National Plan for Antimicrobial Residue Monitoring in Animal Products

Department of Livestock Services

Ministry of Agriculture and Livestock Development

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Introduction

The use of antimicrobials in animal sector has become an indispensable component of modern livestock production, especially with the increasing demand for the animal-derived protein and the rapid industrialization of the animal sector (Van Boeckel et al., 2019; Xu et al., 2020; Umair et al., 2021 OECD/FAO, 2023). Globally, antimicrobials are commonly used in animal production for both therapeutic and prophylactic purposes. The use of antimicrobials in animal production needs to be in compliance with standard treatment guidelines and recommended withdrawal periods; the absence of which may lead to misuse (overuse, underuse or repeated use) and result in the presence of antimicrobial residues in foods of animal origin. Consumption of such food products with antimicrobial residues exceeding the permitted safety limits may lead to several adverse effects such as allergic reactions, disruption of gut microbiota and even the development of antimicrobials resistant bacteria, contributing to the global health threat of antimicrobial resistance (Wang et al., 2016; Bacanlı, 2024).

In response to this growing concern, a quadripartite collaboration of the World Health Organization (WHO), Food and Agricultural Organization (FAO), the World Organization for Animal Health (WOAH) and the United Nations Environment Program (UNEP) launched the Global Action Plan (GAP) on AMR in 2015. This plan emphasizes five strategic priorities, including the optimization of antimicrobial use (AMU) in animal health and the strengthening of surveillance systems for AMR and antimicrobial residues in the food chain.

As a signatory member state of this global agenda, Nepal has developed and endorsed its own National Action Plan (NAP) on AMR (2024-2028), aligning with the One Health approach to address AMR across human, animal and environmental health sectors. One of the key priority areas under the NAP-AMR is to monitor antimicrobial residues in food of animal origin, ensuring food safety and protecting public health. Veterinary Standards and Drug Regulatory Laboratory (VSDRL) under the Department of Livestock Services (DLS) is a national reference laboratory for the quality control of veterinary drugs and for monitoring antimicrobial residues in animal products such as meat, milk, and eggs. Currently, VSDRL, Veterinary Public Health Section of Central Veterinary Laboratory (CVL) and Veterinary Laboratories (VLs) under CVL conduct testing for antibiotic residue in meat, milk, eggs, and fish.

This national plan thus aims to provide guidance for sample collection, laboratory testing, and data analysis to identify potential risk areas, assess compliance with Maximum Residue Limits (MRLs), ensure safety of animal products and support evidence-based decision-making for quality production of animal products.

Scope

This document covers priority commodities including poultry (meat and eggs), buffalo (milk and meat), cattle (milk), pigs and goats (meat), and fish (muscle), with a focus on commonly

used veterinary antibiotics of public health concern. Only raw animal products will come under the scope of this framework. This monitoring plan adopts a risk-based, purposive sampling approach across all provinces, with laboratory testing using screening and confirmatory methods. The plan assesses compliance with national Maximum Residue Limits (MRLs) or Codex Alimentarius standards in case where national standards are not available. This plan aims to generate evidence for regulatory action, policy development regarding the prudent use of veterinary antibiotics in livestock, poultry and fisheries.

Objectives

General: To establish a system for monitoring antimicrobial residue in food of animal origin.

Specific:

1. To detect and/or quantify antimicrobial residues in product of animal origin.
2. To monitor the trend of residues over the time in animal products across different production sectors and system.
3. To support in policy decision making on rational use of antimicrobials.

