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Quantification and trends of antimicrobial use in commercial adult dairy and calves during summer and winter in Pakistan

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ABSTRACT
Background: Surveillance of antimicrobial use (AMU) in food animals is a key strategy to tackle the problem of antimicrobial resistance.
Aim: A cross-sectional period prevalence survey was conducted to collect AMU data from 12 dairy farms in two provinces (Punjab and Khyber Pakhtunkhwa) in Pakistan.
Methods: AMU data were analyzed using three metrics, that is, antimicrobial chemical compound (in kg), (mg/PCU), and [defined daily dose animal (DDDA)/1,000 animal-days].
Results: The total usage in adult dairy cattle in terms of mg/PCU and treatment incidences across both seasons and all provinces were 6.36 mg/PCU and 26.34 DDDA/1,000 animal-days. In adult dairy cattle, the cumulative AMU across both seasons and provinces was 15.23 kg, 6.36 mg/PCU, and 26.34 DDDA/1,000 animal-days, whereas in calves the figures were 1.22 kg, 8.58 mg/PCU, and 36.71 DDDA/1,000 animal-days. Treatment incidences were 23.9% lower in adult dairy cattle and 65% lower in calves in winter than in summer. In adult dairy cattle, 17.35% of antimicrobials used were highest priority critically important antimicrobials (HPCIAs).
Conclusion: These findings highlight the need for countrywide AMU monitoring and regulations on HPCIAs in food animals.
Keywords: Antimicrobial use, Adult dairy cattle, Calves, Critically important antimicrobials, Pakistan.

Introduction

Antimicrobial resistance (AMR) is a complex, multisectoral public health problem associated with the irrational use of antimicrobials. Since AMR is considered a silent pandemic, a One Health approach is necessary to tackle AMR zoonotic threats to humans, animals, and the ecosystem interface (Aslam *et al.*, 2021). According to a recent study, bacterial AMR was associated with 4.95 million deaths worldwide in 2019, with 1.27 million deaths attributable to bacterial AMR (Murray *et al.*, 2022).

Food animal production units, especially in agriculture-based countries, use antimicrobials for the growth and development of food animals, along with their treatment and cure. Rising market demand for meat and associated financial motives are important driving factors for using antimicrobials for commercial ventures. Previous studies have revealed that inadequate training of veterinarians on the prudent use of antimicrobials is a key factor affecting their prescription decisions (Saman *et al.*, 2023; Arshed *et al.*, 2025). Moreover, using antimicrobials as growth promoters in food animals is less effective than management strategies that can prevent illnesses and improve production (Kasimanickam *et al.*, 2021). Global antimicrobial use (AMU) data suggest that the use of antimicrobials in

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animal production sectors is even greater than their consumption in human medicine. Tiseo and colleagues estimated global antimicrobial sales for farm animals in 2017 at 93,309 tonnes, with an expected increase of 11.5% (104,079 tonnes) by 2030 (Tiseo *et al.*, 2020). Despite the growing recognition of the urgency of tackling AMR in many low- and middle-income countries (LMICs), there is a lack of data on the trends and quantities of AMU in food animals both at the country and farm level (Schar *et al.*, 2018; Sanders *et al.*, 2020).

Political commitment to combating AMR has increased significantly since 2015, when the 68th World Health Assembly approved the global action plan (GAP) on AMR (WHO, 2015). Surveillance of AMU in food animals and its reduction in animal husbandry while maintaining animal health and welfare are the key strategic objectives indicated in many regional and global initiatives to address the AMR crisis. This includes the GAP and subsequent plans developed by the Food and Agriculture Organization (FAO) of the United Nations and the World Organization for Animal Health (WOAH) (OIE, 2016; Qiu *et al*., 2024). As a result, many countries, including Pakistan, have drafted their national action plans (NAP) based on the "One Health" approach to combat AMR. Pakistan's NAP on AMR (NAP-AMR) emphasizes AMU surveillance in food animals to address the AMR crisis. The plan aims to promote the rational use of antimicrobials by identifying knowledge gaps in potential areas for dedicated research and stewardship programs in food animal production (GOP, 2017).

Pakistan has an agro-livestock–based economy, with livestock as the largest subsector of agriculture, contributing 60.6% to agricultural gross domestic product (GDP) and 11.7% to national GDP. Livestock contributes to the country's exports and engages more than 8 million rural families, who derive up to 40% of their income from this sector (Mubeen *et al.*, 2024). Due to its high growth potential, the current government has focused on the livestock production sector for economic growth, food security, and rural socioeconomic uplift (GOP, 2020). Most of the cattle and buffalo are kept in small backyard/subsistence herds; for instance, 74% of large ruminants in Punjab province are kept in small herds of 1–10 heads (Zaheer *et al.*, 2020; Khan *et al.*, 2024).

The NAP-AMR has emerged as a comprehensive document describing Pakistan's AMR prevention and control vision. The plan calls for immediate actions to reduce AMU and AMR levels in the human and livestock sectors. In response, point prevalence surveys have been conducted in the commercial broiler sector (Umair et al., 2021b), which provided high-resolution data on farm-level AMU, including seasonal and geographic differences. However, there is no sufficient data on the trends and quantities of AMU in dairy animals in Pakistan.

Umair *et al.* (2020) published the first report of AMU in two large corporate dairy cattle farms in Pakistan, which showed total on-farm AMU to be 47.71 defined daily dose animals (DDDAs) per thousand cow days (DDDA/1,000 cow-days). Oxytetracycline (7.02 DDDA/1,000 cow-days), penicillin G (6.24 DDDA/1,000 cow-days), and cefalonium (4.27 DDDA/1,000 cow-days) were frequently used antimicrobials at the farm level. These figures reveal that these corporate dairy farms in Pakistan used considerably higher amounts of antimicrobials than those used in global studies. Mohsin and colleagues have published the first quantitative analysis of AMU in commercial poultry in Pakistan (Mohsin *et al.*, 2019). The survey was conducted at a single commercial broiler production facility for 5 years, and the farm-level AMU was estimated to be as high as 250 mg/kg of the final flock weight. This figure surpasses the global volumes of antimicrobials used per kilogram of chicken, excluding China (OECD, 2019).

The estimation and reduction of AMU while maintaining animal health is the central goal of Pakistan's NAP on AMR. In March 2020, the Government of Pakistan hosted a Workshop on "Monitoring of the Quantities and Usage Patterns of Antimicrobial Agents Used in Animals" through support from the WOAH and the Fleming Fund Country Grant Pakistan. In the workshop, AMU monitoring in food animals was considered a key factor in understanding the baseline status of AMU in the poultry and dairy sectors. Subsequently, to gain a broader perspective and examine differences in AMU between the two provinces in commercial dairy farming, the Animal Health Commissioner office, in collaboration with the provincial Livestock and Dairy Development Departments of Punjab and Khyber Pakhtunkhwa (KP), along with the National Veterinary Laboratory, initiated a data collection effort for high-resolution quantification of AMU at the farm level in dairy animals across Pakistan. The aim of this study was to conduct a cross-sectional period prevalence study to investigate AMU in the commercial dairy sector and to establish proxy values, identify field challenges, and highlight limitations in quantifying AMU in the context of Pakistan.

Materials and Methods

Study settings

Backyard subsistence farming, characterized by small herd sizes ranging from 1 to 10 animals, presents unique challenges due to the wide geographical distribution of livestock and the involvement of numerous households. Given the constraints in physical and financial resources, a cross-sectional, period prevalence survey was conducted to assess AMU in commercial dairy cattle farms during the summer of 2020, between July and August, and the winter of 2020/2021, between November and January. The farms were selected from the Punjab and KP provinces, holding a share of 61.41% and 10.83% of Pakistan's dairy animals, respectively (GOP, 2010; Zaheer *et al.*, 2020). These farmers maintain few, if any, production or treatment records. Based on available resources, possible challenges, and reliability of collected data, a cross-sectional period prevalence study AMU survey was planned for small- to medium-scale commercial dairy cattle farms, housing n = 100 dairy animals or above. A convenience sampling approach was used, relying on the voluntary participation of dairy farms. A total of 12 dairy cattle farms, six each from Punjab and KP provinces, herding more than 100 exotic/cross-bred animals, were included in the survey (Table 1, Fig. 1). Participation of the farms in the survey was voluntary, and data collection was monitored virtually, once every week via social media applications. The projects were identified in collaboration with the Animal Husbandry Commissioner's Office and the relevant provincial Livestock and Dairy Development Departments. Farms that did not continue for the winter phase of the AMU data collection were replaced with new farms from the same region, and the AMU calculations were based on the total number of farms during each season in each province and not calculated individually by farm.

Data collection

Data were collected using a survey questionnaire/Data collection tool (Supplementary Material). The DCT was designed to capture data on farm demographics, tentative diagnosis, quantities, and volumes of antimicrobials used, and other related information about the animals and herd. The personnel, consisting of one farm employee from each farm, involved in data collection, were trained for data collection on DCT during on-site training. On farm treatments, including the number of animals treated, dosage, and brand names of antimicrobials used, data were recorded on DCT on a daily basis. Trash cans were placed at each farm to enhance the ability of the farms to capture reliable data. The farm supervisors were requested to discard the empty antibiotic vials and bottles in the trash cans. Vials and packaging materials were used to identify the active ingredients and concentrations of the antimicrobials in each brand. The data were collected for 3 months over two cross-sectional surveys, that is, 4 weeks during the summer (July–August 2020) and 8 weeks during the winter phase (November 2020–January 2021). At the end of the survey, the DCT and empty vials were collected from the farms for data compilation and analysis. The data were managed using MS Excel software 2016.

