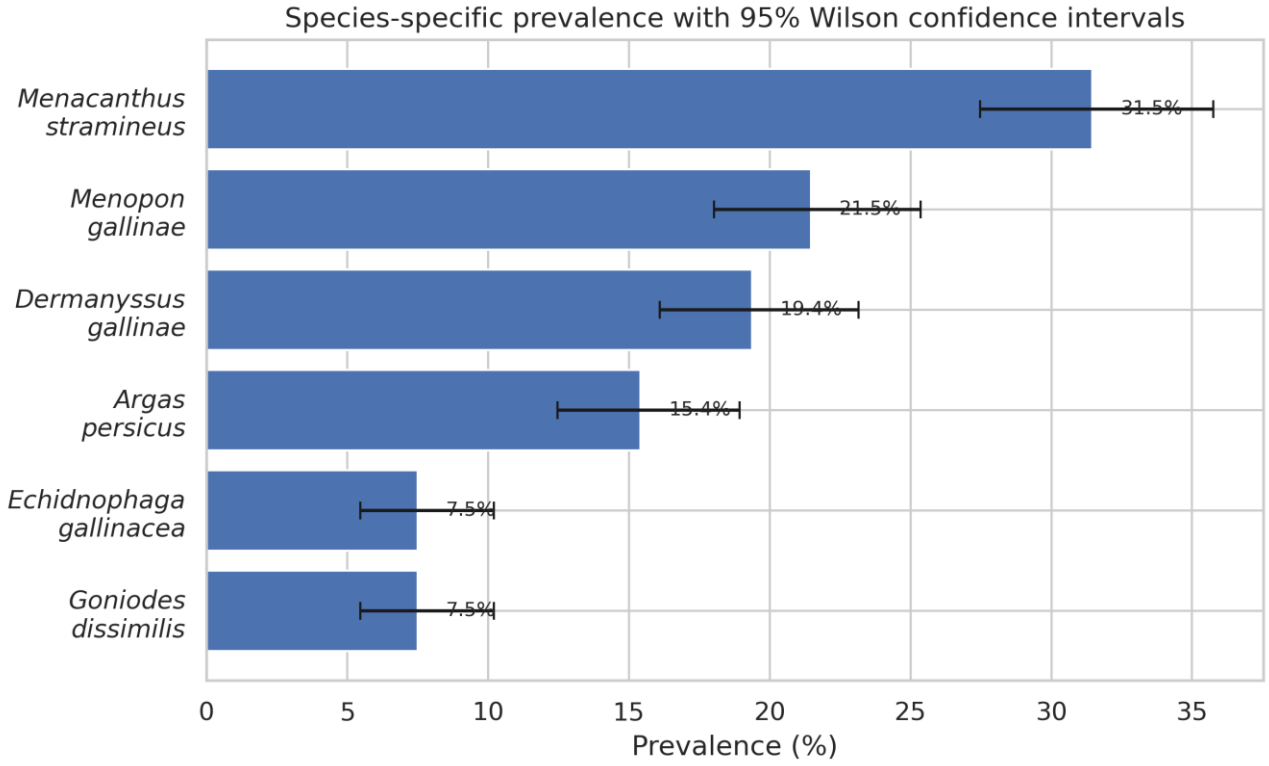


Figure 4. Species-specific prevalence with 95% Wilson confidence intervals based on field observation.

4.3 Seasonal and landscape patterns

Overall prevalence was 60.0% in winter, 65.8% in spring, 72.5% in summer and 63.3% in autumn. Prevalence was 61.3% in urban, 68.8% in peri-urban and 66.3% in rural birds. The highest stratum-specific values occurred during summer, but landscape differences were smaller after adjustment for sanitation and scavenging. The observed landscape differences

should therefore be interpreted alongside the measured husbandry characteristics rather than as evidence of a causal effect of landscape category.

