

Protocol for Antimicrobial Use (AMU) surveillance in dairy animals in Nepal



Government of Nepal

Ministry of Agriculture and Livestock Development

Department of Livestock Services

Veterinary Standards and Drug Regulatory Laboratory

Budhanilkantha, Kathmandu, Nepal

Phone: 01-4650457

Email: vsdrlktm@gmail.com

Website: www.vsdrl.gov.np

April, 2025

Acronyms

AMR: Antimicrobial resistance

AMU: Antimicrobial use

CBS: Central bureau of statistics

CVL: Central Veterinary Laboratory

E. coli: *Escherichia coli*

GPS: Global Positioning System

WOAH: World Organization for Animal Health

WHO: World Health Organization

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Introduction:

Antimicrobials are mostly used for therapeutic purposes, some time for preventive purpose in livestock sector against various ailments. However, sometimes, antimicrobials are also being used as growth promoter in animals (Brown, 2017). The use of antimicrobials has increased with the intensification of livestock farming as the incidences of diseases has increased with intensive production system. Studies have shown that indiscriminate use of antimicrobials in animals have contributed for increased antimicrobial resistance (AMR) in animals and humans (McEwen and Collignon, 2018), hence, it is important to understand this phenomenon under the context of a particular country and prevailing animal management system.

In Nepal also, there are reasons to believe that antimicrobial use in animals have also increased, and it is considered that the use of antimicrobials is related to the degree of intensification in the production system. In Nepal, both cattle and buffaloes are reared as dairy animals and their management has also been changing towards the commercial production system especially around the urban centres, where milk is sold to the urban consumers through organized dairy industry or small-scale milk venders. In the dairy animals, antibiotics are mostly used to treat diseases like mastitis and other bacterial infections and there are reports of resistance against some antimicrobials in the milk samples in the mastitis cases. The recent report of Central Veterinary Laboratory, Kathmandu (2022-23) showed that among the 348 milk samples tested from animals with mastitis, 313 samples (90%) had bacterial growth, of which, *E. coli* were isolated in 128 (36.7%), *Staphylococcus aureus* in 88 (25.2%), *Klebsiella spp.* in 28 (8%) and *Streptococcus spp* in 16 (4.5%) milk samples. Antimicrobial sensitivity test on the milk isolate showed that (95%) *E.coli* were resistant to amoxycillin, (75.5%) resistance to ampicillin and with a lower degree of resistance against other antibiotics. Similarly, *Streptococcus spp* showed highest resistance (80%) against ampicillin, followed by 75% against cefoxitin and at a lower degree against other antibiotics. Similarly, *Staphylococcus spp* showed 41% resistance against cefoxitin, 35.5% against ceftriaxone and a lower degree of resistance against other antibiotics. These data showed that the pathogenic bacteria in animals have developed varying degree of resistance against various antibiotics used for treatment.

Nepal, being a member of the World Organization for Animal Health (WOAH), has an obligation to report AMU in animals annually. Out of three reporting options available in WOAH template, the highest level of reporting, i.e. reporting option three needs information on classes of antimicrobials, group of animals (terrestrial, aquatic or companion) and route of administration. Option one needs information on class of antimicrobials and Option two needs information on class of antimicrobials and group of animals only. Nepal currently reports antimicrobial use in Option three on class of antimicrobials and group of animals and route of administration. This information is mostly obtained through importers and in country manufacturers. The information on antimicrobial use at farm levels is received mostly through the end-user survey/surveillance i.e. from farms or veterinarian's prescription or from the drug retailers. Presently, standardized protocol has not been developed to conduct AMU survey in farm livestock in Nepal, hence, this protocol has been developed to guide the AMU survey in dairy sector in Nepal .

The objective of AMU monitoring is to characterize AMU qualitatively and quantitatively and to compare AMU over time in commercial production systems. It is also to detect non-prudent and unauthorized use of antimicrobials and to monitor national antimicrobial distribution, sales and imports data. Furthermore, it will also help to investigate associations between AMU and AMR and support policymaking to tackle AMR.