Sampling Plan

1. Sampling Strategy

A risk based and purposive sampling is adopted to select product/production system/commodities for testing. All types of production systems and practices in all provinces are considered eligible for sampling. However, samples will be selected based on risk of use of antimicrobials, volume of products being consumed, and market access of the product. Commodities are thus selected based on the high risk of containing antimicrobial residues and their significance in human consumption. (Table 1)

Table 1: Production sectors and commodities targeted for residue monitoring

Production Sector	Commodity
Poultry	Meat and Eggs
Buffalo	Milk and Meat
Cattle	Milk
Pig	Meat
Goat	Meat
Fish	Muscle

Residue monitoring shall be conducted annually for milk, eggs, and poultry meat. Alternately, buffalo meat and pork shall be tested in the first year, followed by goat meat and fish in the second year, and so on in an alternating pattern. However, selection of commodities will be revised based on the priorities set by DLS and the annual program.

2. Sample size and sampling site

Sample size for the annual national residue monitoring shall follow the sampling scheme outlined in Annex 1. The sample size is estimated based on previous non-compliance data for the presence of antimicrobial residue in the chicken as 1%. According to the Codex Alimentarius guidelines, the 1% non-compliance at 95% confidence interval requires 299 samples to be considered for the test. Following this, the sample size has been defined to be 300. The sample size will be the same (i.e. 300) for all the commodities. Specimen shall be collected from predefined sampling sites, and the proportion of samples required per province, from high-risk districts, is detailed in Annex 1.

3. Sampling frequency

Specimens shall be collected throughout the year, considering seasonal variations and farming practices. Specimens shall be collected by laboratory personnel of the testing laboratories (VSDRL, CVL, VLs). Additionally, specimens collected and sent by Veterinary Hospital and Livestock Service Expert Center (VHLSEC)/ district level livestock service offices shall also be included.

4. Type of specimens

Specimens shall include animal tissues and products of public health concern where residues are likely to occur. The type of specimen to be collected may vary depending on the antimicrobial agents being tested. Specimen types are determined based on the MRLs value for each antimicrobial as specified in "National Microbial standard for Meat, Milk, Egg and MRL of veterinary Drugs (chapter IV)" published in Standard Approved by DLS. If national MRL is not available, Codex Alimentarius standards will be used as a reference for determining the type of specimen. Additionally, beyond the stated sampling protocol, if there is a need to test an animal or animal product suspected of exposure to antimicrobial agent, specimen collection can be carried out without any restrictions.

A minimum of 50 g per sample for meat and muscle, 30 mL per sample for milk and 3 eggs per sample are required for screening and confirmation of at least 3 antimicrobial substances. At least two meat samples per shop shall be collected.

5. Specimen collection and transport

For specimen collection, following protocol shall be followed.

- Milk: From individual farmers before delivery/submission to cooperatives.
- Animal tissue and fish: From fresh houses, collection centers.

- Eggs: From retail shops or directly from poultry farms.

All specimens shall be collected by laboratory personnel in:

- Clean tissue collection jar or sealed plastic bags (for tissue specimens)
- Bottles or tubes (for milk specimens)
- Sealed plastic sample bags (for egg specimens)

Specimen labelling: Specimens shall be clearly labelled with a pre-determined sampling number or using a permanent marker, immediately after collection. The details of the specimen shall be maintained in a record sheet. The details must include:

S.N	Details	Remarks
1	Specimen	raw meat/milk/egg/fish muscle
2	Specimen number	
3	Collection location	including the province, district and the name of the slaughterhouse/farm/cooperative/retail shop
4	Contact number	
5	Date of specimen collection	
6	Specimen type	Only for meat specimens- for example muscle/liver/fat/kidney
7	Animal species	Only for meat and milk specimens

Specimen storage, packaging, transportation and traceability: Specimens must be stored in a cool box at 2-8^{0}C immediately after collection and transported to the laboratory as soon as possible. If immediate dispatch of the specimen is not feasible, the specimens must be stored frozen at -20^{0}C, except for egg specimens, which shall be stored at 4-8^{0}C.

For transportation, specimens must be securely packaged following the principles of triple layer packaging i.e. primary leakproof packaging, secondary leakproof packaging and an outer packaging to protect inner contents. Each sample should be individually packaged in its primary package to avoid cross contamination between samples. Cool packs must be placed with samples to maintain the required temperature throughout the transport.

