

RESEARCH NOTE

Open Access



A cross-sectional survey of poultry management systems, practices and antimicrobial use in relation to disease outbreak in Pakistan

Farrukh Saleem^{1,2}, Aqsa Ameer^{1,5}, Farhan Afzal³, Muhammad Usman³, Hamid Irshad⁴, Sadia Sattar¹, Umer Zeeshan Ijaz^{5,6,7*} and Sundus Javed^{1*}

Abstract

Objectives The study aimed to examine how management practices, farming setup and breed influence disease outbreaks. It also sought to investigate the frequency and types of antimicrobials used, as well as the relationship between antimicrobial usage and disease occurrences.

Methods We conducted a survey of 140 poultry farms [Broiler farms = 66; Layer farms = 36; Local (Desi and its crosses) farms = 38] across major poultry producing regions of Pakistan. The gathered information covered demographics as well as the farming associated parameters including size, type of the farms, management practices, breeds raised, disease outbreak and antimicrobials use.

Results Using contingency analyses and log binomial regression models, we identified Broiler control sheds at high risk of disease. Diseases such as Avian Influenza, Newcastle Disease, and Fowl Typhoid were frequently reported and their outbreaks were associated with low cleaning frequency, high stocking density, bedding material using rice husk, and canola as a major feed ingredient. Farmer education was associated with a decrease in disease outbreak. Antimicrobial use was associated with farming experience, farm size, type and breed.

Conclusion High disease incidence is associated with management practices and breed types across various farm setups. Experienced Broiler farmers often report disease outbreaks and use antimicrobials more frequently. Educated farmers, however, experience fewer outbreaks and can better regulate antimicrobial usage.

Keywords Survey, Management practices, Disease outbreak, Antimicrobials, Zoonosis

*Correspondence:

Umer Zeeshan Ijaz

Umer.Ijaz@glasgow.ac.uk

Sundus Javed

sundus.javed@comsats.edu.pk

Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Introduction

Poultry industry is an important sector of Pakistani agriculture and plays a central role in country's economic development, bringing in around 1.5 million jobs annually. Currently, there are around 15,000 to 20,000 farms, producing >2,250-million-kilogram meat and 18,000 million table eggs annually [1], with an annual growth of 7.3% [2]. The industry expanded from 1960 to 1980s, but it was not without its perils. Huge production losses were reported due to outbreaks of different infections such as avian influenza, Newcastle disease, fowl typhoid, Marek's disease, etc. [3–6]. Circumventing these crises prompted a shift in poultry sector towards antimicrobials (AMs) usage as therapeutics and growth promoters [7–9]. In the past decade, antimicrobial resistance (AMR) has posed a major threat to global health due to higher rates of mortality and illnesses in humans and animals [10]. It has been reported that unmediated transmission of AMR bacteria occurs from livestock, especially poultry, to handlers and vice versa [11–13]. The general public is the predominant recipient of AMR bacteria involving various factors [14, 15]. Although farmers are concerned about the health of birds, they are unaware of the risk factors associated with disease outbreaks, transmission of zoonotic infections and AMR [16, 17]. Hence, understanding of farmer perspective and management practices is necessary such as following hygienic measures may enable antimicrobials and disease free rearing of poultry [18, 19].

Using an interview-based survey, the present study was designed to establish baseline information regarding different farming systems, management practices, training and awareness of farmers regarding outbreak, farmer health and antimicrobials use.

Materials and methods

Expert consultation, study design and categorization of farms

A cross-sectional field survey was conducted, with a non-experimental research design where researchers recorded variables and tested their effects on disease outbreak information and antimicrobials usage using statistical methods. Prior to formulation of questionnaire, a panel of 15 poultry experts [veterinarians ($n=5$); veterinary pathologists ($n=3$); veterinary pharmacists ($n=2$) and farmers ($n=5$)] were consulted. Focus group discussions lead to selection of poultry farms and to identify major poultry farming setups according to management practices, based on their field knowledge and experience (Fig. 1). Farms were limited to those that were easily accessible, and were registered with the Poultry Research Institute (PRI) Punjab to ensure availability of verifiable intermittent disease outbreak data, and where the farms

had a historical legacy of routinely submitting their samples to PRI for diagnoses of disease. Furthermore, focus group discussions also led to the final version of questionnaire which was first filled by the experts and were considered as quality control.

The study was conducted in the major poultry producing regions of Punjab and Khyber Pakhtunkhwa including Islamabad capital territory in Pakistan, during February–November 2022. Data was gathered through a questionnaire based on one-on-one interviews of 140 poultry farmers. Majority of the farms included in the survey were individual holdings and not belonging to the corporate sector. The participant farmers were briefed about the broad purpose of the questionnaires *i.e.*, general farm management practices.

Questionnaire design and data collection

Based on expert consultation and literature survey, a broad-range questionnaire was drafted including ~25 closed and open-ended questions (S5 Fig). The conceptual framework for recording major parameters associated with antimicrobials use and disease outbreak is summarized in Fig. 2. An outbreak is previously defined by the death of two birds (at the minimum) of the same species with similar clinical signs in corresponding farms in the same month, one or two months prior. Here, we have followed a strict criterion for considering a disease as an outbreak. The respondents were specifically asked about their past experiences with high mortality (>50% of the flock) and if they know that the disease was also reported in nearby farms. However, the latter criterion could not be strictly fulfilled either due to entry restrictions, reluctant behavior or closures due to COVID-19 pandemic. The in-person interviews with farmers were conducted with the questionnaire administered by a trained veterinarian. The questions were explained in the local language and the interviews were conducted on site to ensure reliability of the data collected. For descriptive questions, particularly on antibiotic usage, the veterinarian synthesized the information into different categories.

Statistical analysis

For the categorical data, to see if any two covariates have a relationship, we constructed a contingency table and used χ^2 test of independence using `chisq.test()` function in R [20]. Based on <http://www.sthda.com/english/wiki/chi-square-test-of-independence-in-r>, and where the relationship existed, we then calculated χ^2 residuals for individual rows and columns of the contingency table. These were drawn using R's `corrplot` [21] package where positive values in cells specify an attraction (positive association; blue) between the corresponding row and column variables whilst negative

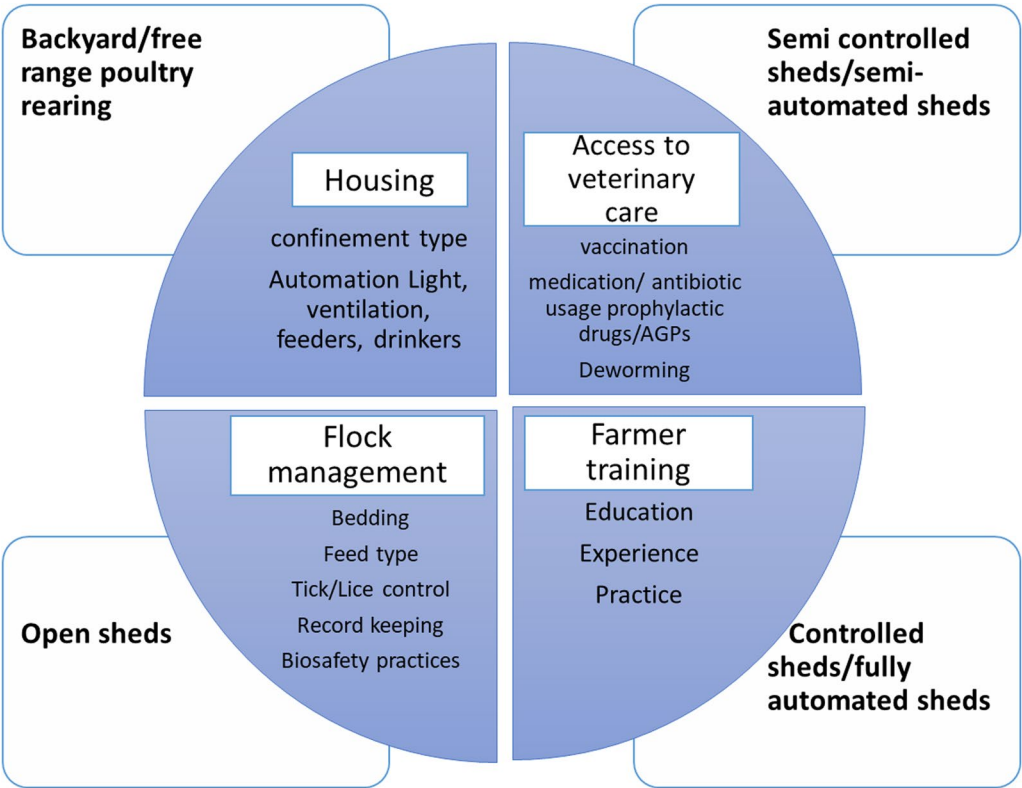


Fig. 1 Comparison of main management parameters in terms of zoonotic transmission risk in different poultry farming setups