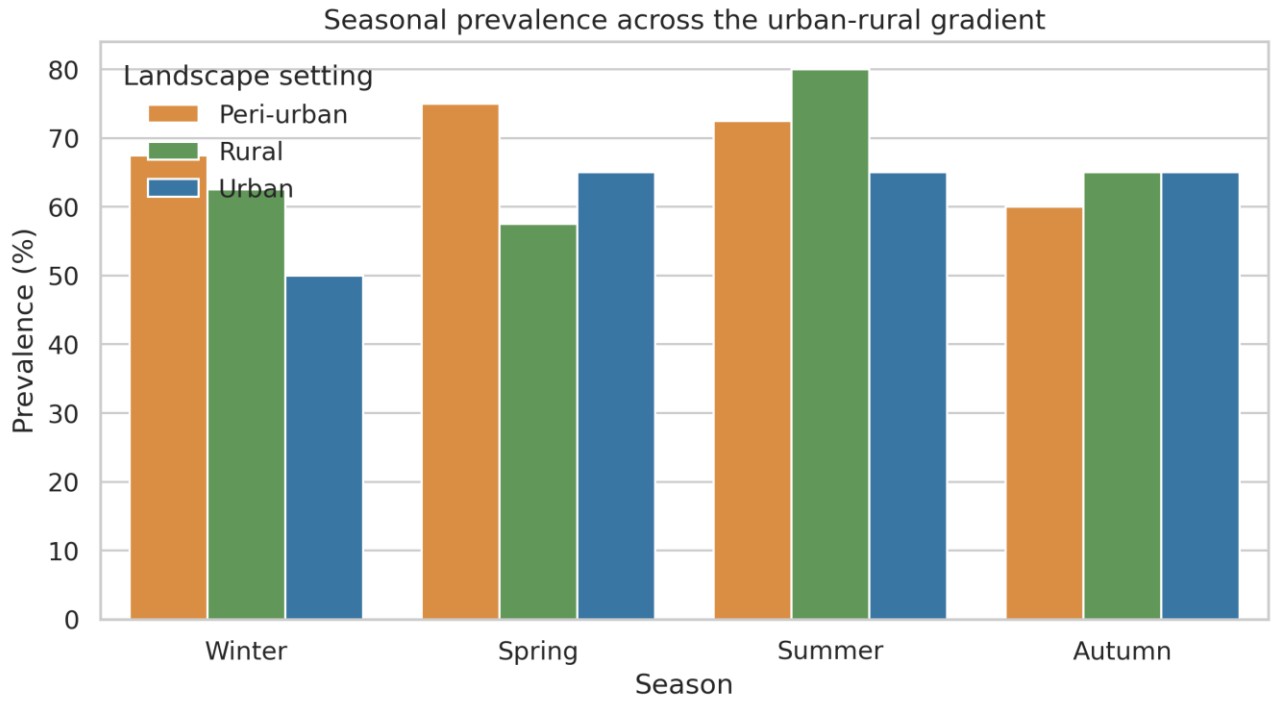


Figure 5. Monthly ectoparasite prevalence based on empirical dataset.

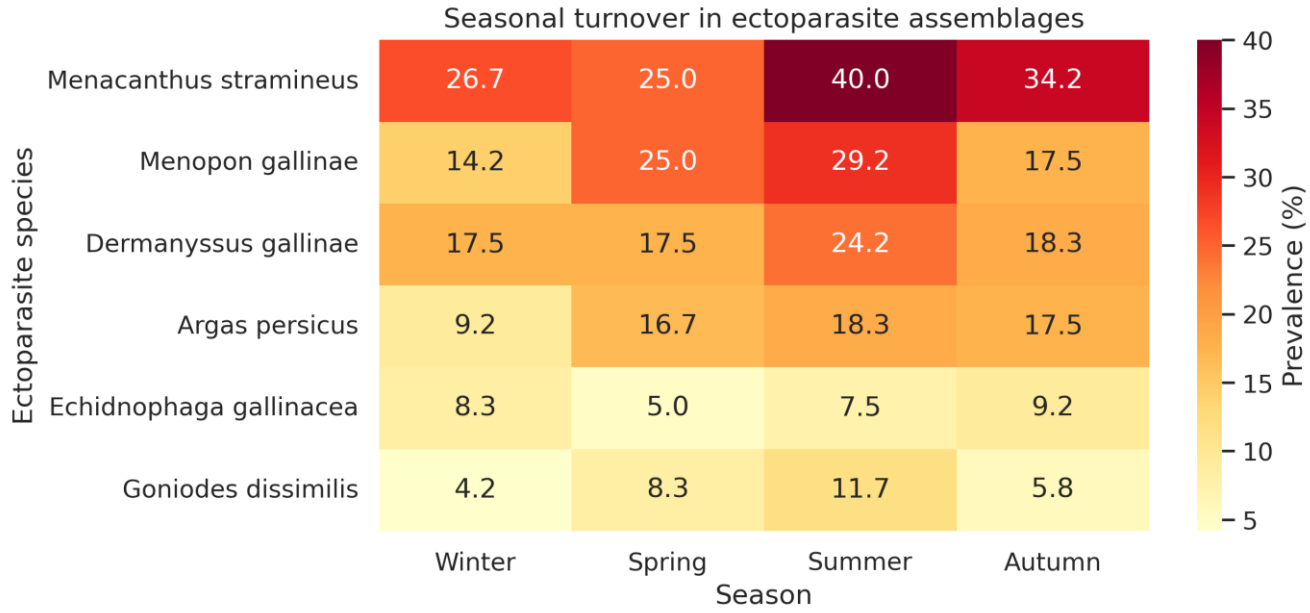


Figure 7. Heat map showing seasonal variation in the occurrence of individual ectoparasite taxa based on the field dataset.