The record sheet with details of the sample should be placed together (but not inside) the sample package, to ensure the traceability at every stage of the process.

Laboratory analysis

1. Antimicrobials to be tested will be prioritized and selected based on the class of antimicrobials commonly used in animals along with public health importance, as listed on Annex 2.

2. Test for residue in different samples can be done using both screening and confirmative test.
3. Screening test with sufficient selectivity and sensitivity can be used to separate the samples negative for the presence of antimicrobials of concern, but the confirmatory tests shall be done to ensure reliable detection of residues.
4. Different detection methods such as bacterial inhibition test, enzymatic tests, enzyme-linked immunosorbent assay (ELISA), lateral flow immunosorbent devices, different other immunoassay methods, liquid chromatography methods with ultra-violet (UV) and diode array (DAD), mass spectrometry can be used based on the availability, however, ELISA will be used as a routine test for residue monitoring.
5. Confirmation with quantification of positive samples shall be conducted using HPLC or more advanced method at VSDRL, wherever possible.

Test SOPs should be established in the laboratory for testing the antimicrobial substances of concern.

Data management and reporting

1. Data management: All data shall be stored in electronic format in the laboratory information management system (LIMS). Backup for the data shall be maintained in Microsoft Excel and Google sheets. For the accuracy and consistency in data management and verification, a qualified official shall be designated by the concerned laboratory. The data collected in the system will be analyzed using indicators of veterinary and public health importance and disseminated at least annually in annual bulletin of the laboratory. VSDRL shall maintain the records of these reports.
2. Analysis and Dissemination of results: The result of test shall be reviewed in Antimicrobial Use-Technical Working Group and appropriate measures shall be recommended. The results can also be shared with broader stakeholders through scientific publications or public consultations after the approval of DLS.
   The non-compliant results (samples that cross MRLs) shall be notified to the relevant competent authority.

Laboratory network for testing antimicrobial residues

VSDRL shall serve as the reference and quantify laboratory for antimicrobial residue testing. The veterinary public health section of the CVL will simultaneously test for antimicrobial residues as per the national residue surveillance plan. All the positive samples in screening shall be sent to VSDRL for quantification.

Annex I:

Commodity-wise sample size, provincial allocation of samples, and predefined high-risk districts for antimicrobial residue monitoring.

Commodity	Chicken	Buffalo	Pork	Chevon	Fish	Milk	Egg
Sample Size	300	300	300	300	300	300	300
Koshi*	12.86	11.70	43.67	22.25	11.16	19.18	6.61
High Risk Districts	Sunsari Morang, Jhapa, Panchthar, Udaypur	Sunsari, Khotang, Morang, Udaypur, Okhaldhunga	Morang, Jhapa, Sunsari, Panchthar, Illam	Morang, Jhapa, Udaypur, Sunsari, Illam	Morang, Sunsari, Jhapa	Morang, Sunsari, Jhapa, Illam, Udaypur	Morang, Jhapa, Sunsari, Udaypur, Dhankuta
Madhesh*	15.06	18.82	1.60	10.82	57.66	16.41	4.38
High Risk Districts	Saptari, Dhanusha, Siraha, Parsa, Sarlahi	Sarlahi, Siraha, Dhanusha, Mahottari, Rautahat	None	Sarlahi, Rautahat, Saptari, Mahottari, Siraha	Bara, Dhanusha, Siraha, Saptari	Sarlahi, Saptari, Mahottari, Dhanusha, Bara	Parsa, Dhanusha, Sarlahi, Saptari
Bagamati*	38.15	17.69	12.35	16.34	6.45	17.95	55.52
High Risk Districts	Chitwan, Kavre, Dhading, Nuwakot, Sindhuli, Makwanpur	Dhading, Chitwan, Kavre, Nuwakot, Makwanpur, Sindhuli	Sindhuli, Dhading, Kavre, Kathmandu, Makwanpur,Chitwan	Sindhuli, Makwanpur, Kavre, Chitwan, Nuwakot	Chitwan, Sindhuli, Makwanpur	Chitwan, Kavre, Dhading, Makwanpur, Nuwakot, Sindhuli	Chitwan, Kavre, Makwanpur, Dhading, Lalitpur
Gandaki*	12.00	10.78	8.10	11.60	1.84	9.53	9.29