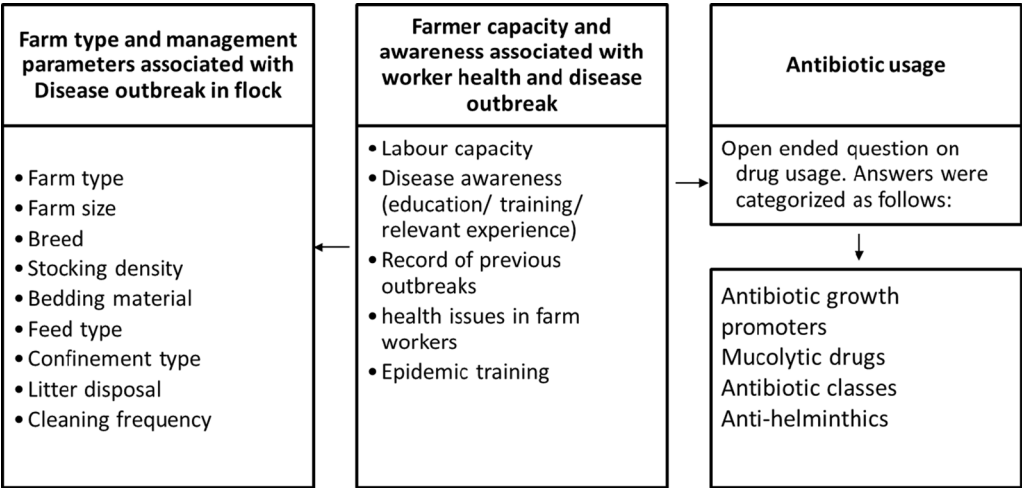


Fig. 2 Framework for recording major parameters associated with antimicrobial use and disease outbreak

values implies a repulsion (negative association; red) between the corresponding row and column variables. To get the relative risks for disease outbreak, we have used generalized linear models (GLMs) with log link functions to binomial data using R's logbin package [22]. To generate the regression tables, we have used

tab_model() function from R's sjPlot package [23] which also facilitated confidence interval display. In some cases, where we had more than two categories in the outcome variable, we have used multinomial logistic regression using multinom() function from R's nnet package [24] with recommendations given in <https://stats.oarc.ucla>.

[edu/r/dae/multinomial-logistic-regression/](https://r/dae/multinomial-logistic-regression/). For UpSet plots, ggupset repository was used: <https://github.com/const-ae/ggupset>.

Results

Respondent characteristics and scope of survey

Based on contingency analysis, we observed significant relationships between various parameters and incidence of disease outbreak (Fig S1). Log binomial regression statistics was used to calculate the prevalence ratios of disease outbreak. Farmers with minimal formal education are more inclined towards farming hybrid breeds and face more health issues in their flocks. 82.86% farmers with higher secondary qualification reported different disease outbreaks including fowl typhoid, avian influenza and Newcastle disease. In contrast, farmers with high education level i.e., graduation and post-graduation, reported less outbreaks (25.81% prevalence with 64% reduction in risk of disease outbreak as compared to those with secondary education) and preferred raising Local (Desi and its crosses) breeds. Furthermore, experienced farmers reported health issues in birds with a large number involved in rearing Broiler birds in intensive setups. No significant association of farmer's training status with disease outbreaks was observed (Table 2; Figs. 3, 4).

Different types of commercial poultry farming setups and disease spread

Major poultry setups, along with backyard farming were identified by the poultry experts, and were based on typical management practices followed locally (Table 1).

Commercial poultry farming setups including open (44.3%), semi-controlled (21.4%) and controlled sheds (34.3%) were targeted. Disease outbreak was observed in 93.33% of controlled sheds which are predominantly involved in Broiler rearing as compared to the open sheds (60% reduced risk of disease outbreak as compared to controlled sheds) or semi controlled (44% reduced risk of disease outbreak as compared to controlled sheds) setups (Table 2).

Furthermore, we categorized the farms as small, medium and large, with maximum disease outbreak reported in large farms. These large farms have greater labor capacity and therefore pose a higher risk of disease spread (93.93% of the farms with labor capacity of more than 5 laborers). Majority of the farms included in the study were raising Broiler (47%), in controlled sheds and are a popular choice due to quick turnover times (Table 2; Fig S2-S3).

Frequency of common disease outbreaks reported with major risk predictors

Majority of the farmers reported Newcastle disease, avian influenza and fowl typhoid outbreaks (Fig. 4B). Farmers also reported few other infections such as Marek's disease, bronchitis, coccidiosis, colibacillosis but they were not sure about the exact mortality and spread so we did not consider them as an outbreak. Out of a total 140 poultry farms, avian influenza (AI), Newcastle disease (ND) and fowl typhoid (FT) outbreaks were reported in 20 (14.29%), 38 (27.14%) and 18 (12.86%) farms, respectively, and found disease risk predictors including education, training status, farm type, breed type, cleaning

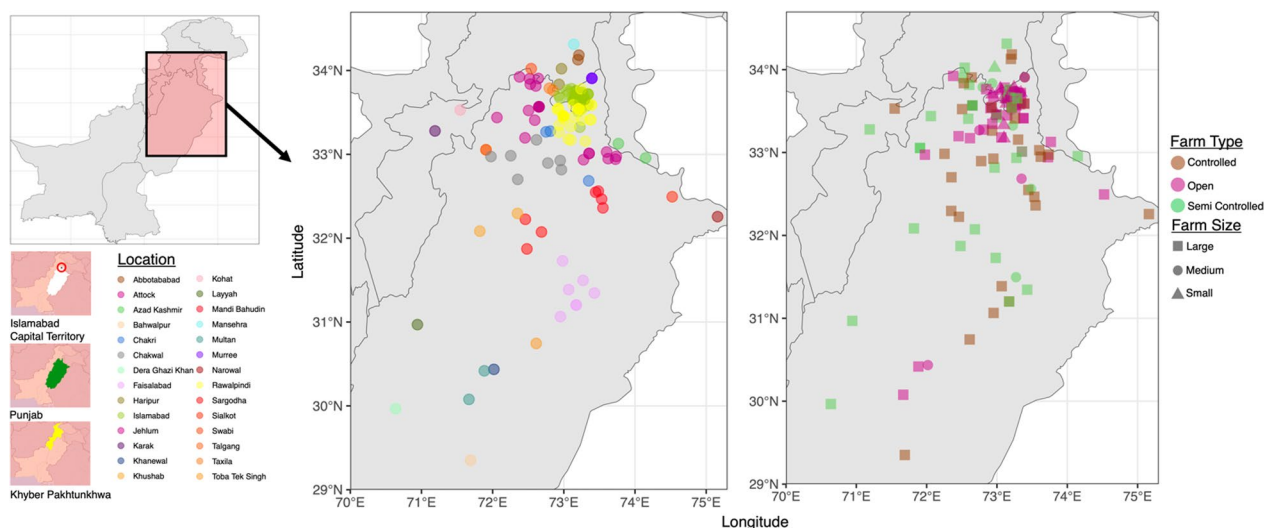


Fig. 3 Location, size, and types of farms included in survey, with majority of them belonging to Punjab region, with some located in Khyber Pakhtunkhwa. Note that these are the major poultry producing regions. Maps created in R using sf package [65] and R's rnatruearth package [66]