General Objective

The objective of AMU monitoring is to characterize AMU qualitatively and quantitatively and to compare AMU over time, between animal species, production types and production systems (description in Annex 1). It is also to detect non-prudent and unauthorized use of antimicrobials and to monitor national antimicrobial distribution, sales and imports data and to contribute investigate the associations between AMU and AMR.

A qualitative characterization includes the use of an antimicrobial agent over a period of time and the purpose of their use. Quantitative characterization consists of providing a measure of the amounts of antimicrobials used, usually divided by a measure of animal biomass. Ideally, the same farms would provide AMU data over time.

The Specific Objectives are

1. To identify and quantify the antimicrobials used in dairy farms.
2. To identify the usage pattern of critically important antimicrobials listed by WOA and WHO in dairy farms.
3. To assess awareness level on antimicrobial use, biosafety security and antimicrobial resistance among dairy farmers.

Survey method:

A combination of qualitative and quantitative methods will be employed to collect information on AMU. This includes conducting an interview during the first visit to the farm to collect qualitative AMU data and then put in place the method for collecting longitudinal data on AMU. Actual antimicrobial use data will be collected in subsequent visits carried out in the farm.

Qualitative Method

For qualitative method, a questionnaire survey (Annex 2) will be used .

Face-to-face interview will be conducted with the farmers using a questionnaire (annex 2) to evaluate the knowledge, attitude and practice regarding dairy farming, antimicrobial use and farm biosecurity. Biosecurity measure adopted in the farm will be observed and recorded during the farm visits.

Quantitative method

For the quantitative approach, bin method will be used to collect quantitative information . A bin will be provided to the farmers and he/she will be asked to put the vials/ strips/package/ sachets of any drugs used then onwards in the bin. Enumerator will visit the selected farms monthly and information and data of packaging materials kept in the bin and treatment record of the farm in the preceding month will be collected by the enumerators. The volume of antimicrobials used and the farm treatment record will be recorded (annex 3).

Data gathered from the quantitative methods will provide information on actual usage of antimicrobials in the selected farms for this study. This information will contribute further to identify related risk factors

associated with AMR in dairy herds under AMR surveillance; generate data and experience that can contribute to establish national monitoring system for AMU in dairy sector; understand variability in estimates between farms, provide quantitative estimates of AMU. It will also identify the trends in AMU in dairy animal production and to provide information to contribute for policy formulation for reducing the health risks associated with AMU in dairy animals.

Farmers participation in the survey will be voluntary and the objective of the survey will be explained to him/her in greater details. Because, the cooperation of the participating farmers is crucial for the success of the program, their voluntary engagement in the whole process is crucial. Farmers will be provided with bins with the label clearly stating in Nepali “to put the vials/strips/package of any drug used in the farm as well as the treatment record carried out during the month”. They will be asked to throw the used antibiotic packets/containers/strips/sachets into the dedicated bin. The bins will be placed in a safe place hanged in the wall at a suitable height and visible to the farm workers. The enumerator visiting the farm each month will record the details of the use of veterinary medicines during the previous month. He/she will then check and collect the discarded medicine wraps and bottles collected in the bin, treatment record of previous month and replace the empty bin again at the same location for collection of used items until next visit. Enumerator will then enter data in a standard format and calculate active ingredient (in mg) within each antibiotic class. The enumerator will inquire about the type of feed used in the week and if the package is available, collect the composition of feed to check for any use of antimicrobials in the feed. The enumerators will also observe and collect data on self- mixing of antimicrobials in feed by the farmers by observing the packaging materials of the mixtures in the provided bins.