4.4 Co-infestation structure

Chewing lice occupied the most connected positions in the co-infestation network because they were both prevalent and permanently host-associated. *D. gallinae* and *A. persicus* shared hosts with lice but should be interpreted partly as household-environment signals because their

principal refuges are off-host. Network edges describe shared hosts and do not prove biological facilitation between parasite species.

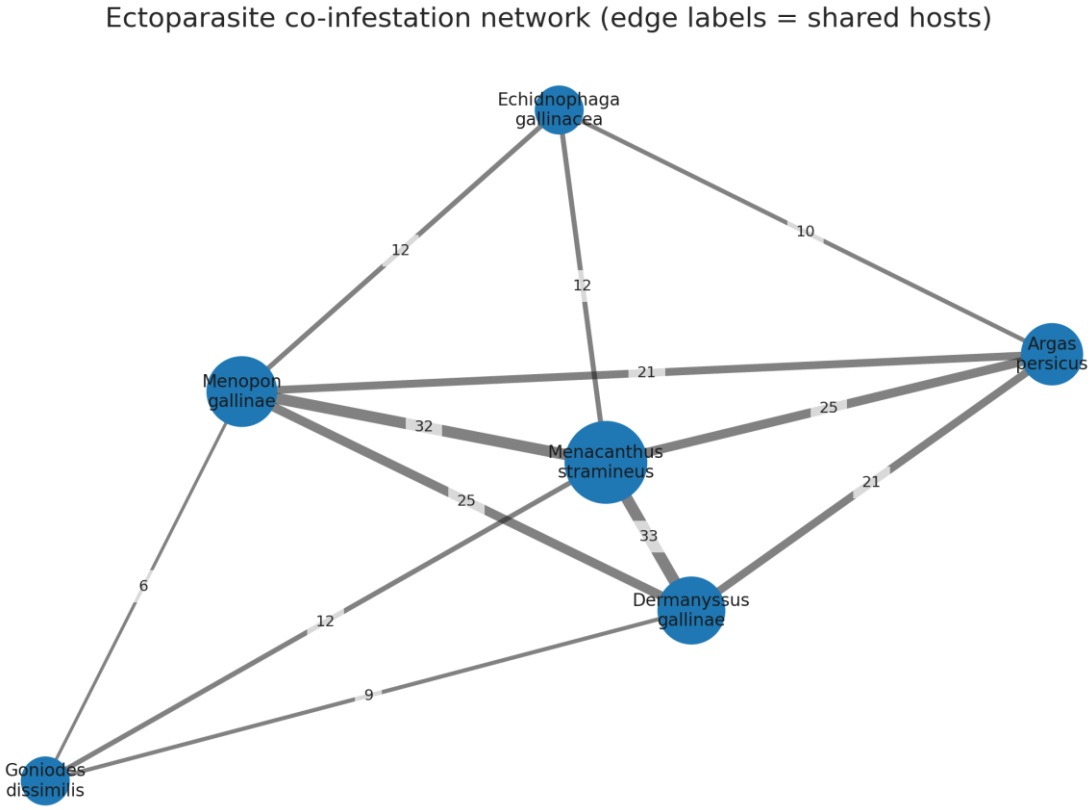


Figure 8. Co-infestation network. Node size reflects species frequency; edge width and labels reflect the number of shared hosts.