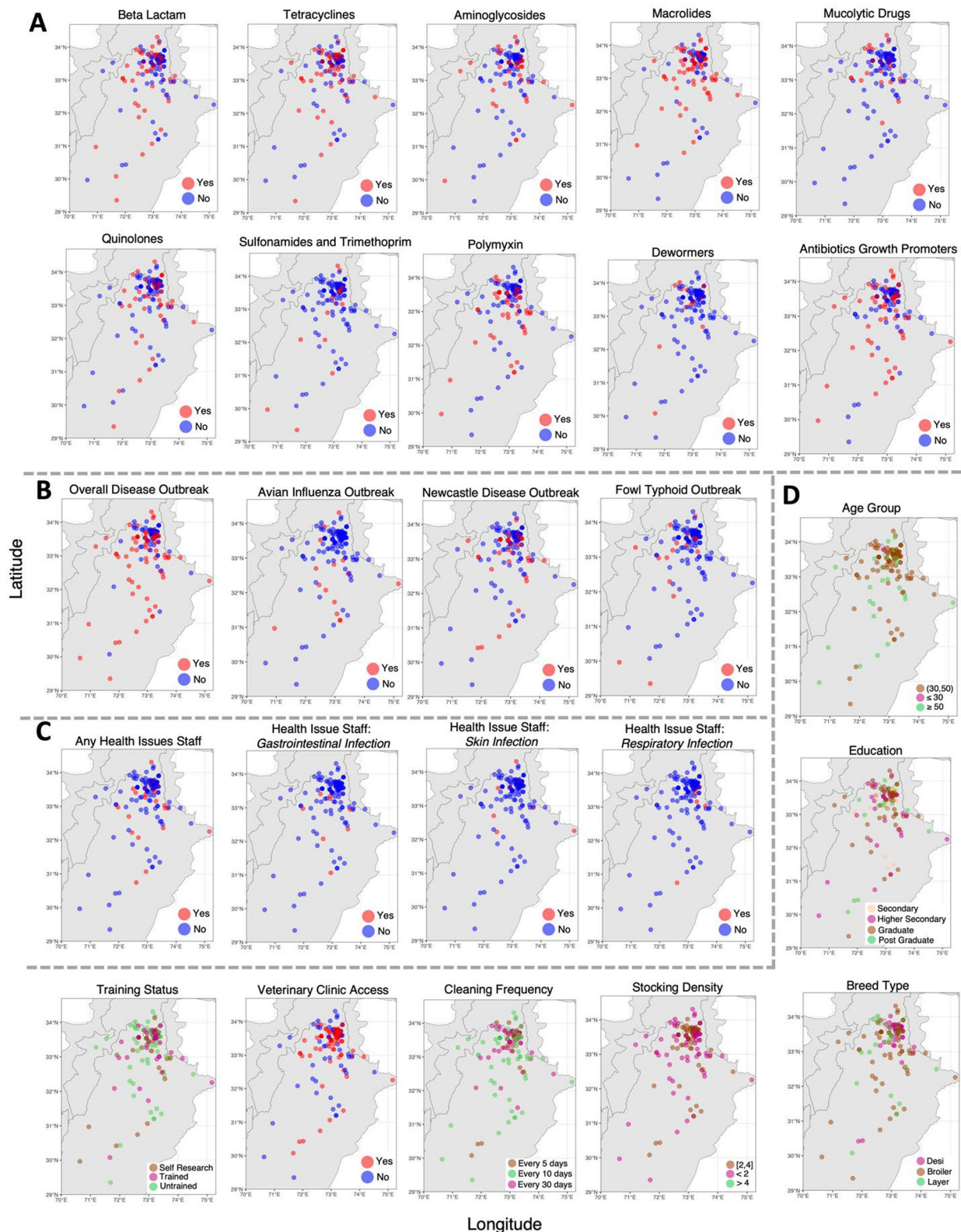


Fig. 4 Distribution of key parameters associated with the farms, categorized under **(A)** veterinary drugs, **(B)** disease outbreak in birds, **(C)** health issues reported in farmer workers, and **(D)** other parameters including farm management. Maps created in R using sf package [65] and R's rnaturalearth package [66]

Table 1 Baseline difference between different poultry farming setups depending on the management practices

Management parameters	Backyard/free range poultry rearing	Open sheds	Semi controlled sheds/semi-automated sheds	Controlled sheds/fully automated sheds
Housing	No proper sheds	Pillars fixed with mesh and small protection wall	Fixed solid walls	Fixed solid walls
Ventilation	Natural	Natural	Manual exhaust fans	Automated ventilation system
Lightening	Sunlight	Sunlight/electric bulbs	Electric bulbs	Electric bulbs
Roaming area	No boundaries	Shed area	Shed area	Shed area
Stocking density	NA	Broiler: 1.5–2 ft ² /bird Layer: 2.5–4 ft ² /bird	Broiler: 1–1.5 ft ² /bird Layer: 2–3 ft ² /bird	Broiler: 0.75–1 ft ² /bird Layer: 1.5–2 ft ² /bird
Drinkers	Manual	Manual	Manual/automated	Automated
Feeders	Manual	Manual	Manual	Automated
Feed type	Kitchen waste, fodder, vegetables, insects	Commercial feed with additives and growth promoters	Commercial feed with additives and growth promoters	Commercial feed with additives and growth promoters
Vaccination	No vaccination	Scheduled vaccination	Scheduled vaccination	Scheduled vaccination
Medication	No medication	Prophylactics and treatment	Prophylactics and treatment	Prophylactics and treatment
Deworming	Not followed	Followed	Followed	Followed
Tick/Lice Control	No control	Controlled	Controlled	Controlled
Workers	No staff	Supervisor and care takers	Veterinarian, supervisor and care takers	Veterinarian, supervisor, electrician, plumber and care takers
Biosecurity	Not implemented	Not implemented	Implemented	Implemented
Record keeping	No	Yes	Yes	Yes

frequency, litter disposal method and feed ingredients and calculated prevalence ratios. Farming experience of over 10 years was associated with a 3.42 folds increase in outbreak reporting (Table 2). It must be noted that the questions asked during the interviews did not indicate any time frame for disease reporting and with greater farming experience, increased chances of encountering a disease outbreak become likely. Further, 32.26% of farms with AI outbreak, 45.16% with ND, and 19.36% with FT also reported health issues in the farm workers (S1–S3 Tables).

Compared to controlled sheds, semi controlled and open sheds reported fewer disease outbreaks. As for the breed types, raising Local breeds and Layer resulted in 43% and 53% reduced risk in disease outbreaks, respectively, compared to raising Broiler (Fig S2–S3). Certain parameters associated with farm management also correlated with risk of disease outbreak e.g., labor capacity of over 5 workers compared to <3 workers was associated with 2.61 fold increase in outbreak risk. Decreasing farm cleaning frequency from every 5 days to every 30 days increased outbreak risk 1.95 times. 72.22% of the farms with stocking density of <2 ft²/bird, reported disease outbreak. In contrast, decreasing stocking density from <2ft² to 2–4 ft², decreases the risk of disease outbreak by 48%. Canola as a feed ingredient, increased the disease risk 2.10 folds and wheat decreased the risk

of disease outbreak by 29% (Fig S4). In contrast to wood shaving, rice husk (bedding material) was associated with high disease risk (Table 2; Fig S2–S3).

Zoonosis emergence with reference to farmer training and management practices

Farmers who reported diseases in their birds also reported concomitant health issues in their staff (increased risk of disease outbreak by 12.21 times). 38.16% of the farms with disease outbreak history coincided with different human infections (Fig. 4C). AI and ND outbreaks were associated with 2.86- and 2.05-folds increase in risk of health issues amongst farm workers, respectively (S1–S2 Tables). The majority of larger farms, with controlled setups and higher labor capacity, are found to be at a higher risk of zoonosis. As compared to controlled sheds, we observed 58% and 56% reduced risk of health issues in farmers associated with semi-controlled and open sheds, respectively. A decreased risk for disease amongst workers was seen with farmers raising Local breeds. (0.37 or 63% reduction in risk as compared to raising Broiler). We did not ask the farmers about any specific disease occurrence, but based on the information gathered, we broadly categorized the infections as respiratory, digestive and skin-related infections (Table 3).

