Wildlife Conservation Society

# Framework and Guidelines for Passive Surveillance of Zoonotic Pathogens in Wildlife.

## Coordination, Protocols, and Health Event Notification Systems.

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## Executive Summary

This document presents a comprehensive framework for passive surveillance of zoonotic pathogens in Bolivia, emphasizing the need for structured monitoring, early detection, and intersectoral coordination to address zoonotic threats associated with wildlife trade, consumption, and traditional practices. Given Bolivia's rich biodiversity and high-risk human-wildlife interactions, a robust surveillance system is essential for identifying, reporting, and mitigating spillover events that could impact public and animal health.

The framework outlines standardized methodologies for passive surveillance, ensuring uniform data collection, analysis, and reporting across human, animal, and environmental health sectors. It provides strategic recommendations on stakeholder coordination mechanisms, data integration, and disease notification workflows to enhance Bolivia's preparedness for emerging zoonotic threats. A key focus is placed on the surveillance of high-risk wildlife species such as primates (Alouatta spp., Ateles spp.), jaguars (Panthera onca), and armadillos (Dasypus novemcinctus), which are known reservoirs of zoonotic pathogens.

A risk-based approach is applied to prioritize surveillance of key zoonotic diseases, including rabies, brucellosis, leptospirosis, salmonellosis, and toxoplasmosis. The document details procedural steps for health event notification and response, ensuring timely intervention and containment of outbreaks. Additionally, it integrates GIS-based risk mapping, remote sensing technologies, and digital reporting systems to strengthen Bolivia's national surveillance infrastructure.

The framework aligns with international best practices, drawing from the World Organisation for Animal Health and the WHO Recommended Surveillance Standard. This framework is also supported by scientific publications and a WCS technical report *"Zoonotic risks associated with the trade in wild animals: pet ownership, meat consumption and traditional medicine in Bolivia*." Altogether, ensuring that Bolivia's passive surveillance system is compatible with global health security initiatives.

This document serves as a technical resource for policymakers, researchers, and stakeholders, providing actionable strategies for reducing zoonotic disease risks, enhancing One Health integration, and safeguarding Bolivia's public and wildlife health through sustainable and adaptive surveillance measures.

## Table of Contents

*Executive Summary ... 1*

*1. ACRONYM ... 3*

*2. INTRODUCTION ... 4*

*SECTION 1: ESTABLISHING A FRAMEWORK FOR A PASSIVE SURVEILLANCE NETWORK AND COORDINATION MECHANISMS ... 5*

1. Stakeholder coordination mechanisms. ... 5
   - 1.1. National Coordination ... 5
   - 1.2. Local Coordination ... 6
   - 1.3. International Collaboration ... 7
2. Framework for passive surveillance ... 10
   - 2.1. Data sources and integration ... 10
   - 2.2. Remote Sensing and GIS Applications ... 13
   - 2.3. Technology and Data Management. ... 15

*SECTION 2: RECOMMENDATIONS ON KEY ZOONOTIC PATHOGENS AND PROTOCOLS FOR THEIR SURVEILLANCE. ... 16*

1. Key zoonotic pathogens and passive surveillance protocols. ... 17
   - 1.1. Passive surveillance for Rabies ... 17
   - 1.2. Passive surveillance for Brucellosis ... 19
   - 1.3. Passive surveillance for Leptospirosis ... 23
   - 1.4. Passive Surveillance for Salmonellosis ... 27
   - 1.5. Passive Surveillance for Toxoplasmosis ... 31

*SECTION 3: RISK ANALYSIS AND MANAGEMENT STRATEGY THAT IDENTIFIES THE MOST EXPOSED INSTANCES, THE PROCESSES WITH THE GREATEST RISK, AND STRATEGIES TO MITIGATE THESE RISKS. ... 35*