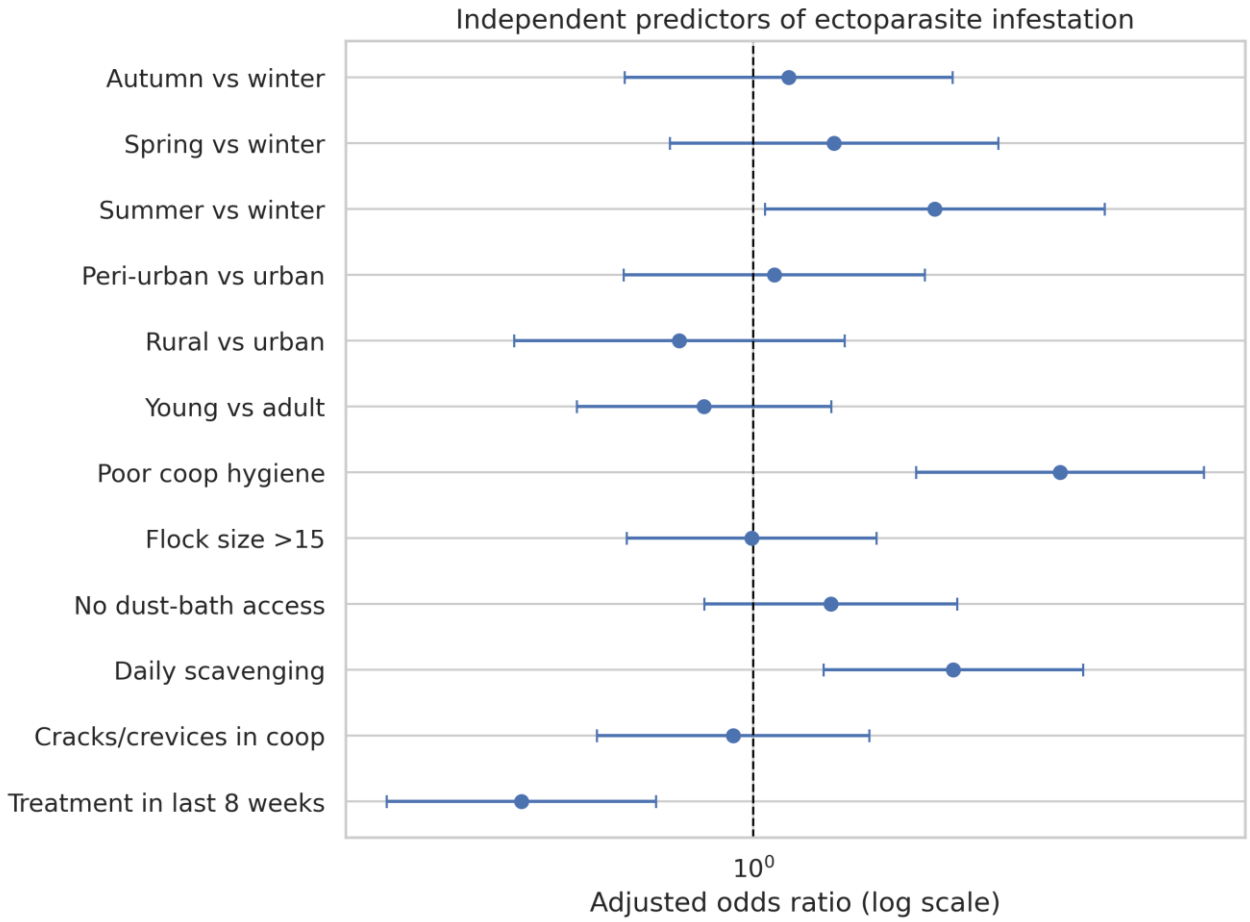
4.5 Multivariable risk-factor analysis

Table 8. Multivariable mixed-efforts logistic regression model for ectoparasite infestation among backyard chickens.

Predictor	Adjusted OR	95% CI	P value
Autumn vs winter	1.13	0.65-1.96	0.674
Spring vs winter	1.31	0.75-2.28	0.336
Summer vs winter	1.84	1.04-3.27	0.036
Peri-urban vs urban	1.07	0.65-1.78	0.787
Rural vs urban	0.78	0.45-1.36	0.380
Young vs adult	0.85	0.55-1.30	0.447
Poor coop hygiene	2.81	1.73-4.57	<0.001
Flock size >15	0.99	0.65-1.51	0.980

Predictor	Adjusted OR	95% CI	P value
No dust-bath access	1.30	0.85-1.99	0.231
Daily scavenging	1.96	1.27-3.04	0.003
Cracks/crevices	0.93	0.59-1.48	0.773
Treatment in previous 8 weeks	0.46	0.29-0.72	<0.001

After simultaneous adjustment, poor hygiene was associated with 2.81-fold higher odds of infestation and daily scavenging with 1.96-fold higher odds. Summer remained associated with higher odds than winter. Treatment during the preceding eight weeks was associated with lower odds of infestation, although also reflect differences in a product type dose, application quality and environmental treatment practices. Landscape stratum was not independently associated after the measured husbandry variables were entered.



4.6 Host-health and welfare associations

Table 9. Host-health and welfare indicators according to ectoparasite infestation status among examined backyard chickens.