Table 2 Major risk predictors associated with disease outbreaks at poultry farms by fitting different regression models represented by M numbers

Predictor	β - coefficients	Count (n = 140)	Disease outbreak reported, n = 76 (54.29%)	Prevalence Ratio (95% CI)	Significance
M1 Intercept		NA		0.72 (0.54—0.96)	*
Farmer's Education	Secondary	18	13 (72.22%)	REF	NA
	Higher secondary	35	29 (82.86%)	1.15 (0.83—1.59)	NS
	Graduation	56	26 (46.43%)	0.64 (0.43—0.96)	*
	Post-graduation	31	8 (25.81%)	0.36 (0.18—0.69)	**
M2 Intercept		NA		0.27 (0.15—0.48)	***
Farming Experience	Less than 5 years	30	8 (26.67%)	REF	NA
	5 to 10 years	65	27 (41.54%)	1.56 (0.81—3.01)	NS
	More than 10 years	45	41 (91.11%)	3.42 (1.87—6.23)	***
M3 Intercept		NA		0.55 (0.44 —0.68)	***
Epidemic Training Status	Untrained	66	36 (54.55%)	REF	NA
	Self-research	23	14 (60.87%)	1.12 (0.75—1.66)	NS
	Trained	51	26 (50.98%)	0.93 (0.66—1.32)	NS
M4 Intercept		NA		0.93	NS
Farm Type	Controlled	30	28 (93.33%)	REF	NA
	Semi controlled	48	25 (52.08%)	0.56 (0.42—0.74)	***
	Open	62	23 (30.09%)	0.40 (0.28—0.56)	***
M5 Intercept		NA		0.36 (0.25—0.52)	***
Labor Capacity	Less than 3	50	18 (36.00%)	REF	NA
	3 to 5	57	27 (47.37%)	1.32 (0.83—2.08)	NS
	More than 5	33	31 (93.93%)	2.61 (1.79—3.81)	***
M6 Intercept		NA		0.73 (0.63—0.84)	***
Breed Type	Broiler	66	48 (72.73%)	REF	NA
	Layer	36	15 (41.67%)	0.57 (0.38—0.87)	**
	Desi and crosses	38	13 (34.21%)	0.47 (0.30—0.75)	**
M7 Intercept		NA		0.33 (0.13—0.84)	*
Confinement Type	No confinement	9	3 (33.33%)	REF	NA
	Open house with mesh	58	22 (37.93%)	1.14 (0.43—3.03)	NS
	Closed house with solid walls	73	51 (69.86%)	2.10 (0.82—5.34)	NS
M8 Intercept		NA		0.68 (0.58—0.79)	***
Flock Management	All-in-all-out	74	50 (67.57%)	REF	NA
	Multiple flocks and all-in-all-out	55	21 (38.18%)	0.57 (0.39—0.82)	**
	Continuous topping	11	5 (45.45%)	0.67 (0.35—1.31)	NS
M9 Intercept		NA		0.36 (0.21—0.61)	***
Cleaning Frequency	Every 5 days	25	9 (36.00%)	REF	NA
	Every 10 days	48	20 (41.67%)	1.16 (0.62—2.15)	NS
	Every 30 days	67	47 (70.15%)	1.95 (1.13—3.36)	*
M10 Intercept		NA		0.67 (0.38—1.17)	NS
Litter Disposal	Open place	6	4 (66.67%)	REF	NA
	Drain	36	23 (68.89%)	0.96 (0.52—1.78)	NS
	Pit	98	49 (50.00%)	0.75 (0.41—1.37)	NS
M11 Intercept		NA		0.72 (0.63—0.83)	***
Stocking Density	Less than 2 ft ² /bird	72	52 (72.22%)	REF	NA
	2 to 4 ft ² /bird	66	23 (38.85%)	0.48 (0.34—0.69)	***
	More than 4 ft ² /bird	2	1 (50.00%)	0.69 (0.17—2.79)	NS

Table 2 (continued)

Predictor	β - coefficients	Count (n = 140)	Disease outbreak reported, n = 76 (54.29%)	Prevalence Ratio (95% CI)	Significance
M12 Intercept		NA		0.36 (0.26—0.48)	***
Major Feed Ingredient: Canola	No	73	26 (35.62%)	REF	NA
	Yes	67	50 (72.63%)	2.10 (1.49—2.94)	***
M13 Intercept		NA		0.67 (0.55—0.82)	***
Major Feed Ingredient: Wheat	No	46	31 (67.39%)	REF	NA
	Yes	94	45 (47.87%)	0.71 (0.53—0.95)	*
M14 Intercept		NA		0.54 (0.42—0.69)	***
Veterinary Clinic Access	No	52	28 (53.85%)	REF	NA
	Yes	88	48 (54.55%)	1.01 (0.74—1.39)	NS
M15 Intercept		NA		0.43 (0.35—0.53)	***
Health Issue Staff	No	109	47 (43.12%)	REF	NA
	Yes	31	29 (93.55%)	2.17 (1.72—2.74)	***

The significant predictors that cause an increase in disease outbreak are shown with a bold italic font, whilst those that cause a decrease in outbreak are shown with an italic font as compared to reference (REF), with the prevalence ratio/risk ratio shown in bold

* NA refers to Not Applicable; NS refers to Non-significant

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Antimicrobial usage in different farm setups

Farmers reported disease outbreaks at their farms even when birds are given feed with added antimicrobials as growth promoters or prophylactics (Fig. 4A; S4). Antimicrobials usage was found to be highest amongst medium to large poultry farms following controlled shed system. Concomitant use of macrolides, tetracyclines, beta lactams and quinolones were reported highest in the open sheds (Fig S7A). Macrolides, tetracyclines, beta lactams and quinolones were most frequently employed by 62%, 52%, 47% and 43.5% of farms, respectively. Meanwhile, usage of polymyxin (33.6%), aminoglycosides (26%), and sulfonamides (12.8%), was limited. 45% of farms reported adding antibiotic growth promoters (primarily lincomycin) to bird feeds. 17.8% and 12% farmers also reported using mucolytic and anti-helminthic (dewormers) drugs. Educated farmers reported greater use of macrolides, sulfonamides and anti-helminthics and decreased use of tetracyclines and polymyxin. Experienced farmers reported higher use of beta lactams (4.87 folds high risk), aminoglycosides (7.14 folds high risk) and AGPs (28.34 folds high risk). Medium and large farms were associated with significantly high macrolide, polymyxin and mucolytic drugs use. Large farms also reported high aminoglycoside usage. Semi-controlled and open shed systems had significantly lower aminoglycoside, macrolide, AGPs and anti-helminthic drug use compared to controlled sheds. Farmers rearing Broiler (reference) reported

high antimicrobial use and rearing Local and Layer was associated with 0.03 (~ 97% reduction) and 0.188 (~ 81% reduction) AGPs usage, respectively (Table 4).

Identification of the gaps in general farmer's practices

The farmers response about knowledge on poultry diseases and general practices was highly variable i.e., Newcastle disease (96.4%); avian influenza (84.3%); fowl typhoid (58.57%); infectious bursal disease (52.86%); infectious bronchitis (52.14%); Colibacillosis (42.86%); Marek's disease (40%); Coccidiosis (37.85%); and Prolapse (11.43%). This made it difficult to discern any viable pattern. However, the knowledge of the diseases was mainly attributable to the breed type reared by farmer. Farmers raising Layer birds had a higher frequency of reporting Marek's disease, Fowl typhoid and IBD. These trends can be seen in the UpSet plots (Fig S6A). Litter disposal included dumping the litter either in pits, open area or drains lying in the vicinity of the farms. Based on UpSet plots, cleaning frequency of 30 days and disposal in pits is contributing to disease outbreaks. These patterns are also coinciding with the antimicrobial usage. All of the top patterns suggest the importance of biosecurity practices in managing outbreaks (Fig S6B).