1. Identification of the most exposed Instances for Zoonotic Disease Transmission in Bolivia. ... 35
2. High-Risk Processes and Factors for Zoonotic Disease Emergence in Bolivia ... 36
3. Risk Management Strategies for Zoonotic Disease Prevention in Bolivia ... 37
   - 3.1. Regulatory and Policy Measures ... 37
   - 3.2. Surveillance and Monitoring ... 38
   - 3.3. Public Awareness and Behavioral Change ... 38
4. Risk Mitigation Strategies ... 39
5. Implementation Plan and Stakeholder Roles ... 40
6. Recommended Monitoring and Evaluation Framework for Zoonotic Disease Prevention in Bolivia ... 40
   - 6.1. Key Performance Indicators (KPIs) ... 41
   - 6.2. Annual Risk Assessments ... 42
   - 6.3. Community Engagement Metrics ... 42

*SECTION 4: MONITORING AND EVALUATION ... 43*

*SECTION 5: KEY CHALLENGES AND CONSIDERATIONS ... 43*

1. Geographic Diversity and Regional Adaptation ... 43
2. Data Reliability and Community Engagement ... 44
3. Sustainability and Institutional Strengthening ... 44

*REFERENCES ... 44*

## 1. ACRONYM.

| | |
|---|---|
| **DGBAP**: | Dirección General de Biodiversidad y Áreas Protegidas |
| **INIAF**: | Instituto Nacional de Innovación Agropecuaria y Forestal |
| **INLASA:** | Instituto Nacional de Laboratorios de Salud |
| **INS**: | Instituto Nacional de Salud |
| **MMAyA**: | Ministerio de Medio Ambiente y Agua |
| **ORAS-CONHU:** | Organismo Andino de Salud - Convenio Hipólito Unanue |
| **POFOMA:** | Policía Forestal y de Medio Ambiente |
| **REDES** | Red de Salud |
| **SENASAG:** | Servicio Nacional de Sanidad Agropecuaria e Inocuidad Alimentaria |
| **SIIS** | Sistema Integral de Información sobre Salud |
| **SINVET:** | Veterinary Surveillance Information System |
| **SIVE**: | Sistema de Vigilancia Epidemiológica |
| **SNIS**: | National Epidemiological Surveillance Information System |
| **UAGRM:** | Universidad Autónoma Gabriel René Moreno |
| **UMSA:** | Universidad Mayor de San Andrés |

## 2. INTRODUCTION

The interconnectedness of human, animal, and environmental health underscores the importance of monitoring zoonotic pathogens [1]. In this context, Bolivia faces unique challenges due to its high biodiversity, cultural practices involving wildlife, and a significant illegal wildlife trade [2,3]. Highlighted species such as jaguars, primates, and reptiles being on high demand create potential hotspots for zoonotic disease transmission. This underscores the need for targeted surveillance systems that integrate local knowledge and scientific approaches.

To promote more proactive management of zoonotic diseases linked to the consumption, trade, and cultural practices of wildlife in Bolivia, it is essential to first establish baseline data and evaluate the potential risks associated with zoonotic spillovers. Research has highlighted the importance of understanding knowledge, attitudes, and practices towards zoonotic risks in Latin America to develop effective interventions. This foundational understanding helps to identify changes or anomalies in disease patterns early, using existing infrastructures to provide timely warnings (World Health Organization. Such an approach allows for the identification of specific areas or transmission patterns that require focused attention, ultimately contributing to more effective zoonotic disease management [4,5].

The goal is to ensure a comprehensive, coordinated approach to tracking and managing zoonotic risks, preventing outbreaks, and safeguarding both human and animal health. In this context, diagnostic investigation plays a crucial role, as it is central to proactive disease management [6]. Diagnostic investigations usually involve a broad spectrum of stakeholders such as hunters, wildlife rangers, community liaisons, citizen scientists, government agencies, wildlife conservation groups, veterinary services, public health organizations, environmental agencies, and international bodies. Rights-holders, including Indigenous Peoples, may also play a key role in these detection networks.

This document presents a framework for a proactive surveillance network, outlining coordination mechanisms and providing recommendations for monitoring key zoonotic pathogens and their monitoring protocols in a Bolivian context. The importance of monitoring and evaluation for effective zoonotic disease management has been emphasized as part of global strategies for disease prevention [7]. Furthermore, guidelines for addressing disease risks in wildlife trade serve as essential tools for strengthening Bolivia's approach to zoonotic disease prevention . This framework also offers guidelines for establishing a notification database for health events related to wildlife or zoonotic diseases tied to the consumption, trade, and cultural practices of important wildlife species, along with a clear process for event notification and response.