AMU calculations

For high-resolution quantification of AMU at dairy cattle farms, three metrics were used that is, antimicrobial chemical compound (ACC, as grams or kilograms), milligrams of ACC used per population correction unit (mg/PCU), and antimicrobial treatment incidence (ATI, as DDDA/1,000 animal-days). ACC and mg/PCU quantify the volumes of antimicrobials used, whereas ATI quantifies treatment frequencies. All calculations were performed separately for both adult dairy cattle and calves for each season and province.

Antimicrobial active compounds

Formulation of each antimicrobial product, that is, ACC/s and their concentrations, was taken from product containers or labels in the trash can contents or online searching of the respective product. Product quantities and ACC concentrations were used to calculate the weight (kg) of ACC for each antimicrobial class (Eq. 1). Calculations for all three metrics were performed against respective ACCs instead of the antimicrobial active chemical moiety (e.g., gentamicin sulfate instead of gentamicin) (WOAH, 2022).

$$ACC_{kg} = \frac{\text{Amount of product used}_{g-ml} \times \text{Conc. of AAC}_{mg/g-ml}}{1000000} \quad (1)$$

Milligrams of ACC per population correction unit

The total amount of ACC used in milligrams was divided by the population correction unit (PCU) for dairy cattle and calves to calculate the mg/PCU for dairy cattle and calves, respectively. The PCU was calculated by multiplying the total number of dairy cattle or calves by 425 or 140 kg, respectively (standardized average weight of dairy cattle and calves at the estimated time of treatment) (ESVAC, 2018) (Eqs. 2 and 3).

$$mg/PCU_{mg/kg} = \frac{\text{Total amount of the ACC used}_{mg}}{\text{Population} \times \text{Standardised weight}_{kg}} \quad (2)$$

The cumulative mg/PCU was calculated as follows:

$$\sum_{n=1}^{N} mg/PCU_{mg/kg} = \frac{\sum_{n=1}^{N}\left(\text{Total amount of the ACC used}_{mg}\right)_N}{\sum_{n=1}^{N}\left(PCU\right)_N} \quad (3)$$

where N is the total number of animals treated.

Antimicrobial treatment incidence

The total number of antimicrobial treatments per 1,000 animals per day was calculated as DDDA per 1,000 animal days. DDDAs (i.e., milligrams of ACC recommended to be administered per animal per day) for products containing single or multiple ACCs were taken from the product containers or labels obtained from the trash can contents or by visiting the manufacturer's website. For long-acting products (to be repeated after 48 hours), the DDDAs for each ACC were divided by two. For intermammary applicators, one applicator was accounted as a single DDDA (EMA, 2015; Firth *et al.*, 2017; Umair *et al.*, 2020) (Eqs. 4–6).

$$ATI_{DDDA/1000\ \text{animal-days}} = TF \times 1000 \quad (4)$$

where TF is the treatment factor, which was calculated as follows:

Table 1. Commercial dairy farm locations and number of animals.

Punjab			KPK		
Summer					
Farm ID	Location (District)	No. of animals	Farm ID	Location (District)	No. of animals
PD-1	Faisalabad	150	KD-1	Peshawar	119
PD-2	Sheikhupura	129	KD-2	Charsadda	140
PD-3	Faisalabad	170	KD-3	Mardan	153
PD-4	Kasur	4100	KD-4	Peshawar	191
PD-5	Gojra	205	--	--	--
PD-6	Shahkot	350	--	--	--
	$\bar{x} = 851; \tilde{x} = 188$			$\bar{x} = 151; \tilde{x} = 147$	
Winter					
PD-1	Faisalabad	154	KD-1	Peshawar	260
PD-2	Sheikhupura	140	KD-2	Charsadda	551
PD-3	Faisalabad	110	KD-5	Charsadda	397
PD-5	Gojra	230	KD-6	Mardan	101
PD-7	Sargodha	300	--	--	--
PD-8	Gujrat	200	--	--	--
	$\bar{x} = 189; \tilde{x} = 177$			$\bar{x} = 327; \tilde{x} = 329$	

$$TF = \frac{\text{Total amount of ACC used}_{mg}}{DDDA_{mg/kg} \times \text{Animal} - \text{Days}} \quad (5)$$

where animal-days is the product of the total number of animals at risk and the number of studied days.

The cumulative ATI was calculated as follows:

$$\sum_{n=1}^{N} ATI = \frac{\sum_{n=1}^{N} (nDDDA)_N}{\sum_{n=1}^{N} (\text{Animal} - \text{Days})_N} \times 1000 \quad (6)$$

where nDDDA is the total number of DDDAs used, and N is the total number of dairy cattle farms.

Calculations of WHO critically important antimicrobials

All antimicrobial drugs used in adult dairy cattle and calves were categorized per the WHO list of critically important antimicrobials (IAs) for human medicine (WHO-CIA) (WHO, 2019). AMU percentages of WHO-CIA classes were calculated for the total ACCs used in adult dairy cattle and calves.

Ethical approval

Not needed for this study.

Results

AMU data collection

Due to voluntary participation, for the summer phase, 10 out of 12 commercial dairy cattle farms completed the DCT treatment record. However, for the winter phase, PD-4 and PD-6 from Punjab and KD-3 and KD-4 from KP did not continue the survey and were replaced with PD-7 and PD-8 and KD-5 and KD-6, respectively.

AMU of adult dairy cattle

In dairy cattle, 29 ACCs belonging to 15 antimicrobial classes were used at the surveyed farms in Punjab and KP during summer and winter. The cumulative AMU in adult cattle across both provinces for summer and winter was 15.23 kg, 6.36 mg/PCU, and 26.34 DDDA/1,000 animal-days. The total ACC weight of adult cattle in each province, during the time periods of study, was 12.61 kg in Punjab and 2.62 kg in KP. The total mg/PCU AMU for adult cattle in Punjab and KP in the studied time period was calculated to be 7.14 and 4.15, respectively. Streptomycin (3.17 mg/PCU), oxytetracycline (1.51 mg/PCU), enrofloxacin (1.38 mg/PCU), penicillin G (1.32 mg/PCU), and amoxicillin (1.09 mg/PCU) were the most heavily used antimicrobials per adult cow PCU in Punjab and KP in both seasons Table 2.

The incidences of antimicrobial treatment for adult cattle in Punjab and KP across both seasons and all antimicrobials were 34.14 and 10.43 DDDA/1,000 animal-days, respectively. With all study treatments of adult cattle totalled, enrofloxacin (5.92 DDA/1,000 animal-days), penicillin G (2.81 DDA/1,000 animal-days), gentamicin (2.73 DDA/1,000 animal-days), oxytetracycline (1.56 DDA/1,000 animal-days), and tylosin (1.48 DDA/1,000 animal-days) were overall the most frequently used antimicrobials. Enrofloxacin, penicillin G, and gentamicin were shared antimicrobials among the most frequently used for adult cattle in

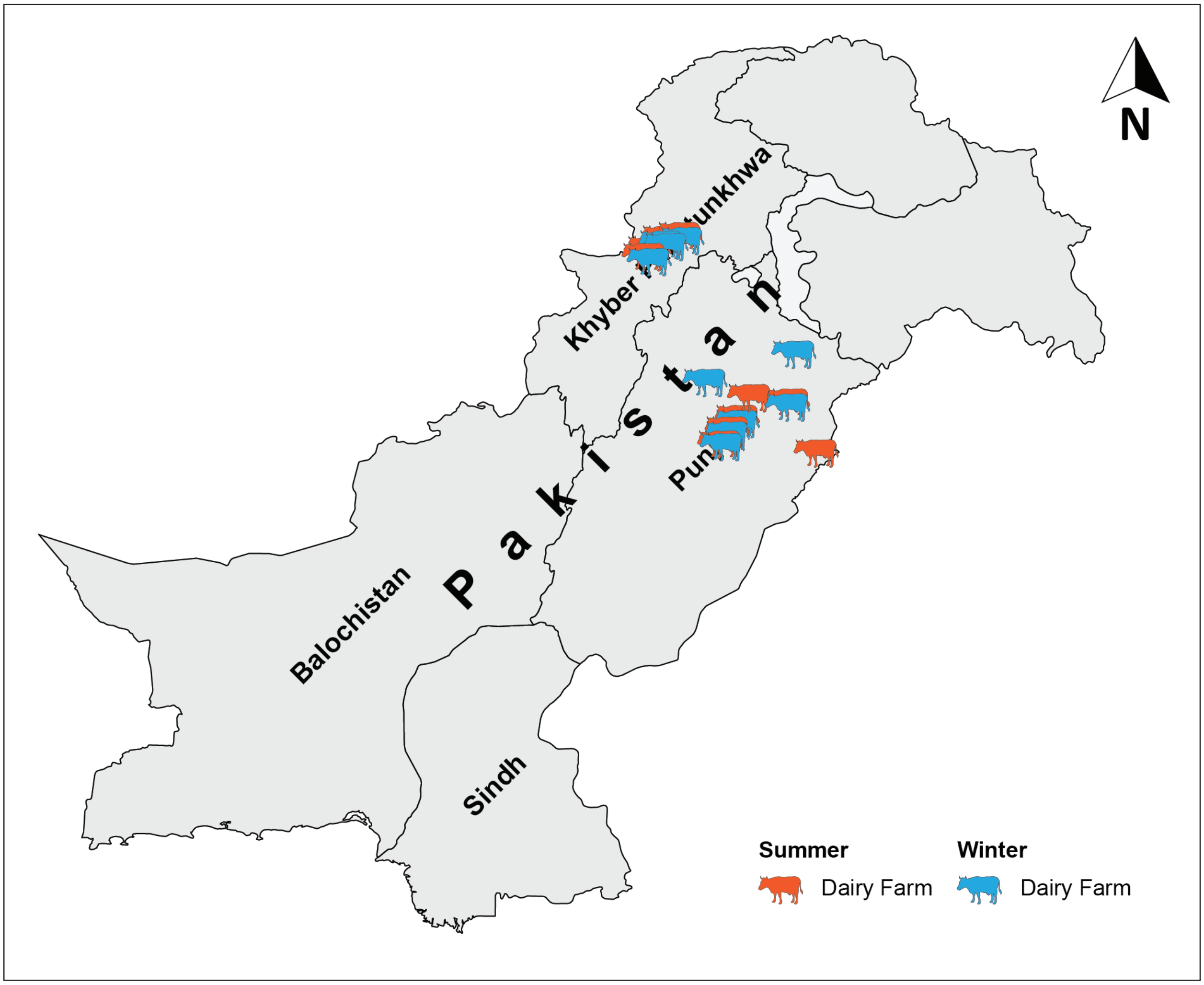

Fig. 1. Geographical locations of dairy farms in Punjab and Khyber Pakhtunkhwa participating in the AMU survey during the summer and winter months.