Discussion

Frequent disease outbreaks and logistic hurdles within the poultry sector have led to major economic losses in Pakistan. Farmers with little or no formal education

Table 3 Major risk predictors associated with health issues in poultry farmers by fitting different regression models represented by M numbers

Predictor	β - coefficients	Count (n = 140)	Health issues staff reported, n = 31 (22.14%)	Prevalence Ratio (95% CI)	Significance
M1 Intercept		NA		0.22 (0.09—0.53)	***
Farmer's Education	Secondary	18	4 (22.22%)	REF	NA
	Higher secondary	35	11 (31.43%)	1.41 (0.52—3.82)	NS
	Graduation	56	10 (17.86%)	0.80 (0.29—2.25)	NS
	Post-graduation	31	6 (19.35%)	0.87 (0.28—2.68)	NS
M2 Intercept		NA		0.07 (0.02—0.25)	***
Farming Experience	Less than 5 years	30	2 (6.67%)	REF	NA
	5 to 10 years	65	14 (21.54%)	3.23 (0.78—13.33)	NS
	More than 10 years	45	15 (33.33%)	5.00 (1.23—20.30)	*
M3 Intercept		NA		0.23 (0.15—0.35)	***
Epidemic Training Status	Untrained	66	15 (22.73%)	REF	NA
	Self-research	23	4 (17.39%)	0.77 (0.28—2.07)	NS
	Trained	51	12 (23.53%)	1.04 (0.53—2.01)	NS
M4 Intercept		NA		0.40	***
Farm Type	Controlled	30	12 (40.00%)	REF	NA
	<i>Semi controlled</i>	48	8 (16.67%)	0.42 (0.19—0.90)	*
	<i>Open</i>	62	11 (17.74%)	0.44 (0.22—0.89)	*
M5 Intercept		NA		0.12 (0.06—0.25)	***
Labor Capacity	Less than 3	50	6 (12.00%)	REF	NA
	3 to 5	57	11 (19.29%)	1.61 (0.64—4.03)	NS
	More than 5	33	14 (42.42%)	3.54 (1.51—8.27)	**
M6 Intercept		NA		0.29 (0.20—0.42)	***
Breed Type	Broiler	66	19 (28.79%)	REF	NA
	Layer	36	8 (22.22%)	0.77 (0.38—1.58)	NS
	<i>Desi and crosses</i>	38	4 (10.51%)	0.37 (0.13—1.00)	*
M7 Intercept		NA		0.27 (0.19—0.39)	***
Flock Management	All-in-all-out	74	20 (20.03%)	REF	NA
	Multiple flocks and all-in-all-out	55	9 (16.36%)	0.61 (0.30—1.23)	NS
	Continuous topping	11	2 (18.18%)	0.67 (0.18—2.49)	NS
M8 Intercept		NA		0.20 (0.09—0.44)	***
Cleaning Frequency	Every 5 days	25	5 (20.00%)	REF	NA
	Every 10 days	48	8 (16.67%)	0.83 (0.30—2.28)	NS
	Every 30 days	67	18 (26.87%)	1.34 (0.56—3.23)	NS
M9 Intercept		NA		0.17 (0.03—1.00)	*
Litter Disposal	Open place	6	1 (16.67%)	REF	NA
	Drain	36	6 (16.67%)	1.00 (0.14—6.91)	NS
	Pit	98	24 (24.49%)	1.47 (0.24—9.09)	NS
M10 Intercept		NA		0.03 (0.01—0.12)	***
Disease Outbreak	No	64	2 (3.13%)	REF	NA
	Yes	76	29 (38.16%)	12.21 (3.03—49.21)	***
M11 Intercept		NA		0.18 (0.12—0.26)	***
Disease Outbreak Avian Influenza	No	120	21 (17.50%)	REF	NA
	Yes	20	10 (50.00%)	2.86 (1.59—5.13)	***
M12 Intercept		NA		0.20 (0.14—0.29)	***
Disease Outbreak Fowl Typhoid	No	122	25 (20.49%)	REF	NA
	Yes	18	6 (33.33%)	1.63 (0.78—3.41)	NS

Table 3 (continued)

The significant predictors that cause an increase in health issues are shown with a bold italic font, whilst those that cause a decrease in health issues, are shown with an italic font as compared to reference (REF), with the prevalence ratio/risk ratio shown in bold

* NA refers to Not Applicable; NS refers to Non-significant

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 4 Major risk predictors associated with antimicrobial use when using outcome variables with more than two categories in multinomial logistic regression

Predictor		Intercept	Beta-lactams	Tetracyclines	Aminoglycosides	Macrolides	Quinolones	Sulfonamides and Trimethoprim	Polymyxin	Mucolytic drugs	Dewormers	Antibiotic Growth Promoters
Farmers Age	<30 years	REF										
	30 to 50 Years	40.662782 ($p=0.0570$ 3986)	1.16255 ($P=0.9308$ 314)	1.3960954 ($P=0.8592$ 171)	55094.23 ($p<0.001$)	1.580960 ($p=0.8239$ 453)	0.3169797 ($p=0.60573$ 48)	21683.53 ($p=0.98390$ 52)	5622.955 ($p=0.97360$ 94)	56755.24 ($p<0.001$)	0.0981145 4 ($p=0.2172$ 116)	2552955178 ($p<0.001$)
	> 50 Years	5.404968 ($p=0.4019$ 9995)	1.96048 ($p=0.7053$ 630)	0.3793958 ($p=0.6181$ 168)	74567.76 ($p<0.001$)	5.218686 ($p=0.4352$ 628)	0.1629598 ($p=0.42314$ 43)	29675.11 ($p=0.98339$ 95)	4918.926 ($p=0.97401$ 81)	44652.26 ($p<0.001$)	0.1154114 0 ($p=0.2729$ 317)	6977641341 ($p<0.001$)
Breed Type	Broiler	REF										
	Desi	16.880814 6 ($p=0.0001$ 641241)	0.6740115 ($p=0.5311$ 314)	0.3362833 ($p=0.1487$ 713)	0.3816093 ($p=0.2324$ 304)	0.4467423 ($p=0.2563$ 097)	0.3989774 ($p=0.16952$ 78)	0.7668179 ($p=0.78980$ 08)	0.1411559 ($p=0.01433$ 528)	0.7301106 ($p=0.7645$ 776)	23.25835 ($p=0.0323$ 877998)	0.0294279 ($p<0.001$)
	Layer	0.6611293 ($p=0.0001$ 641241)	1.5766259 ($p=0.4269$ 025)	1.6241647 ($p=0.5314$ 633)	0.8161518 ($p=0.7970$ 760)	0.6448277 ($p=0.5489$ 349)	1.3162238 ($p=0.65183$ 11)	0.5550270 ($p=0.51896$ 28)	0.4520486 ($p=0.26373$ 313)	2.6082542 ($p=0.1786$ 438)	75.74602 ($p<0.001$)	0.1885887 ($p=5.420306$ e-03)
Education	Secondary	REF										
	Higher Secondary	1.106751 ($p=0.9139$ 1449)	1.3091205 ($p=0.7182$ 133)	0.1586415 6 ($p=0.0670$ 66272)	1.8211256 ($p=0.5206$ 645)	16.418806 ($p=0.0042$ 16337)	0.3831908 ($p=0.24571$ 16)	28273683 ($p<0.001$)	0.46893144 ($p=0.40874$ 1716)	0.6408143 ($p=0.6353$ 490)	3281061 ($p<0.001$)	1.714136 ($p=0.500991$ 1)
	Graduate	3.203003 ($p=0.1759$ 9057)	0.7422006 ($p=0.6810$ 120)	0.1555954 0 ($p=0.0499$ 26907)	2.1355320 ($p=0.3935$ 316)	9.85601 ($p=0.0122$ 22997)	0.8060030 ($p=0.78471$ 46)	28608664 ($p<0.001$)	0.14242028 ($p=0.03119$ 493)	0.8251194 ($p=0.8348$ 529)	6313761 ($p<0.001$)	1.882635 ($p=0.407082$ 6)
	Post Graduate	4.654695 ($p=0.0793$ 4486)	1.0157942 ($p=0.9840$ 843)	0.0405290 1 ($p=0.0024$ 02492)	0.4206581 ($p=0.4395$ 721)	12.384762 ($p=0.0147$ 74141)	1.1633675 ($p=0.86379$ 43)	13534918 ($p<0.001$)	0.02785422 ($p=0.00209$ 4919)	0.4675871 ($p=0.5148$ 848)	8405387 ($p<0.001$)	1.619265 ($p=0.565552$ 4)
Farm Size	Small	REF										
	Medium	0.5550343 ($p=0.3884$ 1149)	0.3464292 ($p=0.2631$ 092)	0.1271057 ($p=0.0667$ 2176)	0.0031295 730 ($p<0.001$)	28.8043 ($p=0.0111$ 37356)	1.797596 ($p=0.56938$ 98)	0.00000005 57 ($p<0.001$)	5909586 ($p<0.001$)	10170681 ($p<0.001$)	5.442871 ($p=0.1788$ 520)	4.366122 ($p=0.148844$ 4)
	Large	0.1621456 ($p=0.0261$ 3346)	1.7515105 ($p=0.5313$ 311)	0.10172173 ($p=0.9862$ 0797)	1.320378e +10 ($p<0.001$)	19.1681 ($p=0.0081$ 71376)	4.139072 ($p=0.13473$ 80)	2.336956 ($p=0.56717$ 23)	95674497 ($p<0.001$)	61111550 ($p<0.001$)	3.338636 ($p=0.3294$ 336)	1.368829 ($p=0.753266$ 4)
Farm Type	Control	REF										
	Semi Control	62.40497 ($p=7.6049$ 46e-04)	1.2690556 ($p=0.7012$ 417)	1.0636772 ($p=0.9400$ 466)	0.2393817 ($p=0.0526$ 2571)	0.1744945 ($p=0.0337$ 8152)	0.6809085 ($p=0.57821$ 10)	0.2864191 ($p=0.15301$ 15)	0.4199594 ($p=0.19851$ 939)	0.6299841 ($p=0.5671$ 869)	41025287 ($p<0.001$)	0.1666341 ($p=2.038986$ e-02)
	Open	142.95718 ($p=7.6508$ 36e-05)	0.7392848 ($p=0.6628$ 815)	0.8724305 ($p=0.8789$ 419)	0.1726017 ($p=0.0397$ 4690)	0.2423449 ($p=0.1151$ 9746)	1.3151799 ($p=0.71389$ 19)	0.4593417 ($p=0.41540$ 57)	0.1867983 ($p=0.03009$ 114)	0.5411177 ($p=0.5027$ 128)	111030910 ($p<0.001$)	0.0293674 ($p<0.001$)
Farming Experience	<5	REF										
	5 to 10	0.6297286 3 ($p=0.3619$ 463081)	2.394874 ($p=0.1057$ 877)	0.7684534 ($p=0.6582$ 616)	2.962216 ($p=0.1555$ 9775)	2.028038 ($p=0.2456$ 845)	2.317104 ($p=0.12744$ 13)	0.9089806 ($p=0.91164$ 40)	0.8840462 ($p=0.85142$ 78)	0.534637 ($p=0.4289$ 052)	0.9726698 ($p=0.9687$ 232)	2.932002 ($P=1.049208$ e-01)
	>10	0.0389131 8 ($p=0.0002$ 212391)	4.870405 ($p=0.0187$ 778)	1.8577515 ($p=0.4497$ 106)	7.141763 ($p=0.0262$ 3148)	1.613546 ($p=0.5333$ 772)	1.176539 ($p=0.80834$ 83)	0.5962 ($p=0.60254$ 21)	2.4830928 ($p=0.23349$ 44)	1.460879 ($p=0.6569$ 907)	0.2634722 ($p=0.2828$ 587)	28.344045 ($p<0.001$)