## SECTION 1: ESTABLISHING A FRAMEWORK FOR A PASSIVE SURVEILLANCE NETWORK AND COORDINATION MECHANISMS

Establishing an effective passive zoonotic surveillance network in Bolivia requires a multi-sectoral coordination framework that leverages the expertise, resources, and authority of key national agencies and stakeholders (see proposed example in Figure 1). Each agency has a defined role within a cohesive structure to ensure early detection, reporting, and response to zoonotic threats. The following paragraphs outline the specific coordination mechanisms and the involvement of various entities.

### 1. Stakeholder coordination mechanisms.

### 1.1. National Coordination

#### 1.1.1. Inter-Ministerial Coordination

The formation of a National Committee or Task Force is vital to oversee and guide the implementation of the passive surveillance framework. This committee should be composed of representatives from key government ministries, including the Ministry of Health, Ministry of Environment and Water, Ministry of Rural Development, and Ministry of Agriculture. Each ministry contributes its unique expertise: the Ministry of Health oversees human health surveillance and epidemiological investigations, the Ministry of Rural Development ensures livestock health monitoring, and the Ministry of Environment and Water provides data on environmental factors that may influence zoonotic pathogen transmission. The Ministry of Agriculture plays a critical role in agricultural practices that may impact livestock health. Additionally, experts in zoonotic diseases, public health, veterinary medicine, epidemiology, NGOs, and international organizations such as the WHO, OIE, and FAO should also be included. The committee would serve as the central governing body, responsible for strategic planning, resource mobilization, policy integration, and regular monitoring and evaluation of the surveillance system [4,7] .

Operationally, the committee would establish specialized subcommittees focused on specific areas such as wildlife, livestock, human health, and environmental surveillance[8]. It would also delegate responsibilities to regional task forces to ensure effective coordination at the departmental and municipal levels [6]. Stakeholder engagement would be a priority, with regular consultations involving NGOs, researchers, and community leaders to foster inclusivity and transparency [9]. This structure would provide a clear mechanism for multi-sectoral collaboration, ensure alignment of efforts across agencies, and enhance the country's preparedness for potential zoonotic outbreaks [4].

#### 1.1.2. Centralized Data Hub

The creation of a centralized data hub is equally critical for integrating and managing data from multiple sectors [7]. This platform would serve as a repository for routine health reports, clinic visit data, environmental indicators, and veterinary and wildlife records [10]. By automating data transfer from regional nodes to the central hub, it would provide real-time analytics for early outbreak detection and trend analysis [5].

The centralized data hub should include user-friendly features such as role-based access to protect data privacy while enabling collaboration among government agencies, NGOs, and researchers [8]. Advanced analytics tools (e.g., TensorFlow, PyTorch, ArcGIS, QGIS, Tableau, EPIPOX, GLEaM, Nextstrain, and CommCare) would enable predictive modeling and anomaly detection, while dashboards with visualization tools like heatmaps and graphs would allow stakeholders to easily interpret data trends [4]. The hub should also support multiple languages to accommodate Bolivia's diverse linguistic communities and ensure accessibility across all regions.

To govern the data hub, the Ministry of Health would serve as the lead agency, coordinating with other ministries and stakeholders [6]. Clear data-sharing agreements would be established to ensure seamless collaboration, while a technical advisory board would oversee system quality and updates [7]. Implementation would begin with a needs assessment to identify existing gaps in data systems, followed by the design and development of the platform in collaboration with technology experts [5]. Training programs would be provided for data contributors, such as clinic staff, veterinarians, and wildlife experts, to ensure accurate and consistent reporting [10]. The system would be piloted in selected regions before scaling up nationwide, with continuous improvements made based on user feedback and advancements in technology [8].

### 1.2. Local Coordination

Effective local coordination is essential to the success of a passive surveillance system for zoonotic diseases, as it bridges the gap between data collection at the community level and centralized analysis [4]. Local health, veterinary, and wildlife authorities must play a pivotal role in gathering and reporting data from their respective regions into the central system [6]. Local health departments, veterinarians, wildlife experts, and agricultural extension agents should be trained extensively in recognizing early clinical signs and environmental indicators of zoonotic diseases in both humans and wild [5].