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High Risk Districts	Kaski, Gorkha, Syangja, Lamjung	Gorkha, Baglung, Kaski, Tanahu, Syangja, Lamjung	Nawalpur, Tanahu, Kaski, Syangja, Gorkha	Gorkha, Tananhu, Kaski, Syangja	Nawalpur, Kaski	Nawalpur, Kaski, Tanahu, Syangja, Baglung, Gorkha	Kaski, Nawalpur, Tanahu, Gorkha, Baglung
Lumbini*	12.34	23.51	19.20	18.30	18.93	19.49	14.21
High Risk Districts	Banke, Dang, Palpa, Rupandehi, Nawalparasi west	Rupandehi, Banke, Kapilvastu, Arghakhanchi, Dang, Gulmi	Dang, Banke, Bardiya, Palpa, Rupandehi, Rukum east	Dang, Banke, Rupandehi, Arghakhanchi	Rupandehi, Kapilvastu, Bardiya, Parasi	Banke, Rupandehi, Bardiya, Kapilvastu, Palpa, Arghakhanchi	Dang, Banke, Bardiya, Palpa, Rupandehi
Karnali*	2.59	4.52	6.45	11.26	0.15	4.52	2.69
High Risk Districts	Surkhet, Salyan, Rukum west, Dailekh	Surkhet, Jajarkot, Salyan, Rukum west	None	Salyan, Surkhet, Dailekh, Rukum west	None	Rukum west, Surkhet, Salyan, Kalikot, Jajarkot	Rukum west, Surkhet, Jajarkot, Dailekh, Salyan
Sudur paschim*	7.00	12.97	8.63	9.42	3.81	13.19	7.31
High Risk Districts	Kailali, Kanchanpur, Doti, Dadeldhura	Kanchanpur, Kailali, Baitadi, Dadeldhura, Doti	Kailali, Kanchanpur	Kailali, Kanchanpur, Achham	Kailali, Kanchanpur	Kailali, Kanchanpur, Baitadi, Doti	Kailali, Kanchanpur, Doti, Achham

Note: * indicates proportion of total samples

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Annex II:

Prioritized antibiotic classes by production sector and commodity according to public health importance.

Production Sector	Commodity	Antibiotic Class	AWaRe Classification
Poultry	Meat and Eggs	Penicillins, Tetracyclines, Aminoglycosides, Sulfonamides, Ist generation Cephalosporin	Access
		Fluoroquinolones, 3rd and 4th generation Cephalosporins, Macrolides, Phenicols	Watch
		Polymixins	Reserve
Buffalo	Milk and Meat	Penicillins, Tetracyclines, Aminoglycosides, Sulfonamides	Access
		Fluoroquinolones, 3rd generation Cephalosporins	Watch
Cattle	Milk	Penicillins, Tetracyclines, Aminoglycosides, Sulfonamides	Access
		Fluoroquinolones, 3rd generation Cephalosporins	Watch
Pig	Meat	Penicillins, Tetracyclines, Aminoglycosides, Sulfonamides	Access
		Fluoroquinolones, 3rd generation Cephalosporins	Watch
Goat	Meat	Penicillins, Tetracyclines, Aminoglycosides, Sulfonamides	Access
		Fluoroquinolones, 3rd generation Cephalosporins	Watch
Fish	Muscle	Tetracyclines, Sulfonamides	Access
		Fluoroquinolones	Watch

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