Pb (Punjab); KPK (Khyber Pakhtunkhwa)

Punjab and KP, with treatment incidences 7.09, 3.54, 3.5 DDDA/1,000 animal-days and 3.53, 1.32, and 1.17 DDDA/1,000 animal-days in Punjab and KP, respectively (Table 2, Fig. 2).

In adult dairy cattle, antimicrobials were most frequently administered via the parenteral route, followed by intrauterine, intramammary, and oral routes. The AMU results for individual provinces and seasons classified according to the route of administration are given in Appendix S1 (Supplementary material).

AMU in calves

In calves, 19 ACCs belonging to 13 antimicrobial classes were used during the study period. Cumulative AMU values for calves across both provinces and seasons were 1.22 kg, 8.58 mg/PCU, and 36.71 DDDA/1,000 animal-days. In calves, cumulative ATI (36.71 DDDA/1,000 animal-days) was higher than in adult dairy cattle (26.34 DDDA/1,000 animal-days). The total ACC weight of calves in Punjab and KP across both seasons was 1.07 and 0.15 kg, respectively. Regarding mg/PCU, the total AMU for calves in Punjab and KP across both seasons was 9.93 and 4.32, respectively. The greatest weights of antimicrobials used per PCU of calf overall in both provinces and seasons were sulphadiazine (1.85 mg/PCU), florfenicol (1.77 mg/PCU), sulphadimidine (1.2 mg/PCU), enrofloxacin (1.05 mg/PCU), and tylosin (0.57 mg/PCU). The ATI total AMU for calves in Punjab and KP across both seasons was 45.91 and 10.69 DDDA/1,000 animal-days, respectively. In all study treatments of calves combined, enrofloxacin (10.7 DDDA/1,000 animal-days), gentamicin (5 DDDA/1,000 animal-days), colistin (3.72 DDDA/1,000 animal-days), tylosin (3.47 DDDA/1,000 animal-days), and sulphadiazine (2.65 DDDA/1,000 animal-days) were overall the most frequently used antimicrobials (Table 3, Fig. 2). In

Table 2. Total AMU in adult dairy cattle divided into seasons (summer and winter) and provinces (Punjab and Khyber Pakhtunkhwa).

WHO-CIA	Antimicrobial class	Antimicrobial	Summer			Winter			ATI	Punjab			Khyber Pakhtunkhwa			Total		
			ACC	mg/PCU	ATI	ACC	mg/PCU	ATI	% Diff*	ACC	mg PCU	ATI	ACC	mg PCU	ATI	ACC	mg PCU	ATI
CIA-HhP	Aminopenicillins	Amoxicillin	610.5	0.38	1.44	484	0.61	1.32	−8.33	790.7	0.45	1.39	303.8	0.48	1.36	1094.5	0.46	1.38
CIA-HhP	Aminopenicillins with beta-lactam inhibitors	Amoxicillin + Clavulanic Acid	630.9	0.39	1.49	0	0	0	−100	630.9	0.36	1.15	0	0	0	630.9	0.26	0.77
CIA-HtP	Cephalosporins 3rd	Ceftiofur	26.5	0.02	0.53	8.8	0.01	0.19	−64.15	35.2	0.02	0.54	0	0	0	35.2	0.01	0.36
HIA	Cephalosporins 1st	Cephalexin	2.8	0.002	0.06	1.4	0.002	0.03	−50	3.2	0.002	0.05	0.2	0.0004	0.01	3.4	0.001	0.04
HIA	Cephalosporins 1st	Cephapirin	0	0	0	0.8	0.001	0.02	NUS	0.8	0.0005	0.01	0	0	0	0.8	0.0003	0.01
CIA-HtP	Quinolones and fluoroquinolones	Ciprofloxacin	1	0.001	0.004	0	0	0	−100	0	0	0	1	0.002	0.01	1	0.0004	0.002
HIA	Penicillins	Cloxacillin	0	0	0	0.6	0.001	0.01	NUS	0	0	0	0.6	0.001	0.01	0.6	0.0003	0.003
CIA-HtP	Polymyxins	Colistin	86.4	0.05	1.83	6.6	0.01	0.25	−86.34	88.5	0.05	1.45	4.5	0.01	0.3	93	0.04	1.07
CIA-HhP	Aminoglycosides	Dihydro-streptomycin	474.8	0.3	1.24	25.2	0.03	0.06	−95.16	500	0.28	0.99	0	0	0	500	0.21	0.67
CIA-HtP	Quinolones and fluoroquinolones	Enrofloxacin	674.6	0.42	5.59	706.5	0.9	6.27	12.16	1,111.1	0.63	7.09	270	0.43	3.53	1,381.1	0.58	5.92
HIA	Amphenicols	Florfenicol	114.6	0.07	0.12	165	0.21	0.18	50	90.6	0.05	0.07	189	0.3	0.31	279.6	0.12	0.15
CIA-HhP	Aminoglycosides	Gentamicin	395.2	0.25	3.38	280.6	0.36	2.04	−39.64	532.9	0.3	3.5	142.9	0.23	1.17	675.8	0.28	2.73
CIA-HhP	Aminoglycosides	Kanamycin	4.5	0.003	0.02	41.4	0.05	0.18	800	0	0	0	45.9	0.07	0.3	45.9	0.02	0.1
HIA	Lincosamides	Lincomycin	95.3	0.06	0.53	354.4	0.45	2.1	296.23	449.7	0.25	1.91	0	0	0	449.7	0.19	1.29
-	Antifungals	Methyl-hydroxybenzoate	2.2	0.001	1.15	0	0	0	−100	2.2	0.001	0.89	0	0	0	2.2	0.001	0.59
CIA-HhP	Aminoglycosides	Neomycin	81.4	0.05	1.36	44.3	0.06	0.24	−82.35	78.8	0.04	1.06	46.9	0.07	0.33	125.7	0.05	0.82
CIA-HtP	Quinolones and fluoroquinolones	Norfloxacin	8	0.005	0.04	0	0	0	−100	0	0	0	8	0.01	0.07	8	0.003	0.02
HIA	Tetracyclines	Oxytetracycline	875.5	0.54	1.73	636.8	0.81	1.38	−20.23	1377.1	0.78	2.12	135.2	0.21	0.42	1,512.3	0.63	1.56
HIA	Penicillins	Penicillin G	731.3	0.45	2.06	589.1	0.75	3.61	75.24	1163.3	0.66	3.54	157.1	0.25	1.32	1,320.4	0.55	2.81
CIA-HhP	Ansamycins	Rifaximin	2.1	0.001	0.02	0	0	0	−100	2.1	0.001	0.01	0	0	0	2.1	0.001	0.01
CIA-HtP	Macrolides and ketolides	Spiramycin	150	0.09	0.5	312.5	0.4	1.11	122	462.5	0.26	1.18	0	0	0	462.5	0.19	0.79

Continued

WHO-CIA	Antimicrobial class	Antimicrobial	Summer			Winter			ATI	Punjab			Khyber Pakhtunkhwa			Total		
			ACC	mg/PCU	ATI	ACC	mg/PCU	ATI	% Diff*	ACC	mg PCU	ATI	ACC	mg PCU	ATI	ACC	mg PCU	ATI
CIA-HhP	Aminoglycosides	Streptomycin	785.2	0.49	0.55	2384	3.02	1.76	220	2,498.7	1.41	1.34	670.5	1.06	0.73	3,169.2	1.32	1.14
HIA	Sulfonamides	Sulphadiazine	480.6	0.3	0.57	587.9	0.75	0.78	36.84	983	0.56	0.92	85.5	0.14	0.16	1,068.5	0.45	0.67
HIA	Sulfonamides	Sulphadimidine	741	0.46	0.12	337.6	0.43	0.06	−50	569.2	0.32	0.07	509.5	0.81	0.13	1,078.7	0.45	0.09
HIA	Sulfonamides	Sulphamethoxy-pyridazine	297	0.18	0.49	0	0	0	−100	288	0.16	0.37	9	0.01	0.02	297	0.12	0.25
HIA	Sulfonamides	Sulphathiazole	7	0.004	0.01	0	0	0	−100	7	0.004	0.01	0	0	0	7	0.003	0.005
HIA	Tetracyclines	Tetracycline	60	0.04	1.32	1.6	0.002	0.04	−96.97	61.6	0.03	1.04	0	0	0	61.6	0.03	0.7
HIA	Dihydrofolate reductase inhibitors	Trimethoprim	148.7	0.09	1.04	115.6	0.15	0.77	−25.96	247.6	0.14	1.28	16.7	0.03	0.16	264.3	0.11	0.91
CIA-HtP	Macrolides and ketolides	Tylosin	577.5	0.36	2.61	85	0.11	0.28	−89.27	639.5	0.36	2.16	23	0.04	0.09	662.5	0.28	1.48
		Total	8,064.6	5.015	29.80	7169.7	9.09	22.68	−23.9	12,614.2	7.14	34.14	2,619.3	4.15	10.43	15,233.5	6.36	26.34

ACC = antimicrobial chemical compound; ATI = antimicrobial treatment incidence; mg/PCU = milligrams per population correction unit; NUS = not used in summer. Units: ACC (g); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days)
*% difference in winter AMU compared to the summer values. Comparisons between winter and summer ACCs are not recommended due to differences in data collection periods.

calves, antimicrobials were frequently administered via the parenteral route, followed by oral administration.