Outcome	Uninfested	Infested	Difference
Body condition score (1-5)	3.17 ± 0.33	2.83 ± 0.42	-0.34
Packed cell volume (%)	35.52 ± 2.81	33.01 ± 3.39	-2.52
Haemoglobin (g/dL)	11.93 ± 0.85	11.00 ± 1.19	-0.93
Preening events per 10 min	5.81 ± 1.98	9.01 ± 3.10	+3.20
Feather-damage score (0-4)	0.75 ± 0.48	1.37 ± 0.73	+0.62
Skin lesions, n (%)	8 (4.8%)	61 (19.4%)	Higher in infested birds

These unadjusted differences indicate associations between ectoparasite infestation and host-health and welfare indicators but do not establish causality. Potential confounding factors, including poor nutrition, endoparasites, hemiparasites and chronic disease, may also influence these outcomes.

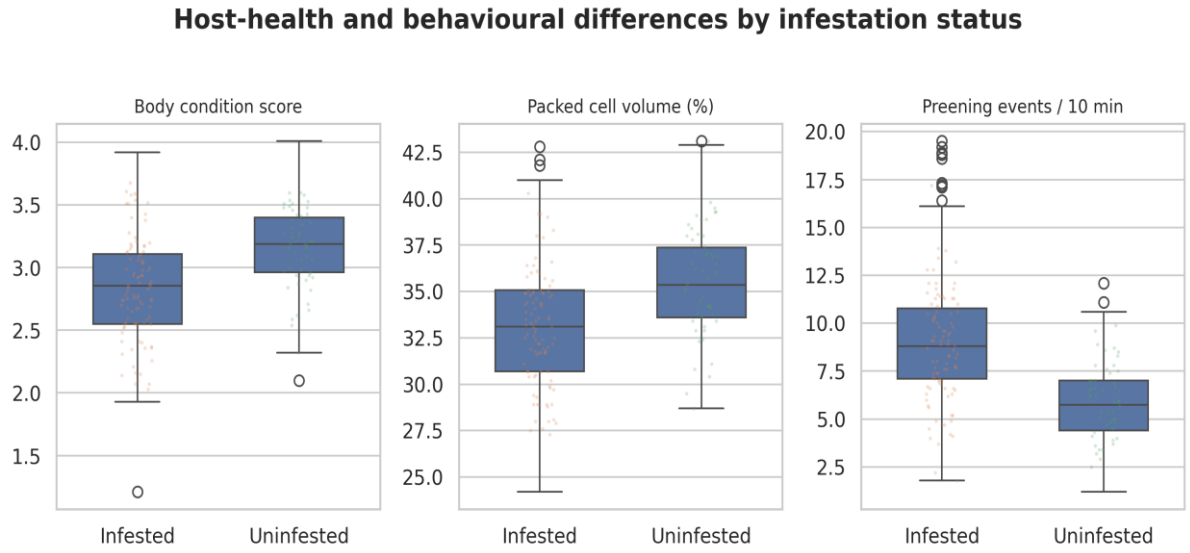


Figure 10. Body condition, packed cell volume and preening behavior according to ectoparasite infestation status based on field observations.