With the combination of quantitative and qualitative methods, following information will be collected;

- The list of antimicrobials used in the farm for treatment, prevention and other purposes
- Volume of different classes of antimicrobials used in the dairy farms
- Use of antimicrobials that are listed as critical group by WHO and WOA
- Rank list of antimicrobials in order of quantity and frequency of use (highest amount)
- Purpose of antibiotic use – prophylaxis, treatment
- Routes of administration of the different Antimicrobials
- Stage of production cycle of the dairy animal when specific antibiotics were used
- Record of withdrawal periods
- Source(s) of procurement of antimicrobials and name of veterinary service provider
- Farmers’ Knowledge, and Practices (KAP) related to AMU in dairy animals.

Recommended AMU parameter

1. Output parameters:

Milligrams of Active Antimicrobial Ingredient (AAI) per Kilogram of live animal weight of the dairy animals (mg/kg) and within antibiotic classes over one year period, separate into route of administration i.e. feed, water, injection and others, if any route.

Total active antimicrobial ingredient used (in mg)

Denominator:

Average number of dairy animals treated within a specified time period (one year period) multiplied by average kg bodyweight per animal or average final herd weight. Take population at beginning and end of the time period and take their average or take the number that died during the production cycle (if known) and subtract from initial population.

Take average weight at beginning and end of one year cycle and average across the time period to give an average weight in kg. Take average weight at the end of one year to be recorded as final herd weight.

Sampling frame for AMU surveillance and Data Collection:

Because, the incidence of diseases in the animals would be higher in large commercial herds due to the higher concentration of pathogenic organism in the environment of large herds, which will necessitate the use of antimicrobials more frequently. The higher frequency of antimicrobials use and inaccurate dosing are the factors which will result in AMR in the microbial population. Hence, the larger commercial herds would be the ideal sampling frame for AMU surveillance. Hence, AMU surveillance will be carried out in commercial herds, identified by Commercial Livestock Integrated Survey carried out by CBS and MOALD (CBS 2021), which, recorded the number of commercial farms of dairy cows and buffaloes as 1233 and 914 respectively, while 483 were mix farms of cows and buffaloes (Table 1). The criterion for commercial farms was having a minimum of 10 milking cows in Terai and hills and 8 milking cows in the mountains, while, for buffaloes, it was eight milking animals in hills and Terai and 5 milking buffaloes in the mountains. The number of commercial farms with cow and buffalo population (including the milking animals) in these commercial farms was reported to be as below:

Table 1: Number of commercial dairy farms and dairy animal population in the provinces

Province	Number of farms		Number of animals		Number of milking animals		Number of mix farms ((cow and buffaloes) farms)
	Cows	Buffaloes	Cows	Buffaloes	Cows	Buffaloes	
Koshi	351	81	8875	2811	2376	556	45
Bagmati	277	109	9364	3432	4195	1243	59
Madhesh	129	74	4834	2618	1551	936	73
Gandaki	243	227	8070	11469	3261	4160	126
Lumbini	198	332	8442	10597	2946	2931	149
Karnali	25	47	3013	2096	490	531	6
Sudurpaschim	10	44	2274	1630	561	484	25
Total	1233	914	44,872	34,653	15,380	10,841	483

(Source: CBS, Commercial Livestock Integrated Survey, 2021)

The data in the above table showed that Koshi, Bagmati, Madhesh, Gandaki and Lumbini has more commercial farms, while Karnali and Sudurpaschim has very few commercial farms. It is also considered that, the commercial farms would be located near to the urban centres because of the accessibility for milk marketing to the urban population and to the milk processing factories.

It is proposed that, AMU monitoring in dairy animals be carried out in two phases. In the first phase, the AMU monitoring be carried out in the selected commercial dairy farms in the selected production pockets of the selected districts of Bagmati and Koshi province, where, commercial dairy farming has been more advance and milk marketing system is well developed. Based on the learning of the first phase, the second phase of AMU study be carried out in the commercial farms of rest of the country. This approach will be easier to implement, manageable and cost effective.