The significant predictors that cause an increase in risk as compared to the reference (REF) of using an antimicrobial are shown with red background whilst those that cause decrease in risk as compared to the reference of using the antimicrobials are shown with a blue background

were mainly involved in raising Broiler at a commercial level using controlled sheds which was associated with increased risk for disease outbreak. This is mainly because those who are associated with this profession are

typically influential landowners in possession of larger areas with substantial capital to invest. Nonetheless, lack of education becomes a hindrance to disease control. On the contrary, educated farmers, which are considerably

fewer, prefer open shed systems and have a mindset that rearing birds in an open environment leads to the best performing birds which reduces disease outbreaks. This corroborates with the previous study [25] which reveals that the education of farmers affects the technical efficiency of poultry farmers in Pakistan.

We found no association between lack of training and prevalence of disease, suggesting on-the-job learning. Previous studies have also demonstrated that high disease risk awareness does not necessarily translate to improved general farming practices [26, 27]. Furthermore, to cut losses, farmers tend to sell their poultry stock as quickly as possible. It has been reported that poultry producers prefer to market alive or depopulated birds in case of known/suspected infection to curb the disease but may result in further disease spread [28].

In open sheds, unrestricted movement of workers, unscheduled vaccination, and improper cleaning contribute to low biosecurity. In contrast, amongst semi-controlled and controlled sheds, movement of workers is restricted due to the automation of feeding and drinking systems. In addition, regular monitoring and record of diseased/dead birds along with proper vaccination schedule is maintained. A previous study from Bangladesh [29] showed substantial decrease in risk of AI outbreaks with the implementation of biosecurity practices. In another study [30], similar biosecurity practices in small scale poultry units also decreased disease risk. It should be noted that the prevalent biosecurity practices typically vary between the farms, and also differ when raising different poultry species [31].

We found that higher stocking density is a major risk factor for disease outbreaks. It has been reported that farmers house their birds in super intensive conditions to increase profit but the overcrowding ultimately increases birds susceptibility to infections and microbial attack [32, 33]. We highlight cleaning frequency and the litter disposal method to be major risk factors for disease spread (also corroborated by a previous survey conducted in Ethiopia and Switzerland [34, 35]). Furthermore, poor poultry waste management may cause health problems in flock, and contamination of land and water. Hence, appropriate farm waste disposal is crucial for protecting environment, human health and poultry welfare [31].

In majority of controlled sheds raising Broiler, where maximum disease outbreak has been reported, and rice husk is used as bedding material. Farmers using wood shaving as bedding material reported less outbreaks, which is supported by the previous findings about antimicrobial properties of wood shaving and improved performance [36, 37].

It is well established that poultry farming relies on antimicrobials usage to control diseases [38]. In this study, antimicrobials use frequency was highest amongst experienced farmers raising Broiler in large, controlled sheds. We observed high macrolide use amongst various farm types. Macrolides are broad spectrum antibiotics that are often used in chickens as therapeutic/prophylactic agents [39, 40]. The rampant use of antimicrobials in farms has resulted in the emergence of multiple antibiotic resistant bacterial strains from poultry sources [41]. We also reported aminoglycosides usage amongst poultry farmers. Aminoglycosides usage in veterinary medicine is associated with increased resistance in bacteria from clinical and animal origin [42, 43]. A similar increase in aminoglycoside resistance amongst bacterial strains of poultry origin from Pakistani farms is also observed [44, 45].

We also observed that farmers frequently supplement feeds with antimicrobials to enhance feed conversion rates. It has been suggested that antimicrobials help birds in gaining weight by various mechanisms such as immune system modulations and less energy uptake by gut bacteria but coincides with emergence of antimicrobial resistant bacteria and severe health challenges [46–48]. As a result, highest antimicrobial resistant zoonotic pathogen burden has been observed in low and middle income countries through poultry [49]. A few recently published studies accentuated high use of antimicrobials in livestock sector, specifically in commercial Broiler in Pakistan [9, 50]. The high antimicrobial usage in Pakistani poultry farms is due to the availability of antimicrobials without prescription, inaccurate diagnostic approaches, or lack of access to diagnostic facility [51, 52].

In the present survey, a high percentage of farmers reported ND, AI and FT outbreaks. Previously, several ND outbreaks have been reported in Pakistan, resulting in huge economic losses [53]. It has been established that the commercial poultry birds are highly susceptible to ND and it is endemic in six continents including Asia [54, 55]. High risk of AI outbreak has also been observed in large scale poultry farms compared to backyard flocks in Thailand [56]. In Pakistan, similar to ND, AI is also endemic and its high prevalence has been reported in various studies [5, 6, 57–59]. Fowl typhoid is caused by *Salmonella gallinarum* which is highly prevalent in Pakistan and other developing countries, leading to huge mortality and subsequent economic losses [60]. ND was found as the most prevalent poultry disease in our survey, mostly within Broiler farms, followed by AI and FT, a similar trend has been observed in previous studies [61, 62]. Despite the limited sample size, owing to inaccessibility of many

major commercial farming setups, the study highlights major gaps in farm management practices associated with the antimicrobial usage and disease spread.

Conclusions

The study highlights major gaps in farmer routine practices on farm, knowledge, and formal education. High disease incidence was associated with poor management practices employed by large farm setups and choice of breed which can be used to design effective intervention strategies to curtail spread of disease while optimizing production in the country. Farmers with higher education, however, have fewer outbreaks and are better able to control the use of antibiotics. The present data can be used as a reference by animal health authorities for surveillance and strategy implementation to optimize quality food production in Pakistan.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13104-025-07220-4>.

Additional file 1.
Additional file 2.
Additional file 3.