AMU seasonal variations

Results from our study indicated that total ATIs for both provinces in adult dairy cattle increased in winter for enrofloxacin, florfenicol, penicillin G, spiramycin, streptomycin, lincomycin, and kanamycin, that is, from 12% increase in enrofloxacin (5.59 in summer–6.27 in winter DDDA/1,000 animal-days) to 900% increase in kanamycin (0.02 in summer–0.18 in winter DDDA/1,000 animal-days). However, the overall ATI for all antimicrobials used for adult cattle in winter decreased by 23.9% from summer values, that is, from 29.8 in summer to 22.68 in winter DDDA/1,000 animal-days (Table 2, Fig. 3).

In contrast, the overall ATI for all antimicrobials decreased by 65% in calves, that is, from 53.48 in summer to 18.73 in winter, DDDA/1,000 animal-days. However, the incidence of treatments increased sequentially for kanamycin (0.02–0.73 DDDA/1,000 animal-days), neomycin (0.48–2.22 DDDA/1,000 animal-days), sulphadimidine (0.53–1.62 DDDA/1,000 animal-days), penicillin G (0.34–0.47 DDDA/1,000 animal-days), sulphadiazine (2.39–2.92 DDDA/1,000 animal-days), and dihydrostreptomycin (0.05 to 0.06 DDDA/1,000 animal-days) (Table 3, Fig. 3).

AMU classification by WHO-CIA

In adult dairy cattle, in terms of ACCs used per PCU (mg/PCU), 17.35% of the antimicrobials belonged to critically IA with the highest priority for human medicine (CIA-HtP), 40.9% belonged to high-priority antimicrobials (CIA-HhP), and 41.75% belonged to IA. CIA-HtP (37.44%) were the most frequently used antimicrobials in terms of ATI, followed by IA (32.96%) and CIA-HhP (29.59%) (Table 2, Fig. 4).

In calves, in terms of ACCs weight IA (63.64%) was the most commonly used antimicrobial, followed by CIA-HtP (24.01%) and CIA-HhP (12.59). Among the ATI values, CIA-HtP (53.91%) was the most frequently used antimicrobial, followed by IA (26.12%) and CIA-HhP (19.97%) (Table 3, Fig. 4).

Discussion

One of the primary goals of the WHO's GAP on AMR is to monitor AMU in food animal production systems (WHO, 2015). Implementing national- and farm-level AMU surveillance programs in many European Union countries has significantly reduced AMU in food animals (EMA, 2015). However, there are few national-level studies on AMU in food animals from LMICs (Cuong *et al.*, 2018). Pakistan lacks formal AMU monitoring of food animals for various reasons, the most important of which is weak legislation and poor implementation of existing legislation.

As such, this study provides quantitative AMU data in support of Pakistan's ongoing national AMR surveillance efforts, and this is the first study to be conducted on a relatively large geographical area, that

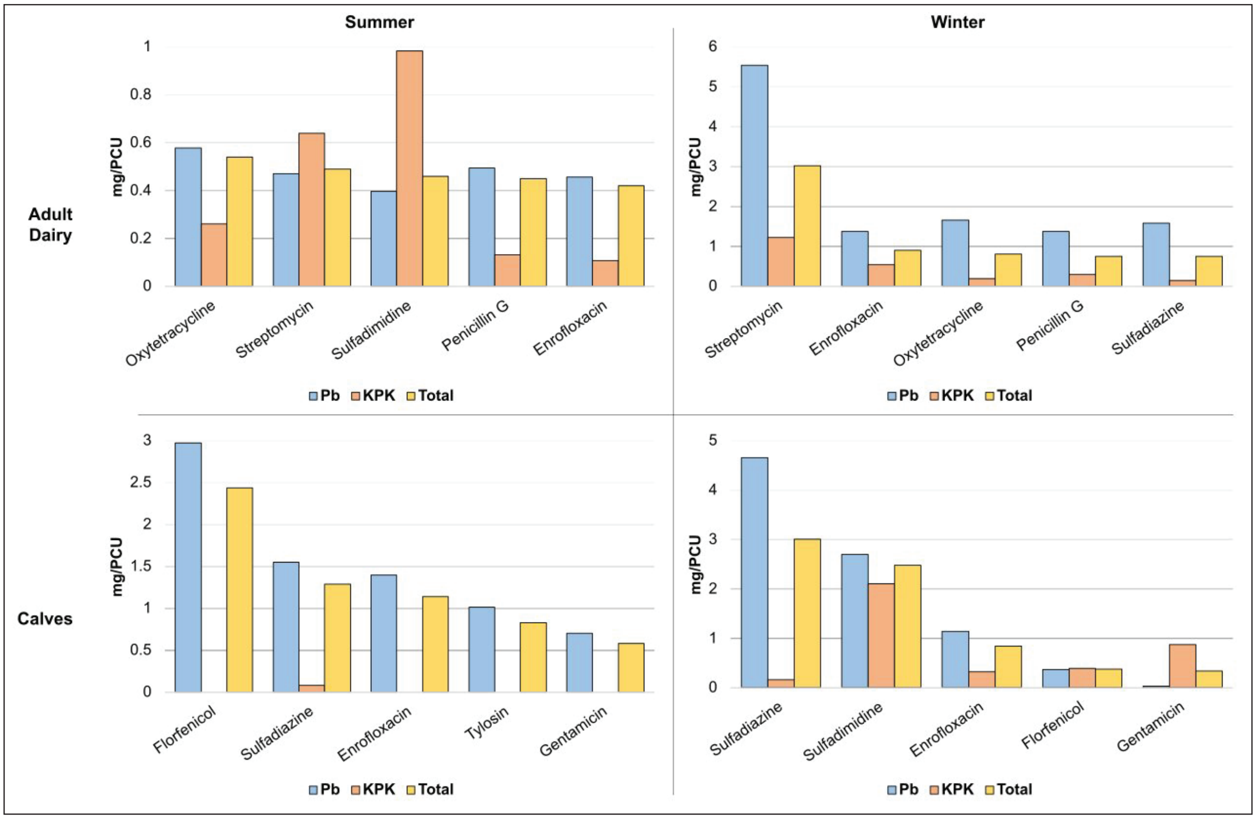

Fig. 2. Antimicrobials most used (mg/PCU) in adult dairy and calves during summer and winter.

is, medium- to large-sized herds from two provinces in Pakistan.

To the best of our knowledge, no other South Asian AMU study has covered such a large cohort and geographical area. The lack of an internationally accepted standardized AMU indicator makes data comparisons across species, production types, and countries difficult (Góchez *et al.*, 2019; Umair *et al.*, 2021a). This study used multiple AMU indicators for dairy cattle (kg, mg/PCU, and DDDA/1,000 cow-days). Multiple AMU indicators are valuable and provide more comprehensiveness and high-resolution AMU data. However, the use of different AMU metrics—such as weight-based measures (e.g., ACC and mg/PCU) and dose-based measures (e.g., DDDA per 1,000 animal-days) – may complicate comparisons of AMU data across regions and countries. These challenges arise because of variations in animal breeds, weights, and production systems across different geographical areas (Brault *et al.*, 2019; Umair *et al.*, 2021a). The newly published guidelines for farm-level AMU will help streamline and strengthen national-level AMU monitoring, and Pakistan can benefit from the guidelines for future monitoring activities (FAO and WOAH, 2023).

This study calculated a lower AMU (26.34 DDDA/1,000 animal-days) in dairy cattle than the previously published study reporting 47.7 DDDA/1,000 animal-days from two commercial dairy cattle farms in Pakistan (Umair *et al.*, 2020). Still, the value is comparable to a Brazilian study reporting 21.9 DDDA/1,000 animal-days (Tomazi and dos Santos, 2020). The variation in AMU levels between the two Pakistani studies may be attributed to differences in study duration, seasonal fluctuations, geographic regions, and the types of dairy farms involved, specifically small- to medium-scale farms versus large corporate commercial dairy farms. The higher treatment frequency observed in calves (36.71 DDDA/1,000 animal-days) was compared to dairy cattle (26.34 DDDA/1,000 animal-days) and may be attributed to suboptimal calf management and welfare practices, as well as an increased incidence of disease (Fentie *et al.*, 2020; Ali *et al.*, 2021).