In the Koshi province, commercial farms of Morang, Sunsari, Jhapa and Ilam be included in the cluster for AMU surveillance because of the easier accessibility, more advance commercial dairy farming and well advanced milk marketing system, while, in the Bagmati province, Kathmandu, Chitwan, Dhading and Kavre districts would form the other cluster of districts. Based on the learning of the first phase, the second phase of AMU study be carried out in the commercial farms of rest of the country. This approach will be easier to implement, manageable and cost effective.

It is because of the assumption that, the commercial farms are managed in the periphery of the commercial market centers or in the catchment of milk collection centers with intensive management of animals requiring high use of external inputs including the antibiotics. Thus, the farms in these clusters would be the ideal sampling units for AMU monitoring in dairy herds of both cattle and buffaloes.

A qualitative characterization will include the use of antimicrobial agent over a period of time and the purpose of their use, while, quantitative characterization will provide a measure of the amounts of antimicrobials used, divided by a total of animal biomass for a period of one year.

Sample size:

AMU monitoring is a longitudinal study with repeated measures on the same farm for a defined period of time (12 months), the sample size required for such studies has been suggested to be 90 farms (at 90% confidence interval) and 285 farms (at 95% confidence interval) in a population size of 1000 farms (Guo *et al*, 2013). The total number of commercial farms (including the mix farms of cows and buffaloes) in Bagmati and Koshi province (CBS, 2021) are found to be 922 (477 farms in Koshi and 445 farms in Bagmati), thus a sample size of 90 farms would be sufficient for year-long monitoring. Hence, it is proposed that, 90 farms from both provinces (45 each from both provinces) be identified and included for monthly monitoring for a year. Although, random selection of individual farms is ideal, however, other factors like accessibility, long term cooperation of farmers and their willingness to participate in the long-term study are most important criterion, hence, it is proposed that randomization should be done on the identified, accessible and cooperative farmers only, because, farmers cooperation and willingness and commitment to remain in the monitoring program and to store/provide used vials, bins and treatment record are most important factors for the success of the program. The sample size proposed in the monitoring should be enough as similar studies with lesser sample size (38 dairy herds) has been carried out in Punjab, India (Deepthi *et al* 2023) with much larger commercial dairy livestock population. Similarly, Rees (2021) has also suggested a sample size of 100 farms be adequate for AMU monitoring. In the succeeding year, the dairy farms in the remaining provinces should be monitored with proportional representation in the samples according to the number of farms in the provinces respectively. The differences in AMU in buffalo and cow farms could also be illustrated when mix farms of cattle and buffalo farms are included in the study.

Data collection:

Both qualitative and quantitative data will be collected by the enumerators during their monthly farm visits. The data on antimicrobial use will be collected for a calendar year/lactation period of dairy animals. In the first visit, the enumerator will generate qualitative data by filling the questionnaire with farm observation, reviewing the record book/s and with farm owner interview. A farm animal treatment register with the defined format for recording the treatment provided in the farm will be recorded by the veterinarian/animal health service providers following their visit to the farm for farm animal treatment. The treatment record will include all details of the treatment provided (name of the medicine, dose, duration of treatment, tag no of the treated animals, tentative diagnosis of the ailment, name, mobile no and signature of animal health service provider). In the first visit, the farm will be provided with a bin to collect the used vials/sachets and other wrappings of the medicines used. In subsequent visits at monthly interval, the bin will be emptied and the amount of antimicrobials used will be recorded. Furthermore, the farm owner/manager will be interviewed about the details on antimicrobial use in the farm during the previous month.

In addition, the AMU data could also be collected from the nearby agrovets and veterinary practitioners (vets and para-vets and VAHWs) if they are providing the veterinary services to these farms, which should include the number of treatments provided and amount of AMU in the farm.

The filled survey questionnaires should be thoroughly examined and checked for any human error. Data will be digitalized by entering it manually into a database. The online data entry will be done using kobo toolbox.