Acknowledgements

We acknowledge the poultry experts from Poultry Research Institute, Rawalpindi and National Veterinary Laboratories, Islamabad for highlighting major poultry farming setups and practices. We thank all the participants including farm owners and workers for giving access to the farms. We also acknowledge Uzma for being part of the training session under which some of the R codes were written by UZI. We are also grateful to them for providing their valuable time and information. For the purpose of open access, the authors have applied a Creative Commons Attribution (CC BY) license to any Author Accepted Manuscript version arising from this submission.

Author contributions

Conceptualization: F. S., A. A., and S. J. Methodology: F. S., A. A., and U. Z. I. Draft writing: F. S., A. A., and U. Z. I. Resources: F. A., M. U., H. I., and U. Z. I. Editing, revising and submission: S. S., F. A., M. U., H. I., S. J.

Funding

AA acknowledges support from International Research Support Initiative Program from Higher Education Commission, Pakistan Project No. 1–8/HEC/HRD/2023/12790. UZI is funded by EPSRC (EP/V030515/1).

Data availability

The relevant data is provided as supplementary files i.e., Additional file 2 and Additional file 3.

Declarations

Ethics approval and consent to participate

The PREPARE guidelines [63] were followed for study design, and ARRIVE guidelines [64] were followed for reporting. The protocol and procedures employed were reviewed and approved by the Ethics Review Board (ERB) at COMSATS University Islamabad (ERB No. CUI/Bio/ERB/4–21/17) and study was

carried out in accordance with their recommendations, as well as with the Declaration of Helsinki. All participants provided written informed consent to participate in the study, and to use the data in research publication.

Competing interest

The authors declare no competing interests.

Author details

¹Department of Biosciences, COMSATS University Islamabad, Islamabad, Pakistan. ²National Veterinary Laboratories, Ministry of National Food Security and Research, Islamabad, Pakistan. ³Poultry Research Institute Punjab, Rawalpindi, Pakistan. ⁴Animal Sciences Institute, National Agricultural Research Center, Islamabad, Pakistan. ⁵Water & Environment Research Group, Mazumdar-Shaw Advanced Research Centre, University of Glasgow, Glasgow, UK. ⁶Department of Molecular and Clinical Cancer Medicine, University of Liverpool, Liverpool, UK. ⁷College of Science and Engineering, University of Galway, Galway, Ireland.

Received: 16 September 2024 Accepted: 31 March 2025

Published online: 08 April 2025

References

1. PPA. Poultry Status – Pakistan Poultry Association. <https://pakistanpoultryassociation.com.pk/poultry-status/>. Accessed 3 Jul 2023
2. GOP. Economic survey of Pakistan. Economic Advisory Wing, Finance Division, Islamabad. 2023;
3. Umar S, Teillaud A, Bin AH, Guerin JL, Ducatez MF. Molecular epidemiology of respiratory viruses in commercial chicken flocks in Pakistan from 2014 through to 2016. *BMC Vet Res*. 2019;15(1):1–12.
4. Parveen S, Mahmood A, Azad A, Umar S, Shoukat N, Azam MMA, et al. Prevalence of concurrent infections in broiler population of district Chakwal Pakistan. *Sarhad J Agric*. 2022;38(2):480–8.
5. Channa AA, Tariq M, Nizamani ZA, Kalhoro NH. Prevalence of avian influenza H5, H7 and H9 viruses in commercial broilers at Karachi Pakistan. *J Anim Heal Prod*. 2022;10(1):29–34.
6. Channa AA, Tariq M, Nizamani ZA, Kalhoro NH. Prevalence of avian influenza H5, H7, and H9 viruses in commercial layers in Karachi, Pakistan. *Iran J Vet Res*. 2021;22(4):352.
7. Azam M, Mohsin M, Sajjad-Ur-Rahman M, Saleemi MK. Virulence-associated genes and antimicrobial resistance among avian pathogenic *Escherichia coli* from colibacillosis affected broilers in Pakistan. *Trop Anim Health Prod*. 2019. <https://doi.org/10.1007/s11250-019-01823-3>.
8. Mohsin M, Umair M. Trends in antimicrobial use in livestock animals in Pakistan. *Int J Infect Dis*. 2020;101(51):17–8.
9. Umair M, Tahir MF, Ullah RW, Ali J, Siddique N, Rasheed A, et al. Quantification and trends of antimicrobial use in commercial broiler chicken production in Pakistan. *Antibiot*. 2021. <https://doi.org/10.3390/antibiotic10050598>.
10. World Health Organization. Antimicrobial resistance: a manual for developing national action plans. Geneva: World Health Organization; 2020.
11. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duinkerken E, Heederik D. Methicillin resistant staphylococcus aureus ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS ONE*. 2010;5(6):e10990. <https://doi.org/10.1371/journal.pone.0010990>.
12. Pires J, Bernasconi OJ, Kasraian S, Hilty M, Perreten V, Endimiani A. Intestinal colonisation with extended-spectrum cephalosporin-resistant *Escherichia coli* in Swiss pets: molecular features, risk factors and transmission with owners. *Int J Antimicrob Agents*. 2016;48(6):759–60.
13. Zhang XF, Doi Y, Huang X, Li HY, Zhong LL, Zeng KJ, et al. Possible Transmission of mcr-1–harboring *Escherichia coli* between companion animals and human. *Emerg Infect Dis*. 2016;22(9):1679.
14. Stanton IC, Bethel A, Leonard AFC, Gaze WH, Garside R. Existing evidence on antibiotic resistance exposure and transmission to humans from the environment: a systematic map. *Environ Evid*. 2022;11(1):1–24. <https://doi.org/10.1186/s13750-022-00262-2>.