However, 26.34 DDDA/1,000 animal-days is much higher compared to AMU figures from developed countries, that is, 4.2 and 14.88 DDDA/1,000 animal-days reported from Pennsylvania and Wisconsin, United States, respectively (Pol and Ruegg, 2007; Redding *et al.*, 2019), 16.05 DDDA/1,000 animal-days from the Netherlands (Kuipers *et al.*, 2016), 20.78 DDDA/1,000 animal-days from Belgium (Stevens *et al.*, 2016), and 8.87 DDDA/1,000 animal-days from the United Kingdom (Hyde *et al.*, 2017).

Table 3. Total AMU in calves divided into seasons (summer and winter) and provinces (Punjab and Khyber Pakhtunkhwa).

WHO-CIA	Antimicrobial Class	Antimicrobial	Summer			Winter			ATI	Punjab			Khyber Pakhtunkhwa			Total		
			ACC	mg/PCU	ATI	ACC	mg/PCU	ATI	% Diff*	ACC	mg/PCU	ATI	ACC	mg/PCU	ATI	ACC	mg/PCU	ATI
CIA-HhP	Aminopenicillins	Amoxicillin	9.6	0.1	0.48	0.3	0.01	0.02	−95.83	6	0.06	0.2	3.9	0.11	0.4	9.9	0.07	0.26
CIA-HhP	Aminopenicillins with beta- lactam inhibitors	Amoxicillin + Clavulanic Acid	0.9	0.01	0.03	0	0	0	−100	0.9	0.01	0.02	0	0	0	0.9	0.01	0.02
CIA-HtP	Cephalosporins 3rd	Ceftiofur	4.5	0.05	1.55	0	0	0	−100	4.5	0.04	1.09	0	0	0	4.5	0.03	0.8
CIA-HtP	Polymyxins	Colistin	18.3	0.19	6.52	1	0.02	0.73	−88.8	18.3	0.17	4.55	1	0.03	1.38	19.3	0.14	3.72
CIA-HhP	Aminoglycosides	Dihydro-streptomycin	2.7	0.03	0.05	1.6	0.03	0.06	20	4.3	0.04	0.07	0	0	0	4.3	0.03	0.05
CIA-HtP	Quinolones and fluoroquinolones	Enrofloxacin	109.6	1.14	15.26	38.9	0.84	5.81	−61.93	143	1.33	13.95	5.5	0.16	1.52	148.5	1.05	10.7
HIA	Amphenicols	Florfenicol	233.7	2.44	4.07	17.4	0.38	0.32	−92.14	244.5	2.27	2.98	6.6	0.19	0.23	251.1	1.77	2.26
CIA-HhP	Aminoglycosides	Gentamicin	55.2	0.58	8.21	15.7	0.34	1.55	−81.12	56.1	0.52	5.85	14.8	0.43	2.59	70.8	0.5	5
CIA-HhP	Aminoglycosides	Kanamycin	0.3	0.003	0.02	9.8	0.21	0.73	3,550	0	0	0	10	0.29	1.38	10	0.07	0.36
HIA	Lincosamides	Lincomycin	21.5	0.22	1.99	1.5	0.03	0.15	−92.46	23	0.21	1.49	0	0	0	23	0.16	1.1
CIA-HhP	Aminoglycosides	Neomycin	1.8	0.02	0.48	13.6	0.29	2.22	362.5	5.2	0.05	1.26	10.2	0.3	1.48	15.4	0.11	1.32
HIA	Tetracyclines	Oxytetracycline	19.3	0.2	0.64	5.2	0.11	0.2	−68.75	19.7	0.18	0.47	4.8	0.14	0.32	24.5	0.17	0.43
HIA	Penicillins	Penicillin G	7.7	0.08	0.34	4.6	0.1	0.47	38.24	7.4	0.07	0.38	4.8	0.14	0.46	12.2	0.09	0.4
CIA-HtP	Macrolides and ketolides	Spiramycin	35.8	0.37	1.99	2.5	0.05	0.15	−92.46	38.3	0.35	1.49	0	0	0	38.3	0.27	1.1
CIA-HhP	Aminoglycosides	Streptomycin	25.5	0.27	0.33	15.4	0.33	0.31	−6.06	20.8	0.19	0.27	20	0.59	0.46	40.8	0.29	0.32
HIA	Sulfonamides	Sulphadiazine	123.3	1.29	2.39	139.2	3.01	2.92	22.18	258.2	2.4	3.55	4.3	0.12	0.1	262.5	1.85	2.65
HIA	Sulfonamides	Sulphadimidine	55.4	0.58	0.53	114.5	2.48	1.62	205.66	108.3	1	1.3	61.7	1.81	0.37	169.9	1.2	1.06
HIA	Dihydrofolate reductase inhibitors	Trimethoprim	18.5	0.19	1.93	12.8	0.28	1.43	−25.91	31.3	0.29	2.29	0	0	0	31.3	0.22	1.69
CIA-HtP	Macrolides and ketolides	Tylosin	79.7	0.83	6.67	0.6	0.01	0.04	−99.4	80.3	0.74	4.7	0	0	0	80.3	0.57	3.47
		Total	823.3	8.6	53.48	394.6	8.54	18.73	−64.98	1,070.1	9.93	45.91	147.6	4.32	10.69	1,217.5	8.58	36.71

ACC = antimicrobial chemical compound; ATI = antimicrobial treatment incidence; mg/PCU = milligrams per population correction unit. Units: ACC (g); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days)

*% difference in winter AMU compared to the summer values. Comparisons between winter and summer ACCs are not recommended due to differences in data collection periods.

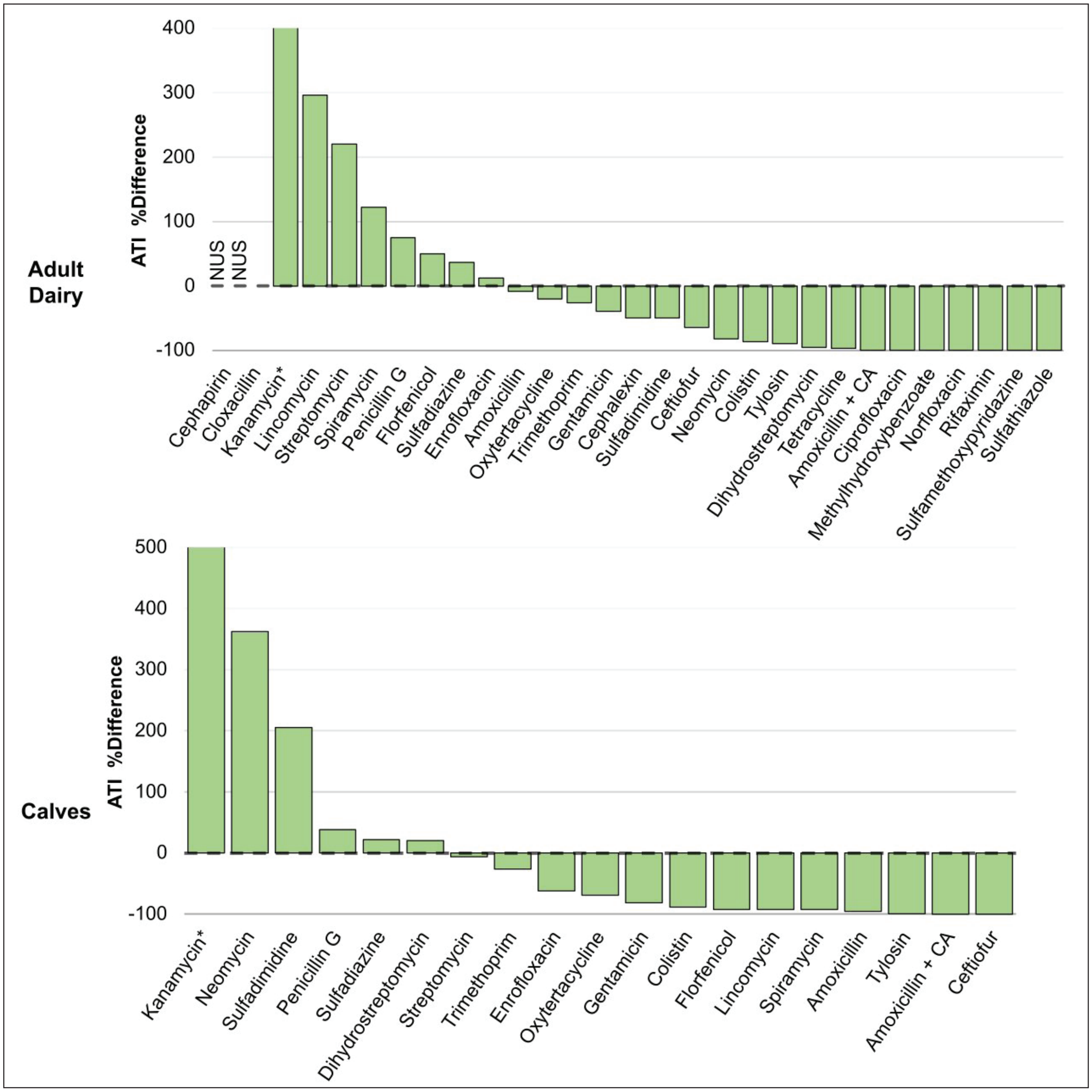

Fig. 3. Percentage difference in antimicrobial treatment incidence (ATI) in adult dairy cattle and calves during winter compared to summer. NUS = not used in summer. *Value exceeding graph limit–referred to Table 3.