Community involvement further enhances the reach and efficiency of the surveillance system [9] . Rural and indigenous communities often possess unique local knowledge, allowing them to detect early signs of unusual animal behavior, illness, or environmental changes that might signal a potential outbreak [7].

Here are some specific examples:

- *Indigenous farmers in rural areas might notice abnormal behavior in wild animals, such as aggression, excessive salivation, or unprovoked attacks, which are characteristic signs of rabies. Early reporting of these behaviors can lead to timely investigations and interventions.*
- *Small-scale poultry farmers might report sudden, unexplained deaths among their chickens, which could indicate an outbreak of avian influenza. Their familiarity with normal seasonal patterns in bird health makes them effective at recognizing anomalies.*

- *Communities involved in forest-based livelihoods may report increased encounters with displaced wildlife due to deforestation or land-use changes, which can heighten zoonotic disease risks.*
- *Forest-dependent communities may report skin lesions or other symptoms associated with leishmaniasis following increased sandfly bites in recently disturbed forest areas.*

These communities should be actively engaged through educational campaigns and participatory monitoring programs. Field-based training for veterinarians, biologists, and community health workers will build local capacity for disease detection. Risk-based surveillance strategies should prioritize high-risk areas, such as Santa Cruz (high livestock density) and Beni (wildlife-human interaction) . Reporting mechanisms, such as mobile apps or designated community health workers, can be established to make the reporting process accessible and straightforward. This grassroots-level engagement not only improves the quality of data but also fosters a sense of ownership and trust within the community, encouraging their sustained participation in disease surveillance.

Collaboration with the private sector is another critical component of local coordination. Private clinics, agricultural companies, and research institutions represent valuable sources of data on zoonotic diseases. These entities often encounter disease cases, environmental trends, or wildlife health issues that may go unreported in public systems. Partnerships should be formalized to facilitate data sharing while ensuring confidentiality and adherence to ethical standards. Agricultural companies, for example, can contribute insights on disease trends in livestock populations, wildlife and laboratory organizations can report diseases trends in wild populations, while private clinics can report human health cases that may have zoonotic origins. Academic institutions can support these efforts through targeted research and predictive modeling. By integrating the private sector into the surveillance framework, the system gains a more comprehensive and multidimensional understanding of zoonotic disease dynamics.

### 1.3. International Collaboration

International collaboration is a crucial component of Bolivia's passive surveillance framework for zoonotic diseases, given the transboundary nature of many emerging pathogens and the country's geographic position in South America [6]. Bolivia shares borders with five countries—Brazil, Argentina, Peru, Chile, and Paraguay—creating significant potential for cross-border disease transmission through wildlife migration, livestock trade, and human movement [10].

Establishing a cross-border surveillance network is essential for the early detection of zoonotic diseases and effective containment. This can be achieved through partnerships facilitated by regional and international organizations such as the Pan American Health Organization (PAHO), the World Health Organization (WHO), and the Andean Health Organization (ORAS-CONHU). Collaborative efforts should include harmonized disease reporting systems, joint outbreak investigations, and coordinated responses to shared threats. For instance, developing standardized protocols for reporting zoonotic diseases across borders can help ensure timely and accurate communication of emerging risks. Regular cross-border workshops and simulations could also build trust and enhance operational readiness among neighboring countries.

In the Bolivian context, international collaboration should focus on leveraging existing regional agreements and health initiatives. Bolivia is part of MERCOSUR and other trade blocks where health and agricultural policies intersect, providing a platform for integrated zoonotic disease monitoring. For example, shared surveillance activities could target high-risk areas such as the Amazon Basin, a biodiversity hotspot shared with Brazil and Peru that is a known reservoir for zoonotic pathogens. Collaborative environmental monitoring projects in this region could involve the collection of data on wildlife health and environmental changes that may signal emerging threats. Additionally, border regions with significant livestock trade, such as those shared with Argentina and Paraguay, require coordinated veterinary health monitoring systems to track and mitigate the spread of diseases like leptospirosis.