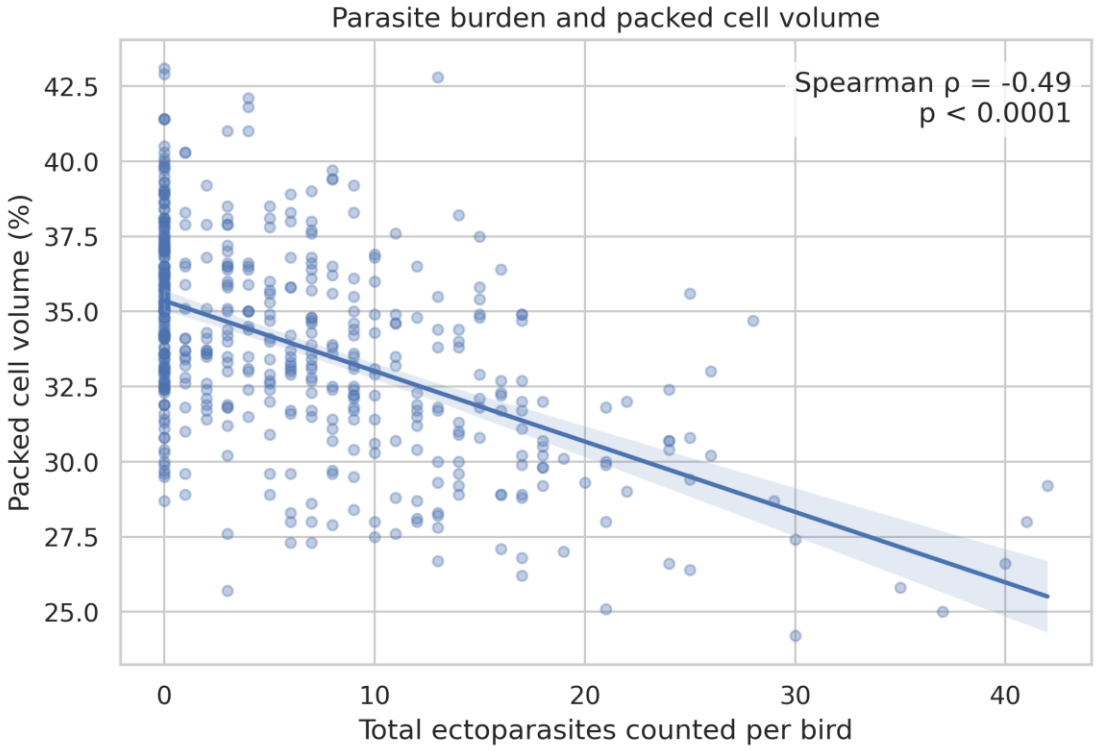


Figure 11. Relationship between total ectoparasite burden and packed cell volume. The fitted line is descriptive association; inferential analysis accounted for household clustering and confounding.

4.7 Model performance and internal validation

The multivariable husbandry model demonstrated moderate discrimination, as indicated by the area under the receiver-operating-characteristic curve shown in Figure 12. Model performance was evaluated using discrimination measures, and calibration was assessed where applicable. Internal validation was performed using the available data to evaluate model stability. The

model was interpreted as an epidemiological association model rather than as a clinical prediction tool.