This could be due to poor management and husbandry practices and inadequate knowledge, attitudes, and practices of veterinary stakeholders (veterinarians, farm owners, farm managers, veterinary pharmacies, and pharmaceutical companies) in Pakistan regarding AMU and AMR. In addition, there was a decrease in cumulative AMU from summer to winter in both adult dairy cattle (29.80 to 22.68 DDDA/1,000 animal days) and calves (53.48 to 18.73 DDDA/1,000 animal days), which could be attributed to higher disease incidence in summer rather than winter, likely due to housing conditions (Sanders *et al.*, 2009).

Moreover, Saman and colleagues reported that the majority of antibiotics prescribed by veterinarians in Pakistan were CIAs, with a sizable proportion of CIA-HhP and CIA-HtP (Saman *et al.*, 2023). Our findings showed that CIAs in food animals contained a considerable amount of CIA-HtP, with 17.35% in adult dairy cattle and 24.01% in calves. These findings are consistent with a recent study from Pakistan, which reported the country is importing

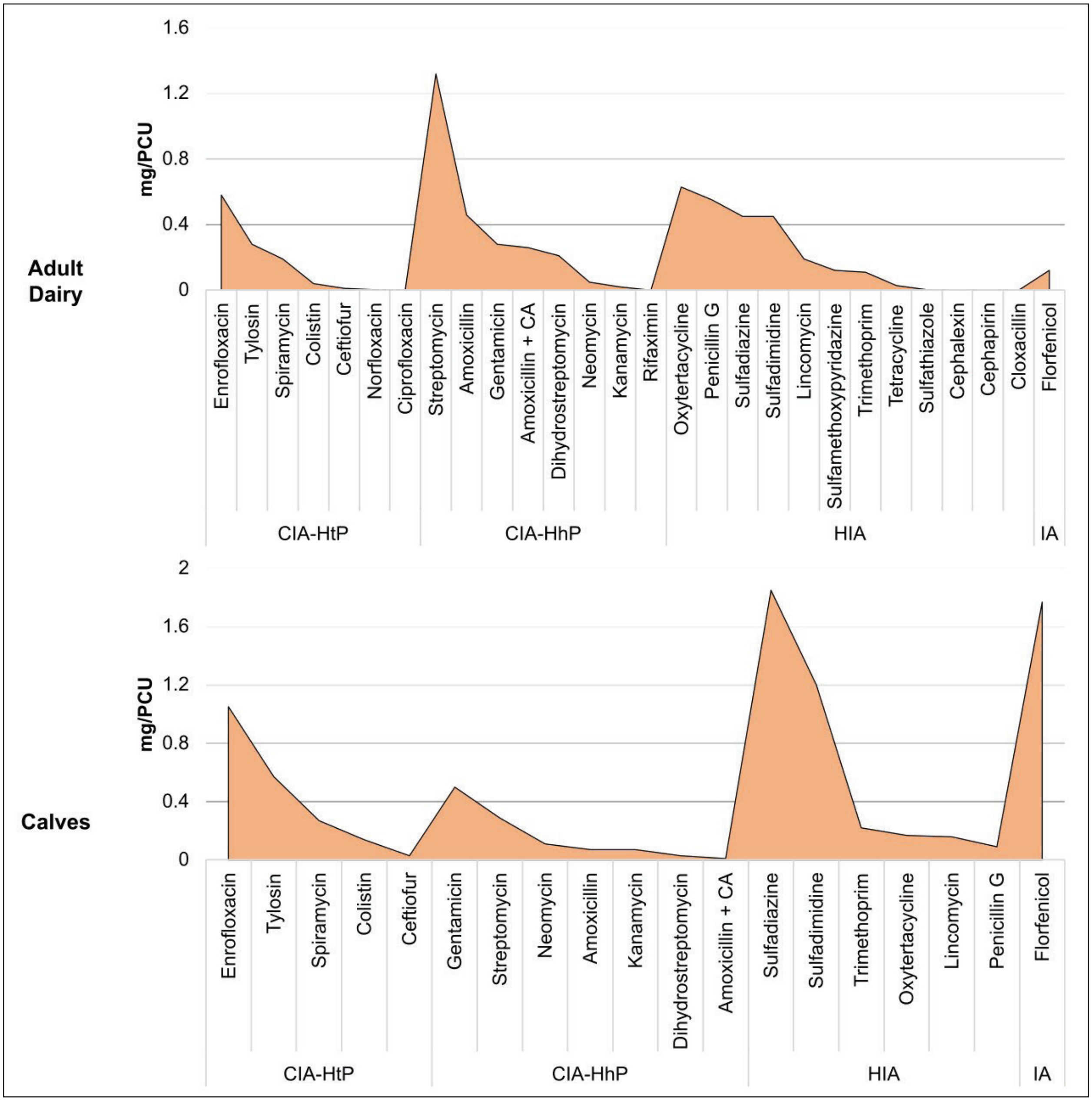

Fig. 4. Area plots for various antimicrobials used overall (mg/PCU) during summer and winter were categorized as per WHO-CIA for human use classification. CIA-HtP = critically important antimicrobial with the highest priority; CIA-HhP = critically important antimicrobials with high priority; HIA = highly important antimicrobials; IA = important antimicrobials.

large volumes of CIA-HtP for use in food animals (Umair *et al.*, 2022), but contradict an earlier one (Umair *et al.*, 2020).

The use of CIA-HtPs, for example, 3rd generation cephalosporins, quinolones, and polymyxins, in food-producing animals is concerning because they are effective in treating human infections, and pathogens resistant to these last-line antimicrobials can lead to untreatable fatal infections. This can be more threatening in LMICs, such as Pakistan, where AMU/AMR surveillance is insufficient, where humans are highly exposed to animals, and herd workers' knowledge of zoonosis is limited (Saman *et al.*, 2023).

The AMU estimates from this first study on dairy animals in Pakistan provide valuable insights for policymakers and veterinary professionals to move toward more prudent use of antimicrobials in food animals in Pakistan. Moreover, these findings are a reminder to conduct large-scale studies on different production systems and seasons to understand the differences and develop tailored strategies. It is important to gather data over longer time periods to provide more representative baseline data, including other provinces, animal species, and farming systems. This study can guide future AMU surveillance that involves a larger number of herds to provide representative baseline data and monitor future AMU trends.

Limitations

One of the primary limitations of this study is its reliance on a convenience sample of farms, which may not be fully representative of the country's commercial dairy sector. Additionally, farm participation varied across seasons in both provinces, potentially influencing the generalizability of our findings. Therefore, our results are not generalizable to the rest of the Pakistani dairy sector. Our data represents 3 months in dairy cattle, accounting for the summer (1 month) and winter (2 months) seasons. A longitudinal study that includes additional farms, farming systems, and animal species is required for sustainable monitoring to better understand AMU patterns in livestock in Pakistan.

Moreover, the under- or overdosing of animals with antimicrobials (i.e., not following the recommended guidelines) may result in the over- or underestimation of DDDAs. As in other studies, AMU metrics using the PCU denominator were greatly dictated by the animal weight standards. For example, adult dairy cattle can weigh 600 kg (Jensen *et al.*, 2004) or 425 kg (ESVAC, 2018). This discrepancy provides an unreliable comparison between studies. Unlike Europe, Pakistan has not established the average weight standards of different livestock. We calculated AMU based on ESVAC's weight standards for dairy adults and calves because these are the latest recommended standards and because most of the exotic breeds in the surveyed farms were exotic cattle imported from Europe. These limitations in calculating animal weights and dose rates present challenges when comparing our results with those from other countries.

Conclusion

The current cross-sectional period prevalence study highlights the need for a robust and well-planned AMU surveillance program for food animals in Pakistan. This study also emphasizes the need to establish national-level animal PCUs to improve the accuracy of weight-based AMU calculations. Our results show high farm-level usage of critically IA in human medicine. In the future, AMU in food animals should be more strongly regulated to reduce the risk of AMR development.

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Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization, M.F.T., M.U.Z., and M.AK.; data curation, M.U. and J.A.; formal analysis, M.U.; funding acquisition, Q.A.; investigation, M.M.; methodology, M.U.; project administration, M.F.T. and M.AK.; resources, M.F.T., R.W.U., and M.U.Z.; supervision, M.M.; validation, J.A. and M.U.Z.; visualization, R.W.U., J.A., M.AB., writing—original draft, M.U., M.M., R.M.A., and S.U.K.B; writing—review and editing, J.A., M.AB., and M.U.Z. All authors have read and agreed to the published version of the manuscript.

Data availability

The data presented in this study are not publicly available.

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Appendix I: Tables

Table S1. Antimicrobial use in dairy cattle (summer).