International research partnerships also represent a significant opportunity for Bolivia to enhance its passive surveillance capabilities. Collaborating with global disease monitoring organizations such as the World Organisation for Animal Health (OIE), the Global Health Security Agenda (GHSA), and academic institutions can facilitate the exchange of knowledge, technology, and best practices. For example, Bolivia could partner with international research groups to access advanced genomic sequencing technologies for pathogen identification and tracking, enabling the country to quickly identify zoonotic disease outbreaks. Collaborations could also focus on training Bolivian researchers and health professionals in advanced epidemiological methods and data analysis techniques. This would help strengthen local capacities while ensuring that Bolivia remains integrated into global surveillance networks.

Furthermore, Bolivia's participation in global early warning systems, such as the FAO's Global Animal Disease Information System (EMPRES-i) or WHO's Epidemic Intelligence from Open Sources (EIOS), would allow the country to monitor and share data on zoonotic disease threats in real time. For example, Bolivia could use these platforms to report outbreaks of diseases like hantavirus or rabies, which are endemic to the region, while gaining access to international alerts and recommendations on emerging pathogens.

To operationalize international collaboration, Bolivia should establish a dedicated liaison office within the Ministry of Health or Ministry of Agriculture to coordinate with international organizations and neighboring. By fostering international partnerships and aligning its surveillance framework with regional and global initiatives, Bolivia can strengthen its capacity to detect and respond to zoonotic diseases while contributing to global health security [4].

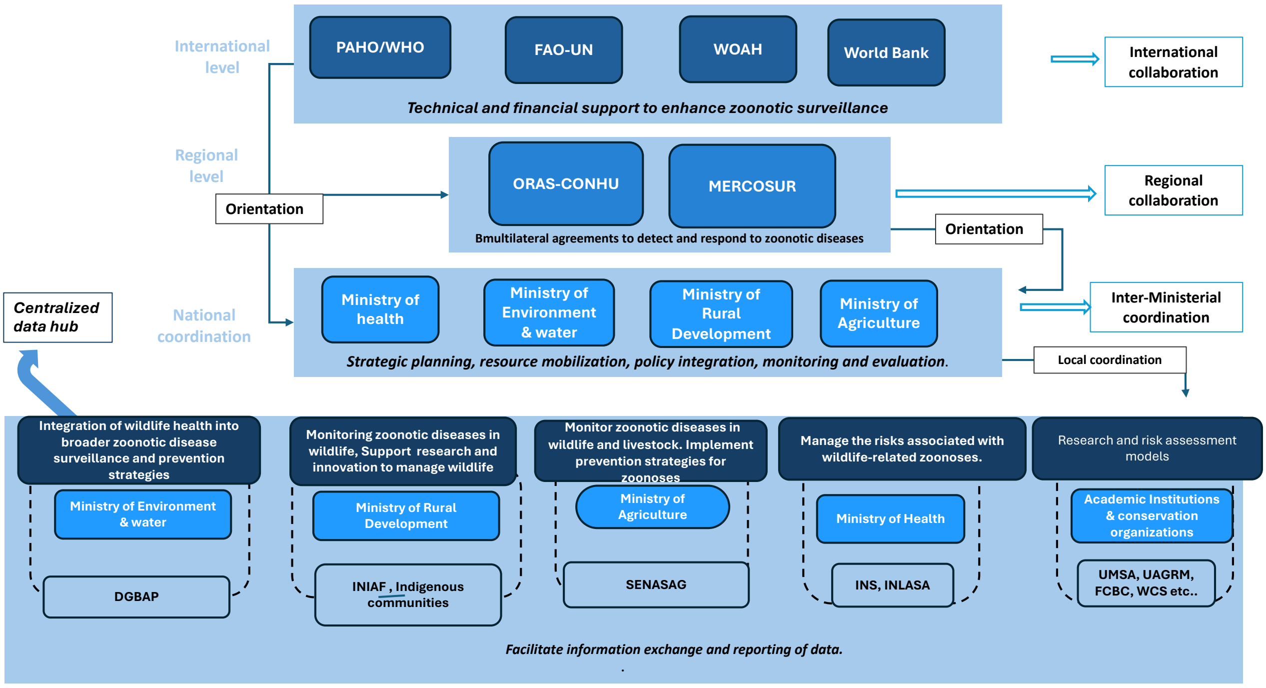


Fig 1. Recommended framework for passive surveillance network for zoontoic pathogens in Bolivia

### 1.4. Summarized approach for implementing the stakeholder coordination mechanisms in Bolivia's passive zoonotic disease surveillance network.