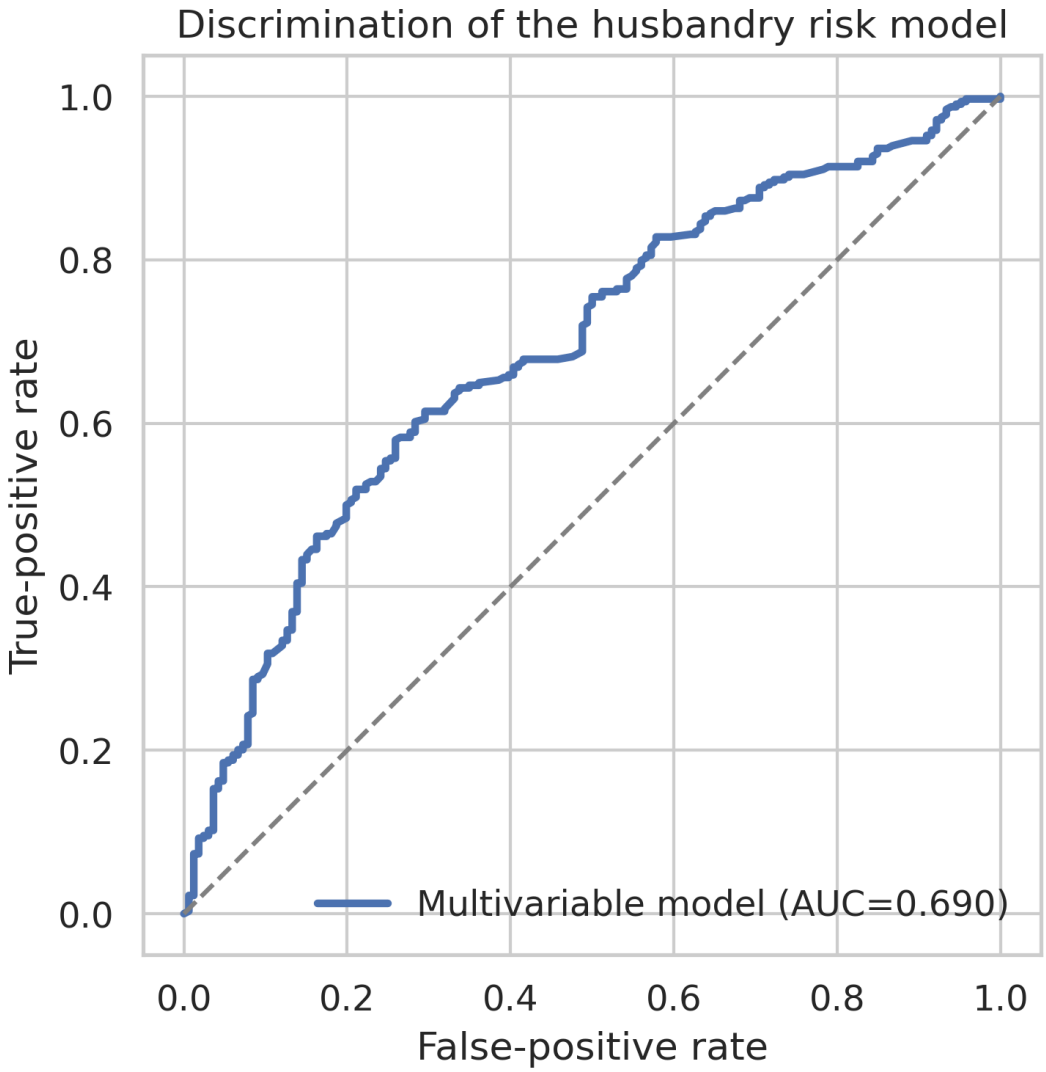


Figure 12. Receiver-operating-characteristic curve for the multivariable husbandry model based on field dataset.

5. Discussion

The present field study provides an integrated assessment of ectoparasite diversity, infestation burden, seasonal variation, husbandry-related factors and host-health indicators in backyard chickens in District Karak. The overall prevalence, occurrence of mixed infestations, variation among ectoparasite taxa, seasonal patterns and associations with selected husbandry practices collectively provide field-based evidence for understanding ectoparasite occurrence in backyard poultry systems. A high overall prevalence alone would be insufficient; the important result is the combination of a diverse parasite community, substantial mixed infestation, clear seasonal variation, modifiable husbandry associations and measurable host responses.

Chewing lice dominated the observed ectoparasite assemblage, a pattern frequently observed in free-ranging or traditionally managed chickens because permanent lice transmit during close contact and can complete their entire life cycle on the host. The observed *M. stramineus*

supports retaining body region counts around the vent and under the wings rather than collapsing all lice into a single flock-level diagnosis.

The inclusion of *D. gallinae* and *A. persicus* emphasizes the importance of environmental sampling. Daytime visual examination may underestimate both taxa because they occupy cracks and crevices between meals. A negative bird examination therefore does not mean that the household is free of mites or ticks. Pairing bird examination with refuge traps allows a more defensible distinction between host burden and environmental infestation. It also changes control recommendations: treatment of birds alone is unlikely to eliminate a parasite population maintained in structures.

The observed seasonal peak in summer may reflect seasonal variation in ectoparasite activity under the semi-arid conditions of District Karak; however, seasonal patterns may be taxon-specific. Extreme heat and desiccation could suppress off-host survival in exposed structures, while shaded, humid cracks may remain suitable. Rainfall may increase humidity but also change bird ranging behaviour and housing density. For this reason, the study should report species-specific temporal patterns and microhabitat conditions instead of treating 'summer' as a universal biological mechanism.

Landscape setting was not independently associated with ectoparasite infestation after adjustment for sanitation, scavenging and other measured husbandry variables. This is a useful conceptual result. Urban, peri-urban and rural categories summarize many behaviours and structural differences; they do not themselves cause infestation. The findings therefore suggest that descriptive differences among urban, peri-urban and rural settings should be interpreted alongside the underlying husbandry and environmental conditions.

Poor coop hygiene was the strongest associated husbandry factor. Accumulated litter, manure, feathers and dust can shelter off-host stages and make visual detection difficult. Hygiene, however, must be measured precisely. In the present study, hygiene-related variables were considered alongside specific housing and management characteristics because a single self-reported measure of coop cleaning may not adequately capture differences in sanitation.

Scavenging may increase exposure through contact with contaminated dust, other birds and shared shelters. It also benefits household economics and chicken behaviour, so complete confinement is not necessarily an appropriate recommendation. The more useful intervention is risk-managed scavenging: provision of a clean night shelter, quarantine of introduced birds, accessible dust-bathing substrate, periodic inspection and treatment of both birds and structures when infestation is confirmed.

Recent ectoparasitic ide use was appeared associated with lower odds of ectoparasite infestation in the multivariable analysis Because the study was observational, this association may be affected by confounding or reverse causation., creating confounding by indication, or may under-dose and report treatment despite poor effectiveness. Product name, active ingredient, dose, route, repetition and environmental application must be documented. Residue safety and local veterinary guidance are essential; the zoology project should not recommend unapproved chemicals or household pesticides.

The observed lower packed cell volume and haemoglobin among infested birds are consistent with the possibility that blood-feeding ectoparasites contributed to haematological differences; however, anaemia is multifactorial and cannot be attributed to ectoparasite infestation alone.