Antimicrobial Class	Antimicrobial	Parenteral						Intramammary			Intrauterine						Oral						Total		
		AI		mg/PCU		ATI		AI	mg/PCU	ATI	AI		mg/PCU		ATI		AI		mg/PCU		ATI		AI	mg/PCU	ATI
		Pb	KPK	Pb	KPK	Pb	KPK	Pb	Pb	Pb	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK			
Aminopenicillins	Amoxicillin	0.5315	0.075	0.37	0.43	1.36	2.05	0.004	0.003	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0.6105	0.38	1.44
Aminopenicillins with beta-lactam inhibitor	Amoxicillin + Clavulanic Acid	0.5661	0	0.39	0	1.5	0	0.0648	0.05	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0.6309	0.39	1.49
Cephalosporins 3rd	Ceftiofur	0.02	0	0.01	0	0.45	0	0.0045	0.003	0.1	0.002	0	0.001	0	0.04	0	0	0	0	0	0	0	0.0265	0.02	0.53
Cephalosporins 1st	Cephalexin	0	0	0	0	0	0	0.0028	0.002	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0.0028	0.002	0.06
Quinolones and fluoroquinolones	Ciprofloxacin	0	0.001	0	0.01	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001	0.001	0.004
Polymyxins	Colistin	0.086	0.0004	0.06	0.002	2.03	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0864	0.05	1.83
Aminoglycosides	Dihydrostreptomycin	0.4588	0	0.32	0	1.34	0	0.016	0.01	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0.4748	0.3	1.24
Quinolones and fluoroquinolones	Enrofloxacin	0.6496	0.0185	0.45	0.11	6.03	1.43	0.0035	0.002	0.03	0	0	0	0	0	0	0.003	0	0.002	0	0.03	0	0.6746	0.42	5.59
Amphenicols	Florfenicol	0.0906	0.024	0.06	0.14	0.11	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1146	0.07	0.12
Aminoglycosides	Gentamicin	0.3817	0.0081	0.27	0.05	3.64	0.37	0.0054	0.003	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0.3952	0.25	3.38
Aminoglycosides	Kanamycin	0	0.0045	0	0.03	0	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0045	0.003	0.02
Lincosamides	Lincomycin	0.0953	0	0.07	0	0.59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0953	0.06	0.53
Antifungals	Methylhydroxybenzoate	0.0022	0	0.002	0	1.24	0	0.0001	0.0001	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0.0022	0.001	1.15
Aminoglycosides	Neomycin	0	0.0045	0	0.03	0	0.17	0.075	0.05	1.48	0	0	0	0	0	0	0.0018	0.0002	0.001	0.001	0.02	0.04	0.0814	0.05	1.36
Quinolones and fluoroquinolones	Norfloxacin	0	0.008	0	0.05	0	0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.008	0.005	0.04
Tetracyclines	Oxytetracycline	0.716	0.045	0.5	0.26	1.59	0.82	0.0005	0.0003	0	0.114	0	0.08	0	0.24	0	0	0	0	0	0	0	0.8755	0.54	1.73
Penicillins	Penicillin G	0.6733	0.0208	0.47	0.12	2.12	0.56	0.0352	0.02	0.11	0.0002	0.0018	0.0002	0.01	0.01	0.05	0	0	0	0	0	0	0.7313	0.45	2.06
Ansamycins	Rifaximin	0	0	0	0	0	0	0	0	0	0.0021	0	0.001	0	0.02	0	0	0	0	0	0	0	0.0021	0.001	0.02
Macrolides and ketolides	Spiramycin	0.15	0	0.1	0	0.56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.15	0.09	0.5
Aminoglycosides	Streptomycin	0.595	0.1025	0.41	0.59	0.46	0.66	0.08	0.06	0.06	0.0002	0.0075	0.0001	0.04	0.01	0.05	0	0	0	0	0	0	0.7852	0.49	0.55
Sulfonamides	Sulfadiazine	0.316	0.0185	0.22	0.11	0.44	0.19	0.112	0.08	0.16	0	0	0	0	0	0	0.033	0.0036	0.02	0.02	0.02	0.04	0.4806	0.3	0.57
Sulfonamides	Sulfadimidine	0.2098	0	0.15	0	0.03	0	0	0	0	0	0	0	0	0	0	0.3594	0.1693	0.25	0.98	0.07	0.25	0.741	0.46	0.12
Sulfonamides	Sulfamethoxypyridazine	0.288	0.009	0.2	0.05	0.53	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.297	0.18	0.49
Sulfonamides	Sulfathiazole	0	0	0	0	0	0	0	0	0	0.007	0	0.005	0	0.01	0	0	0	0	0	0	0	0.007	0.004	0.01
Tetracyclines	Tetracycline	0	0	0	0	0	0	0.06	0.04	1.48	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0.04	1.32
Dihydrofolate reductase inhibitors	Trimethoprim	0.1208	0.0055	0.08	0.03	0.97	0.31	0.0224	0.02	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0.1487	0.09	1.04
Macrolides and ketolides	Tylosin	0.5665	0.011	0.39	0.06	2.88	0.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5775	0.36	2.61
	Total	**6.5172**	**0.3563**	**4.54**	**2.07**	**27.87**	**7.99**	**0.4862**	**0.34**	**4.02**	**0.1255**	**0.0093**	**0.087**	**0.05**	**0.33**	**0.1**	**0.3972**	**0.1731**	**0.28**	**1.001**	**0.14**	**0.33**	**8.0646**	**5.01**	**29.8**

AI = active ingredient; ATI = antimicrobial treatment incidence; KPK = Khyber Pakhtunkhwa; mg/PCU = milligrams per population unit; Pb = Punjab. Units: AI (kg); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days).

Table S2. Antimicrobial use in dairy cattle (winter).

Antimicrobial Class	Antimicrobial	Parenteral						Intramammary						Intrauterine			Oral			Total		
		AI		mg/PCU		ATI		AI		mg/PCU		ATI		AI	mg/PCU	ATI	AI	mg/PCU	ATI	AI	mg/PCU	ATI
		Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	Pb	Pb	KPK	KPK	KPK			
Aminopenicillins	Amoxicillin	0.2477	0.2288	0.75	0.5	1.38	1.25	0.0075	0	0.02	0	0.06	0	0	0	0	0	0	0	0.484	0.61	1.32
Cephalosporins 3rd	Ceftiofur	0.0073	0	0.02	0	0.37	0	0.0005	0	0.002	0	0.03	0	0.001	0.003	0.03	0	0	0	0.0088	0.01	0.19
Cephalosporins 1st	Cephalexin	0	0	0	0	0	0	0.0004	0.0002	0.001	0.0005	0.02	0.01	0	0	0	0	0	0	0.0014	0.002	0.03
Cephalosporins 1st	Cephapirin	0.0008	0	0.002	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0008	0.001	0.02
Penicillins	Cloxacillin	0	0	0	0	0	0	0	0.0006	0	0.001	0	0.01	0	0	0	0	0	0	0.0006	0.001	0.01
Polymyxins	Colistin	0.0025	0.0041	0.01	0.01	0.15	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0.0066	0.01	0.25
Aminoglycosides	Dihydrostreptomycin	0.0252	0	0.08	0	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0252	0.03	0.06
Quinolones and fluoroquinolones	Enrofloxacin	0.329	0.2515	1	0.55	6.65	4.06	0	0	0	0	0	0	0.126	0.38	2.55	0	0	0	0.7065	0.9	6.27
Amphenicols	Florfenicol	0	0.165	0	0.36	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0.165	0.21	0.18
Aminoglycosides	Gentamicin	0.1456	0.1348	0.44	0.29	2.92	1.36	0.0002	0	0.001	0	0.02	0	0	0	0	0	0	0	0.2806	0.36	2.04
Aminoglycosides	Kanamycin	0	0.0414	0	0.09	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0.0414	0.05	0.18
Lincosamides	Lincomycin	0.3499	0	1.06	0	4.72	0	0.0045	0	0.01	0	0.06	0	0	0	0	0	0	0	0.3544	0.45	2.1
Aminoglycosides	Neomycin	0	0.0414	0	0.09	0	0.33	0	0.0009	0	0.002	0	0.03	0.002	0.01	0.09	0	0	0	0.0443	0.06	0.24
Tetracyclines	Oxytetracycline	0.0358	0.0902	0.11	0.2	0.24	0.34	0	0	0	0	0	0	0.5108	1.55	2.47	0	0	0	0.6368	0.81	1.38
Penicillins	Penicillin G	0.1418	0.1345	0.43	0.29	1.94	1.51	0.0048	0	0.01	0	0.07	0	0.3079	0.93	4.32	0	0	0	0.5891	0.75	3.61
Macrolides and ketolides	Spiramycin	0.0888	0	0.27	0	0.72	0	0.0163	0	0.05	0	0.13	0	0.2075	0.63	1.68	0	0	0	0.3125	0.4	1.11
Aminoglycosides	Streptomycin	0.5205	0.5605	1.58	1.22	0.88	0.75	0.0216	0	0.07	0	0.12	0	1.283	3.89	2.16	0	0	0	2.384	3.02	1.76
Sulfonamides	Sulfadiazine	0.522	0.056	1.58	0.12	1.58	0.14	0	0	0	0	0	0	0	0	0	0.0099	0.02	0.02	0.5879	0.75	0.78
Sulfonamides	Sulfadimidine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3376	0.74	0.11	0.3376	0.43	0.06
Tetracyclines	Tetracycline	0	0	0	0	0	0	0.0016	0	0.0049	0	0.0859	0	0	0	0	0	0	0	0.0016	0.002	0.04
Dihydrofolate reductase inhibitors	Trimethoprim	0.1044	0.0112	0.31	0.02	1.58	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0.1156	0.15	0.77
Macrolides and ketolides	Tylosin	0.073	0.012	0.22	0.03	0.57	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0.085	0.11	0.28
	Total	**2.5943**	**1.7314**	**7.87**	**3.77**	**23.87**	**10.92**	**0.0574**	**0.0017**	**0.17**	**0.004**	**0.6**	**0.05**	**2.4382**	**7.39**	**13.3**	**0.3475**	**0.76**	**0.13**	**7.1697**	**9.09**	**22.68**

AI = active ingredient; ATI = antimicrobial treatment incidence; KPK = Khyber Pakhtunkhwa; mg/PCU = milligrams per population unit; Pb = Punjab. Units: AI (kg); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days).

Table S3. Antimicrobial use in calves (summer).