**Step 1:** Formation of a Multisectoral Coordinating Body

**Step 2:** Develop a communication and Data-Sharing Framework.

**Step 3:** Establish a legal framework for communication and data sharing, defining access levels and data privacy protocols, ensuring compliance with Bolivian regulations and international standards such as the WHO International Health Regulations (IHR).

**Step 4:** Formalize operational Guidelines for Intersectoral Collaboration outlining roles, responsibilities, and workflows for all stakeholders.

**Step 5:** Implement an Incident Command System (ICS) to manage outbreaks, assigning clear leadership roles such as Incident Commander, Liaison Officer, and Operations Officer to ensure coordinated and efficient responses.

**Step 6:** Establish Cross-border coordination mechanisms to address shared zoonotic disease risks with neighboring countries.

**Step 7:** Establish Local coordination units to engage communities and indigenous leaders in surveillance efforts.

**Step 8:** Implement Community education campaigns to raise awareness of zoonotic diseases and encourage proactive reporting of unusual animal deaths or human symptoms.

**Step 9:** Develop An integrated Monitoring and Evaluation (M&E) system to assess the effectiveness of the coordination mechanisms. Key Performance Indicators (KPIs) should include metrics such as the timeliness of reporting, the number of intersectoral meetings, and community participation rates.

**Step 10:** Establish Feedback loops to incorporate lessons learned from evaluations into policy updates.

| **Step 11**: | Implement a diversified funding strategy (from government and organizations like the World Bank and the Global Fund) to ensure long-term sustainability. |
|---|---|
| **Step 12**: | Conducted regular cost-benefit analyses to optimize resource allocation. |

### 1.5. Implementation Timeline

| Activities | Timeline of activities (months) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | >12 |
| **Phase 1:** Formation of a Multisectoral Coordinating Body; development of data-sharing agreements, and stakeholder mapping. | | | | | | | | | | | | | |
| **Phase 2:** Training programs for locals and health, veterinary and wildlife experts; pilot testing of the digital platform, and development of operational guidelines. | | | | | | | | | | | | | |
| **Phase 3:** Expansion of surveillance activities to high-risk regions, integration of community reporting mechanisms, and initiation of cross-border collaborations. | | | | | | | | | | | | | |
| **Phase 4:** Nationwide scaling of the network, routine evaluations, and continuous refinement based on stakeholder feedback. | | | | | | | | | | | | | |

## 2. Framework for passive surveillance

This section outlines a structured framework for passive zoonotic disease surveillance in Bolivia, emphasizing data integration, technological advancements, and priority diseases. It describes the implementation of interoperable health information systems, automated early warning mechanisms, and secure data-sharing platforms to improve reporting accuracy, enhance response efficiency, and strengthen national preparedness and mitigation efforts.

### 2.1. Data sources and integration

A passive surveillance network for zoonotic diseases in Bolivia requires an integrated approach using data from human health, animal health, and environmental monitoring sectors. Combining these datasets will support early detection, risk assessment, and policy responses to zoonotic disease threats. The following paragraphs provide details on the different sources of data and how this could be integrated in a single surveillance system to minimize and mitigate threats associated with zoonotic spillivers of wildlife origin.

#### 2.1.1. Human Health Data

The integration of human health data is essential for detecting zoonotic diseases linked to wildlife consumption, trade, and cultural practices in Bolivia. The Bolivian Ministry of Health's Surveillance System, supported by SIVE, REDES and SIIS, will serve as the primary data repository, aggregating information from hospitals, clinics, and indigenous health centers. Given the widespread use of wildlife in traditional medicine, religious rituals, and local dietary practices, a centralized Electronic Health Records (EHR) system must be implemented to ensure real-time reporting and cross-sectoral integration of zoonotic disease cases.