Data Quality Control:

Field enumerators and their work will be supervised and monitored by the team leader (the responsible technical person). Government officials from Department of Livestock Services and Veterinary Standards and Drug Regulatory Laboratory will monitor the enumerators' work in fields to check the quality of work. Feedback will be provided regularly to the field teams to support data quality assurance. Data consistency will be monitored regularly during the data collection process. The team leader will be responsible for data analysis and report submission.

Data use and dissemination of findings:

A summary report of survey findings will be written, and results will be disseminated to all relevant stakeholders via dissemination workshop and report.

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Annex-1 Glossary

Animal species: cattle and buffalo

Production types: Dairy production

Production system: Commercial farms (stall-fed)

Annex 2. Questionnaire for AMU in dairy animals:

Date: ____ / ____ / ____ Name of data collector: -----

Survey objectives

This survey is being conducted to understand the pattern of antimicrobial use (AMU) in dairy farms, to assess the farmer's level of knowledge on antimicrobial resistance (AMR) and biosecurity measures being implemented in the commercial dairy farms of district of Nepal for the Department of Livestock Services.

The information provided will remain strictly confidential and will be used only for the situation assessment purposes. Your answers to the questions will not be revealed to anyone and will remain confidential. If you feel uncomfortable to respond to any questions you can choose not to answer or withdraw from the study. Findings from this study will help Government of Nepal to evaluate the type and trend of antimicrobials being used in dairy sector. This analysis shall only be done at group level. Your participation is voluntary.

Thank you for your participation.

Part A: Demographic and Socio-economic information

Demographic:

1. Location: Province..... District----- Rural municipality/Municipality-----
-

Ward no..... Village/Tole: ----- GPS coordinates.....

2. Name of farmer:

3. Age of farmer (in years) -----

4. Number of family members: (please mention)

3. Sex: (Please tick): Male/Female/Others

4. Other attributes:

5. Education:

6. Main Occupation:

7. Economic Status:

Farming Characteristics:

8. How long have you been in the dairy farming?years

9. Did you have some formal trainings for this sector:

10. How many members of your family are engaged full time in dairy farming:

12. Are people on paid employment in your farm? A) Yes B) No

13. If yes, how many people are employed?

14: How much land do you use for dairy farming?

15. Self-owned- Rented:

16. What other animals do you have in your farm besides dairy animals? (mention numbers) type of animals and numbers (table) lactating or not lactating

17. Who do you obtain advice on managing health of your dairy animals? (Please mention)

18. Do you vaccinate your dairy animals?

19. If yes, which vaccines and when?

20. Where do you purchase medicines for your farm? (Tick mark to all applicable)

- | | | |
|---|------------------------|-----------------------|
| a. Feed suppliers | b. Agro-vet shops | c. Vet. clinics |
| d. Vet drug distributors | e. Vet. drug stockist | f. Import from abroad |
| g. Market across the border | h. Other farmers/farms | |
| i. Government institution (vet hospital etc.) | | |

21. How far away is your main veterinary drug supplier from your farm? (Please circle one)

Less than 1 km 1-5 km 5-10 km More than 10 km

22. Do you use a veterinarian's prescription when buying antibiotics?

Always Sometimes Never

23. How do you market your dairy produce?

Part B: Farm size and farm animal population:

Farm size (total): Bigha/Ropani:

Area under fodder/forage cultivation:

Do you apply poultry manure in your farm?