Antimicrobial Class	Antimicrobial	Parenteral						Oral						Total		
		AI		mg/PCU		ATI		AI		mg/PCU		ATI		AI	mg/ PCU	ATI
		Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK			
Aminopenicillins	Amoxicillin	0.0057	0.0039	0.07	0.23	0.33	1.13	0	0	0	0	0	0	0.0096	0.1	0.48
Aminopenicillins with beta-lactam inhibitor	Amoxicillin + Clavulanic Acid	0.0009	0	0.01	0	0.04	0	0	0	0	0	0	0	0.0009	0.01	0.03
Cephalosporins 3rd	Ceftiofur	0.0045	0	0.06	0	1.89	0	0	0	0	0	0	0	0.0045	0.05	1.55
Polymyxins	Colistin	0.0183	0.00002	0.23	0.001	7.93	0.1	0	0	0	0	0	0	0.0183	0.19	6.52
Aminoglycosides	Dihydrostreptomycin	0.0027	0	0.03	0	0.06	0	0	0	0	0	0	0	0.0027	0.03	0.05
Quinolones and fluoroquinolones	Enrofloxacin	0.1096	0	1.4	0	18.61	0	0	0	0	0	0	0	0.1096	1.14	15.26
Amphenicols	Florfenicol	0.2337	0	2.98	0	4.96	0	0	0	0	0	0	0	0.2337	2.44	4.07
Aminoglycosides	Gentamicin	0.0552	0	0.7	0	10.01	0	0	0	0	0	0	0	0.0552	0.58	8.21
Aminoglycosides	Kanamycin	0	0.0003	0	0.01	0	0.1	0	0	0	0	0	0	0.0003	0.003	0.02
Lincosamides	Lincomycin	0.0215	0	0.27	0	2.43	0	0	0	0	0	0	0	0.0215	0.22	1.99
Aminoglycosides	Neomycin	0	0.0003	0	0.01	0	0.1	0.0015	0.0001	0.02	0.004	0.53	0.15	0.0018	0.02	0.48
Tetracyclines	Oxytetracycline	0.019	0.0003	0.24	0.01	0.76	0.07	0	0	0	0	0	0	0.0193	0.2	0.64
Penicillins	Penicillin G	0.0029	0.0048	0.04	0.28	0.13	1.29	0	0	0	0	0	0	0.0077	0.08	0.34
Macrolides and ketolides	Spiramycin	0.0358	0	0.46	0	2.43	0	0	0	0	0	0	0	0.0358	0.37	1.99
Aminoglycosides	Streptomycin	0.005	0.02	0.06	1.16	0.07	1.29	0.0005	0	0.01	0	0.05	0	0.0255	0.27	0.33
Sulfonamides	Sulfadiazine	0.0924	0.0014	1.18	0.08	2.35	0.15	0.0295	0	0.38	0	0.53	0	0.1233	1.29	2.39
Sulfonamides	Sulfadimidine	0.0053	0.0261	0.07	1.52	0.02	0.48	0.024	0	0.31	0	0.53	0	0.0554	0.58	0.53
Dihydrofolate reductase inhibitors	Trimethoprim	0.0185	0	0.24	0	2.35	0	0	0	0	0	0	0	0.0185	0.19	1.93
Macrolides and ketolides	Tylosin	0.0797	0	1.01	0	8.14	0	0	0	0	0	0	0	0.0797	0.83	6.67
	Total	**0.7107**	**0.0571**	**9.05**	**3.32**	**62.51**	**4.71**	**0.0555**	**0.0001**	**0.71**	**0.01**	**1.64**	**0.15**	**0.8233**	**8.6**	**53.48**

AI = active ingredient; ATI = antimicrobial treatment incidence; KPK = Khyber Pakhtunkhwa; mg/PCU = milligrams per population unit; Pb = Punjab. Units: AI (kg); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days).

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Table S4. Antimicrobial use in calves (winter).

Antimicrobial Class	Antimicrobial	Parenteral						Oral						Total		
		AI		mg/PCU		ATI		AI		mg/PCU		ATI		AI	mg/PCU	ATI
		Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK	Pb	KPK			
Aminopenicillins	Amoxicillin	0.0003	0	0.01	0	0.03	0	0	0	0	0	0	0	0.0003	0.01	0.02
Polymyxins	Colistin	0	0.001	0	0.06	0	2.13	0	0	0	0	0	0	0.001	0.02	0.73
Aminoglycosides	Dihydrostreptomycin	0.0016	0	0.05	0	0.09	0	0	0	0	0	0	0	0.0016	0.03	0.06
Quinolones and fluoroquinolones	Enrofloxacin	0.0334	0.003	1.14	0.18	7.61	1.31	0	0.0025	0	0.15	0	1.1	0.0389	0.84	5.81
Amphenicols	Florfenicol	0.0108	0.0066	0.37	0.39	0.31	0.36	0	0	0	0	0	0	0.0174	0.38	0.32
Aminoglycosides	Gentamicin	0.0009	0.0148	0.03	0.87	0.23	4.1	0	0	0	0	0	0	0.0157	0.34	1.55
Aminoglycosides	Kanamycin	0	0.0098	0	0.58	0	2.13	0	0	0	0	0	0	0.0098	0.21	0.73
Lincosamides	Lincomycin	0.0015	0	0.05	0	0.23	0	0	0	0	0	0	0	0.0015	0.03	0.15
Aminoglycosides	Neomycin	0	0.0098	0	0.58	0	2.13	0.0037	0.0001	0.13	0.01	2.24	0.07	0.0136	0.29	2.22
Tetracyclines	Oxytetracycline	0.0007	0.0045	0.02	0.27	0.07	0.47	0	0	0	0	0	0	0.0052	0.11	0.2
Penicillins	Penicillin G	0.0046	0	0.16	0	0.71	0	0	0	0	0	0	0	0.0046	0.1	0.47
Macrolides and ketolides	Spiramycin	0.0025	0	0.09	0	0.23	0	0	0	0	0	0	0	0.0025	0.05	0.15
Aminoglycosides	Streptomycin	0.014	0	0.48	0	0.27	0	0.0014	0	0.05	0	0.21	0	0.0154	0.33	0.31
Sulfonamides	Sulfadiazine	0.064	0	2.19	0	2.19	0	0.0723	0.0028	2.47	0.17	2.24	0.01	0.1392	3.01	2.92
Sulfonamides	Sulfadimidine	0	0	0	0	0	0	0.079	0.0356	2.7	2.1	2.31	0.32	0.1145	2.48	1.62
Dihydrofolate reductase inhibitors	Trimethoprim	0.0128	0	0.44	0	2.19	0	0	0	0	0	0	0	0.0128	0.28	1.43
Macrolides and ketolides	Tylosin	0.0006	0	0.02	0	0.06	0	0	0	0	0	0	0	0.0006	0.01	0.04
	Total	**0.1477**	**0.0495**	**5.05**	**2.92**	**14.22**	**12.63**	**0.1564**	**0.041**	**5.34**	**2.42**	**7**	**1.5**	**0.3946**	**8.54**	**18.73**

AI = active ingredient; ATI = antimicrobial treatment incidence; KPK = Khyber Pakhtunkhwa; mg/PCU = milligrams per population unit; Pb = Punjab. Units: AI (kg); mg/PCU (mg/kg); ATI (DDDA/1,000 animal-days).

Appendix S2. Data collection tool.

Antimicrobial Use Data Collection Form (Dairy)		
Demography		
A. Correspondent/ Data Collector		
1	Name of correspondent	
2	Designation	
3	Contact number	
4	E-mail address	
5	Education	
6	Experience in related farming (Years)	
B. Farm		
1	Name of Farm	
2	Established (Year)	
3	Address	
4	City	
5	District	
6	Province	
C. Dairy Farm		
1	Type of animal kept	☐ Exotic ☐ Local ☐ Crossbreed
2	Name of breed kept	
3	Type farming operation	☐ Controlled ☐ Semi ☐ Open
4	Herd size (number of animals)	Lactating ____________ Dry ____________ Heifers ____________ Calves ____________ Total ____________
5	Average milk per cow (liters)	

Antimicrobial Use Data Collection Sheet (Dairy)

Date of Record Initiation ___/___/______

Date	Type of Animal				Total	Tentative Diagnosis	Treatment per animal (Brand; Quantity used in ml; Route of administration: IM, SC, IV, IMm, IU, O, Oc)								
تاریخ	جانور کی قِسم				کل جانور	تشخیص	ایک جانور کا علاج (دوائی کا نام، ایک جانور پر استعمال اور دوائی کا راستہ)								
	C	H	L	D			Brand	Dose	Route	Brand	Dose	Route	Brand	Dose	Route
25/03/2020		H			2	Mastitis	Inj. Nugmentan	30 ml	IM	Inj. Penbiotic	2 Vials	IM	Mastijet Forte	2 Tubes	IMm
26/03/2020	C				4	Diarrhea	Inj. Sulphadimidine	5 ml	IM	Scour-X	20 ml	O			
__/__/____															
__/__/____															
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Category: Calves (C), Lactating (L), Heifers (H), Dry Cows (D)

Quantity Used: Bolus (numbers), Injection (ml), Intramammary tubes (numbers), Intravaginal pessaries (numbers), Oral solutions (ml), Powder (mg), Vials (numbers and gm or ml per vial)
Note: Quantities administered in International units must be recorded as IU.

Route Codes: Intramuscular (IM), Subcutaneous (SC) Intravenous (IV), , Intramammary (IMm), Intrauterine (IU), Oral (O), Ocular (Oc)