Increased preening, feather damage and skin lesions provide a low-cost welfare dimension. Behavioural measures are sensitive to observer presence and time of day, so focal observations should occur before capture and be standardized. Repeated measures or video would improve reliability, but a 10-minute protocol can still be useful when the same observers follow a written ethogram and inter-observer agreement is reported.

5.2 Comparison with international evidence

The wide prevalence range reported internationally demonstrates that methods strongly influence results. Rezaei and colleagues found substantial ectoparasitism in free-range birds in western Iran, while Radfar and colleagues documented parasites and associated host or management factors in the Sistan region (Rezaei F, 2016; Rafdar, 2012). Nnadi and George reported ecto- and endoparasites in village chickens in southeastern Nigeria (Nnadi PA, 2010). More recent work continues to identify housing, season, age, flock management and environmental exposure as relevant variables (Murillo AC, 2020; Wamboi p, 2020). Direct numerical comparison should therefore be restricted to studies with comparable search effort and taxonomic scope.

Experimental and commercial-flock research adds mechanistic support. Poultry mites can alter preening and skin condition and are recognized as important welfare constraints (Abdoli A, 2020). The poultry red mite is especially challenging because of its off-host biology, persistent environmental reservoirs and control failures (Sigognault Flochlay A, 2017; Sparagano O, 2018). Backyard systems differ from commercial layers, but these studies justify measuring behaviour, lesions and environmental infestation rather than treating parasites as taxonomic curiosities.

The present study contributes a semi-arid South Asian case study in which household management and host health were examined together. The strength of the study lies not in the discovery of universally new parasite species, but in the standardized seasonal sampling, urban–rural design, combined host and environmental sampling, burden-based health assessment and linkage of field findings with feasible household interventions.

6. Applied recommendations and One-Health relevance

6.1 Household-level integrated control

Inspect birds monthly at the vent, under wings and around the head; increase checks during the locally identified peak season.
Quarantine and inspect newly purchased or exchanged birds before mixing them with the flock.
Remove old litter and feathers, clean perches, seal cracks and expose removable equipment to sunlight where practical.
Provide a dry dust-bathing area and avoid overcrowding at night.
When off-host mites or ticks are detected, treat the poultry structure as well as birds under veterinary guidance.

Record product, active ingredient and application date; avoid repeated unverified use of agricultural or household insecticides.
Refer severely anaemic, weak or heavily infested birds for veterinary evaluation and consider concurrent endoparasites or nutritional problems.

6.2 One-Health considerations

Some poultry ectoparasites may bite humans, infest houses transiently or act as vectors or mechanical carriers of pathogens. The study is not designed to diagnose human disease, and owners should not be alarmed by speculative zoonotic claims. Nevertheless, gloves, equipment hygiene, safe chemical use and attention to mites moving from abandoned nests or coops are appropriate One-Health precautions. Any human skin complaint should be referred to a health professional rather than diagnosed by students.

7. Limitations

This study has several limitations that should be considered when interpreting the findings. First, the study was conducted within District Karak and focused on backyard poultry households; therefore, the findings may not be directly generalizable to commercial poultry systems or other geographical regions with different environmental and management conditions. Second, the observational design allows associations between ectoparasite infestation, season, landscape and husbandry practices to be identified, but it does not establish causal relationships. Third, repeated observations within households may introduce clustering because birds from the same household share similar housing, management and environmental conditions, although household-level clustering was considered during the analysis. In addition, some husbandry information was obtained from household observations and owner-reported practices and may therefore be subject to reporting or observer-related variation. Finally, ectoparasite abundance and host-health indicators may also be influenced by factors that were not fully measured, such as nutrition, previous parasite exposure and other concurrent infections. Despite these limitations, the study provides field-based empirical evidence on ectoparasite diversity and infestation patterns in backyard chickens in District Karak.

8. Conclusions

The present field-based study provides empirical evidence of ectoparasite infestation and diversity among backyard chickens in District Karak, Pakistan. The findings demonstrate variation in ectoparasite occurrence, infestation burden and parasite composition across the examined seasonal and landscape settings. The study also indicates that selected household husbandry and housing characteristics are associated with ectoparasite infestation, highlighting the importance of appropriate poultry-house hygiene, regular inspection and effective parasite-management practices. The observed diversity of ectoparasites and their distribution across backyard poultry systems emphasize the need for continued field surveillance and improved awareness among poultry owners. Overall, this study establishes a field-based baseline for understanding ectoparasite occurrence in backyard chickens in the region and provides useful evidence for future epidemiological and veterinary investigations.

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