- *<u>Standardized Data Collection and Surveillance of Wildlife-Associated Zoonoses</u>*

Health facilities across Bolivia will standardize the reporting of zoonotic diseases by utilizing a digital EHR system within SIIS. This system will allow healthcare providers to instantly flag suspected zoonotic infections based on clinical presentations linked to wildlife exposure. For example, in regions such as Beni and Pando, where wild meat from armadillos *(Dasypus novemcinctus)* is frequently consumed, health workers will document cases of fevers of unknown origin, muscle pain, and jaundice, which could indicate leptospirosis. Similarly, in Cochabamba and La Paz, where flamingo fat and jaguar teeth are used in traditional medicine, reports of neurological symptoms or respiratory distress will be cross-referenced with known zoonotic pathogens carried by these species [11].

- *<u>Automated Reporting and Early Detection of Outbreaks</u>*

A real-time automated reporting system will enhance Bolivia's ability to detect emerging zoonotic threats. Currently, many healthcare facilities still rely on paper-based records, causing delays and inconsistencies in data collection. By transitioning to an integrated digital system, any suspected case matching predefined zoonotic disease criteria will automatically generate an alert [12] . For example, if a hospital in Santa Cruz detects a cluster of hemorrhagic symptoms in individuals who recently consumed howler monkey meat, the EHR system will flag the cases and notify epidemiologists at the Ministry of Health. This automated notification process eliminates manual data entry errors and ensures immediate intervention, such as field investigations and contact tracing.

- *<u>Mobile Data Reporting in Remote and Indigenous Communities</u>*

To bridge the gap between rural and urban health infrastructure, Bolivia must deploy mobile data collection applications that allow community health workers, veterinarians, and indigenous health providers to report zoonotic disease cases in real-time In Amazonian regions such as Madidi National Park and the Gran Chaco, where some indigenous groups may involve in the consumption, cultural practices, and trade of Psittacine birds (*Amazona spp.*, *Ara spp.*, *Aratinga spp.*) and deer (Mazama americana) frontline health workers could use offline-capable mobile applications to log symptoms and suspected zoonotic cases. These applications will automatically sync data once internet connectivity is restored, ensuring that disease surveillance is continuous, even in remote locations. *(Smith et al., 2024)*.

- *<u>Cross-Sectoral Integration of Human Health and Wildlife Data</u>*

To fully integrate human health data into Bolivia's One Health approach, the SIIS system must be linked with veterinary and environmental surveillance networks. For instance, if unexplained deaths in vicuñas or guanacosoccur in the Andean highlands, health workers should be immediately alerted to monitor for brucellosis cases among herders and market workers handling these animals. Likewise, data from wildlife trafficking seizures in border areas like Cobija and Puerto Suárez should be analyzed alongside hospital records to assess potential zoonotic disease spillovers from illegally traded species. A systematic review on integration mechanisms in human and animal health surveillance highlights the need for coordinated actions and data sharing between sectors to effectively monitor and respond to zoonotic diseases [13].

By implementing standardized electronic reporting, automated alerts, mobile health applications, and cross-sectoral integration, Bolivia would be better equiped to monitor, detect, and respond to zoonotic disease threats associated with wildlife consumption, trade, and cultural practices. These advancements will ensure faster outbreak containment, improved data accuracy, and enhanced public health preparedness in both urban centers and remote indigenous territories.

#### *2.1.2. Animal Health Data.*

Passive surveillance of animal health is critical for the early detection of zoonotic spillover events, particularly in Bolivia, where wildlife is frequently used for meat, traditional medicine, and cultural practices, increasing the risk of disease transmission. The Ministry of Agriculture, in collaboration with SENASAG, will oversee data collection from veterinarians, local farmers, and wildlife biologists to monitor potential zoonotic threats. Passive surveillance will rely on reports of unusual animal deaths, behavioral changes, or disease outbreaks, which are often the first indicators of a potential spillover event. For instance, the World Organisation for Animal Health (WOAH) emphasizes that unusual morbidity or mortality events in wildlife can serve as early warning signals for zoonotic diseases [14].