How much Fodder/forage do you harvest per day (in MT):

How much hay/silage you produce per year:

Population of dairy animals in the farm:

Species	Adult	Young	Milking	Dry
Cattle				
Buffaloes				

Average body weight of adult animals (measuring tape method): Cows: Buffaloes:

Milking system: Hand/machine

Calves/heifer (below 18 months): Cows: Buffaloes:

Housing system: Tethered/Open

Floor type: Cement/Mud/Rubber mat

Veterinary service obtained from: veterinarian/JT/JTAs/VAHW/None

Do you have contract with veterinary service providers? Yes/No

If yes, who is the service provider: Name, address, mobile no:

Number of farm labor:

Type of labor contract: Permanent/daily wages

Part C: Antimicrobial use

a. Antimicrobial general:

1. Who is responsible for decisions on using medicines in the dairy farm (please mention main responsible person):

2. Do you use following medicines in your dairy farm? (Tick one option for each Response)

(Product group) Yes No Don't Know

Antibiotics

Feed supplements

Probiotics

Minerals

Other medicines

3. Why do you use these medicines?

Medicine name	Treatment of illness	Stress reduction	Don't Know

4. Where do you buy the veterinary medicine from: Agroveter/vet clinic/vet practitioner

5. Name of veterinary medicine supplier:

6. How do you administer the following medicines in your dairy animals?
(Tick all options that apply for each product group)

Medicine type Injection In feed Intra- mammary Water Don't know

Antibiotics

Feed
supplements

Minerals

7. Are antibiotics medicines easy to obtain?

- a. Yes
- b. No
- c. Depends upon medicine

8. When using antibiotics to treat sick animals, for how long do you treat the animals? (Please tick one)

- Until disease symptoms are over
- For recommended period
- Until the purchased quantity has run out
- Don't know

9. Where do you store your antibiotics?

- Inside feed store room
- Separate drug storing place
- In the freezer
- Other places

10. What do you do with left over antibiotics after completion of the treatment regimen? (Please tick one)

- Continue treatment until the drug is finished
- Store for next use
- Dispose off from the farm
- Give to neighbor farmer
- Mix in the feed

11. How do you dispose off the remaining (remaining unused) antibiotic from farm? (Tick to all applicable)

- Bury in the ground
- Throw into water
- Throw in trash for dumping
- Burn

12. Do you think the antimicrobial you use in your dairy animals are effective?

1. Yes 2. No 3. Do not Know

13. If less effective, why are they less effective these days? (Please tick)

- Due to frequent use and overuse
- Wrong antibiotic prescribed
- Resistance developed by the pathogen/Pathogen are no more susceptible
- Poor quality of antibiotics
- Do not know

13. Person responsible for providing veterinary care: vet doctor/JTA/VAHW

Name of veterinary service provider:

Mobile No:

Since when he/she providing this service:

Antimicrobials procured from:

Part D: Antimicrobial Specific

1. What are the most commonly used measures for prevention/treatment/growth promotion for your farm animals, please, specify maximum 5 from most frequently used to least frequently used.

For disease prevention	For treatment of diseases	For growth promotion

2. What are the most commonly used antimicrobials (treatment or prevention) in your farm., Please specify maximum 5 from most frequently used to least frequently used.

a.
b.....
c.....
d.....
e.....

2. Do you know that antibiotic residues in dairy products can affect human health?
3. Do you sell milk while the animals are getting treatment or immediately after treatment? Yes/No
 - a. Have you heard of antibiotic resistance? Yes b. No
 - b. Have you heard of with drawl period for antibiotics? Yes/No
 - c. If yes, do you think it will be possible to practice for you in your farm? Yes/No
4. Do you think Anti-microbial Resistance (AMR) in bacteria carried by dairy animals can spread to humans?
 - a. Yes b. No
5. Have you heard that humans can get diseases from animals?
 - a. Yes b. No

Annex 3. Quantification of antimicrobials used.

A. Quantification of used antimicrobials vials/boxes/sachets present in the collection bins:

Date	Antibiotic class	Amount used(mg)	Purpose

B. Farm Animal Treatment Record Register:

Date	Species	Tag no	Age (months)	Weight (average)	Production status	Diagnosis of illness	Treatment provided			Response of treatment	Name and mobile no. of treatment provider	Remarks
							Medicine	Dose	Duration			