15. Allel K, Day L, Hamilton A, Lin L, Furuya-Kanamori L, Moore CE, et al. Global antimicrobial-resistance drivers: an ecological country-level study at the human–animal interface. *Lancet Planet Heal*. 2023;7(4):e291–303.
16. Stull JW, Peregrine AS, Sargeant JM, Weese JS. Household knowledge, attitudes and practices related to pet contact and associated zoonoses in Ontario Canada. *BMC Public Health*. 2012;12(1):1–15. <https://doi.org/10.1186/1471-2458-12-553>.
17. Stull JW, Peregrine AS, Sargeant JM, Weese JS. Pet husbandry and infection control practices related to zoonotic disease risks in Ontario Canada. *BMC Public Health*. 2013;13(1):1–15. <https://doi.org/10.1186/1471-2458-13-520>.
18. Røssvoll E, Langsrud S, Bloomfield S, Moen B, Heir E, Mørseth T. The effects of different hygiene procedures in reducing bacterial contamination in a model domestic kitchen. *J Appl Microbiol*. 2015;119(2):582–93.
19. Funk S, Salathé M, Jansen VAA. Modelling the influence of human behaviour on the spread of infectious diseases: a review. *J R Soc Interface*. 2010;7(50):1247–56.
20. Team. RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org>. 2024
21. Wei T, Simko V. R package “corplot”: Visualization of a Correlation Matrix (Version 0.84). 2017;
22. Donoghoe MW, Marschner IC. Flexible regression models for rate differences, risk differences and relative risks. *Int J Biostat*. 2015;11(1):91–108.
23. Lüdtke D. sjPlot: Data visualization for statistics in social science. R Package version. 2018;2(1).
24. Kemp F. Modern applied statistics with S. *J R Stat Soc Ser D Stat*. 2003;52(4):704–5.
25. Khan NA, Ali M, Ahmad N, Abid MA, Kusch-Brandt S. Technical efficiency analysis of layer and broiler poultry farmers in Pakistan. *Agric*. 2022;12(10):1742.
26. Chowdhury VS, Han G, Eltahan HM, Haraguchi S, Gilbert ER, Cline MA, et al. Potential role of amino acids in the adaptation of chicks and market-age broilers to heat stress. *Front Vet Sci*. 2021;8(7):1109.
27. Scott AB, Singh M, Groves P, Hernandez-Jover M, Barnes B, Glass K, et al. Biosecurity practices on Australian commercial layer and meat chicken farms: Performance and perceptions of farmers. *PLoS ONE*. 2018. <https://doi.org/10.1371/journal.pone.0195582>.
28. Delabougli A, Le Thanh NT, Ai Xuyen HT, Nguyen-Van-yen B, Tuyet PN, Lam HM, et al. Poultry farmer response to disease outbreaks in smallholder farming systems in Southern Vietnam. *Elife*. 2020;1(9):1–44.
29. Islam A, Rahman MZ, Hassan MM, Epstein JH, Klaassen M. Farm biosecurity practices affecting avian influenza virus circulation in commercial chicken farms in Bangladesh. *One Heal*. 2024;1(18): 100681.
30. Grace D, Knight-Jones TJD, Melaku A, Alders R, Jemberu WT. The public health importance and management of infectious poultry diseases in smallholder systems in Africa. *Foods*. 2024;13(3):411.
31. Tilli G, Laconi A, Galuppo F, Mughini-Gras L, Piccirillo A. Assessing biosecurity compliance in poultry farms: a survey in a densely populated poultry area in North East Italy. *Animals*. 2022;12(11):1409.
32. Sugiharto S. Dietary strategies to alleviate high-stocking-density-induced stress in broiler chickens—a comprehensive review. *Arch Anim Breed*. 2022;65(1):21–36.
33. Altaf MT, Mahmud A, Mehmood S, Saima. Effects of supplemented growth promoters on performance and intestinal morphology in broilers reared under different stocking densities. *Braz J Poult Sci*. 2019;21(4):1073.
34. Mijinyawa Y, Dlamini BJ. Livestock and poultry wastes management in Swaziland. 2006
35. Melkamu BY, Berhan TM, Ashenafi MW. Disease management and biosecurity measures of small-scale commercial poultry farms in and around Debre Markos, Amhara Region Ethiopia. *J Vet Med Anim Heal*. 2016;8(10):136–44.
36. Munir MT, Belloncle C, Irle M, Federighi M. Wood-based litter in poultry production: a review. *Worlds Poult Sci J*. 2019;75(1):5–16.
37. Gomes B, Pena P, Cervantes R, Dias M, Viegas C. Microbial contamination of bedding material: one health in poultry production. *Int J Environ Res Public Health*. 2022;19(24):16508.
38. Sirdar MM, Picard J, Bisschop S, Gummow B. A questionnaire survey of poultry layer farmers in Khartoum State, Sudan, to study their antimicrobial awareness and usage patterns. *Onderstepoort J Vet Res*. 2012. <https://doi.org/10.4102/ojrv.79i1.361>.
39. Gaskins HR, Collier CT, Anderson DB. Antibiotics as growth promotants: mode of action. *Animal Biotechnol*. 2006;13(1):29–42. <https://doi.org/10.1081/ABIO-120005768>.
40. Gibreel A, Taylor DE. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J Antimicrob Chemother*. 2006;58(2):243–55. <https://doi.org/10.1093/jac/dkl210>.
41. Jalil A, Masood S, Ain Q, Andleeb S, Dudley EG, Adnan F. High resistance of fluoroquinolone and macrolide reported in avian pathogenic *Escherichia coli* isolates from the humid subtropical regions of Pakistan. *J Glob Antimicrob Resist*. 2023;1(33):5–17.
42. Van Duijkeren E, Schwarz C, Bouchard D, Catry B, Pomba C, Baptiste KE, et al. The use of aminoglycosides in animals within the EU: development of resistance in animals and possible impact on human and animal health: a review. *J Antimicrob Chemother*. 2019;74(9):2480–96. <https://doi.org/10.1093/jac/dkz161>.
43. Zhang X, Zhou Q, Tang M, Pu J, Zhang J, Lu J, et al. Aminoglycoside resistance and possible mechanisms in *Campylobacter* Spp. isolated from chicken and swine in Jiangsu China. *Front Microbiol*. 2021;12:716185.
44. Wajid M, Saleemi MK, Sarwar Y, Ali A. Detection and characterization of multidrug-resistant *Salmonella enterica* serovar Infantis as an emerging threat in poultry farms of Faisalabad Pakistan. *J Appl Microbiol*. 2019;127(1):248–61. <https://doi.org/10.1111/jam.14282>.
45. Tagar S, Qambrani NA. Bacteriological quality assessment of poultry chicken meat and meat contact surfaces for the presence of targeted bacteria and determination of antibiotic resistance of *Salmonella* spp. *Pakistan Food Control*. 2023;1(151): 109786.
46. Feighner SD, Dashkevich MP. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytase activity. *Appl Environ Microbiol*. 1987;53(2):331–6. <https://doi.org/10.1128/aem.53.2.331-336.1987>.
47. Hafez HM. Governmental regulations and concept behind eradication and control of some important poultry diseases. *Worlds Poult Sci J*. 2005;61(4):569–82.
48. Mehdi Y, Létourneau-Montminy MP, Lou GM, Chorfi Y, Suresh G, Rouissi T, et al. Use of antibiotics in broiler production: global impacts and alternatives. *Anim Nutr*. 2018;4(2):170–8.
49. Criscuolo NG, Pires J, Zhao C, Van Boeckel TP. resistancebank.org, an open-access repository for surveys of antimicrobial resistance in animals. *Sci Data*. 2021;8(1):1–10.
50. Umair M, Hassan B, Farzana R, Ali Q, Sands K, Mathias J, et al. International manufacturing and trade in colistin, its implications in colistin resistance and One Health global policies: a microbiological, economic, and anthropological study. *The Lancet Microbe*. 2023;4(4):e264–76.
51. Sartelli M, Hardcastle TC, Catena F, Chichom-Mefire A, Coccolini F, Dhinra S, et al. Antibiotic use in low and middle-income countries and the challenges of antimicrobial resistance in surgery. *Antibiotics*. 2020;9(8):1–12.
52. Habiba UE, Khan A, Mmbaga EJ, Green IR, Asaduzzaman M. Use of antibiotics in poultry and poultry farmers- a cross-sectional survey in Pakistan. *Front Public Heal*. 2023. <https://doi.org/10.3389/fpubh.2023.1154668>.
53. Rehan M, Aslam A, Khan MR, Abid M, Hussain S, Umer J, et al. Potential economic impact of Newcastle disease virus isolated from wild birds on commercial poultry industry of Pakistan: a review. *Hosts and Viruses*. 2019. <https://doi.org/10.17582/journal.hv/2019/6.1.1.15>.
54. Miller PJ, Decanini EL, Afonso CL. Newcastle disease: Evolution of genotypes and the related diagnostic challenges. *Infect Genet Evol*. 2010;10(1):26–35.
55. Shabbir MZ. Review article Newcastle disease virus : disease appraisal with global and Pakistan perspectives. *J Infect Mol Biol*. 2013;1(4):52–7.
56. Graham JP, Leibler JH, Price LB, Otte JM, Pfeiffer DU, Tiensin T, et al. The animal-human interface and infectious disease in industrial food animal production: rethinking biosecurity and biocontainment. *Public Health Reports*. 2008;123(3):282–99. <https://doi.org/10.1177/003335490812300309>.
57. Ahmed A, Khan TA, Kanwal B, Raza Y, Akram M, Rehmani SF, et al. Molecular identification of agents causing respiratory infections in chickens from southern region of Pakistan from October 2007 to February 2008. *Int J Agric Biol*. 2009;11(3):325–8.
58. Naeem K, Hussain M. An outbreak of avian influenza in poultry in Pakistan. *Vet Rec*. 1995;137(17):439.
59. Naeem K, Siddique N, Ayaz M, Jalalee MA. Avian influenza in Pakistan: outbreaks of low- and high-pathogenicity avian influenza in Pakistan during 2003–2006. *Avian Dis*. 2007;51(1):189–93.

60. Munir A, Ilyas SZ, Tahir H, Basit A, Haider Z, Rehman S. PCR based early detection and antibiotic resistance pattern of *Salmonella Gallinarum* isolates from Pakistan poultry. *J Microbiol Methods*. 2023;208:106709.
61. Abbas G, Khan SH, Hassan M, Mahmood S, Naz S, Gilani SS. Incidence of poultry diseases in different seasons in Khushab district Pakistan. *J Adv Vet Anim Res*. 2015;2(2):141–5.
62. Yunus AW, Nasir MK, Aziz T, Böhm J. Prevalence of poultry diseases in district chakwal and their interaction with mycotoxicosis: 2. effects of season and feed. *J Anim Plant Sci*. 2009;19(1):1–5.
63. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018;52(2):135–41.
64. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *J Cereb Blood Flow Metab*. 2020;40(9):1769–77.
65. Pebesma E. Simple features for R: standardized support for spatial vector data. *R J*. 2018;10(1):439–46.
66. South A. rnaturalearth: World map data from Natural Earth. 2017;1–14. Available from: <https://cran.r-project.org/package=rnaturalearth>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.