The following are some examples in animal behavioral changes:

- Wildlife such howler monkeys in the Amazon, suddenly appearing in villages or behaving tamely can signal neurological infections like lyssaviruses or toxoplasmosis.
- Unusual sounds or excessive vocalization in birds can indicate distress due to neurological or respiratory infections like avian influenza.
- Sloths, anteaters, or rodents appearing sluggish and failing to respond to threats may be suffering from diseases like Leptospirosis
- Rodents becoming more active during the day instead of night could indicate environmental stress or infections such as hantavirus, which has been linked to rodent population surges in Bolivia’s rural areas.

Additionally, the trade of live animals in markets, such as those in El Alto or Santa Cruz, presents a risk of disease transmission. Live animal markets have been identified as potential hotspots for zoonotic spillover due to the close contact between humans and various animal species [15]. To address these risks, SENASAG will coordinate with veterinary research institutions, wildlife conservation organizations, and environmental agencies to systematically record cases of wildlife morbidity and mortality. Special emphasis will be placed on rural and remote areas, such as the Yungas and Gran Chaco, where human-wildlife interactions are frequent due to hunting, farming, and deforestation-driven habitat encroachment. Data collected from local farmers reporting unusual livestock illnesses, such as Brucella infections in cattle or Trypanosoma cruzi (Chagas disease) in domestic animals, will also contribute to identifying disease emergence hotspots. Engaging local communities in surveillance efforts is crucial, as their observations can provide valuable insights into emerging health threats [9].

#### *2.1.3. Environmental Data*

Environmental changes such as deforestation, habitat fragmentation, flooding, and climate variability significantly influence zoonotic disease transmission in Bolivia. Deforestation in regions like the Amazon, Andean highlands, and Chaco plains forces wildlife into closer contact with humans, thereby increasing the risk of spillover events. For instance, deforestation has been linked to the emergence of zoonotic viruses in South America, as it disrupts natural habitats and facilitates closer interactions between wildlife and humans [16]. Similarly, habitat fragmentation in Bolivia's lowlands has altered ecosystems, potentially affecting disease dynamics [17].

Seasonal flooding in the Beni wetlands heightens the risk of leptospirosis, as animals such as capybaras, peccaries, and tapirs can contaminate water sources with Leptospira bacteria, which may subsequently expose humans through contact and traditional hunting activities. Additionally, the trade and consumption of wildlife further contribute to zoonotic disease transmission; turtles, caimans, and iguanas, commonly consumed in Amazonian communities, frequently carry Salmonella, while howler monkeys, reservoirs of yellow fever, pose risks through hunting and habitat disturbance. Wild ungulates like deer and vicuñas can carry echinococcosis, a parasitic disease contracted through contaminated meat or water.

To mitigate these threats, Bolivia should consider the integration of hydrological data and GIS-based risk models to monitor deforestation, flooding, vector habitats, and areas where wildlife is traded, consumed, and used for cultural practices. This approach aims to enhance early detection and enable targeted interventions to reduce zoonotic disease transmission.

## 2.2. Remote Sensing and GIS Applications

Remote sensing and GIS will boost passive surveillance in Bolivia, particularly for zoonotic disease risks linked to wildlife trade, consumption, and cultural use. Remote sensing and GIS have been effectively utilized in monitoring environmental changes that influence disease transmission, providing valuable tools for surveillance and control [18].

### 2.2.1. Wildlife Trade and Market Surveillance

Wildlife trade, both legal and illegal, presents a significant zoonotic disease risk in Bolivia, particularly in urban markets such as Mercado Rodríguez in La Paz and Los Pozos in Santa Cruz, where live animals and bushmeat are sold. Remote sensing can help identify supply chain sources by mapping wildlife extraction zones in biodiversity hotspots like the Madidi National Park and Chiquitano Dry Forest. GIS tools can track transport routes connecting these areas to urban markets, where close interactions between humans and stressed, immunocompromised animals increase the risk of disease spillover.

For example, species like peccaries (*Tayassu pecari*) and deer (*Mazama americana*), commonly hunted for meat in Bolivia's Amazon and Gran Chaco regions, may harbor Brucella *spp*., a bacterial pathogen that causes brucellosis in humans. GIS mapping can help track regions where brucellosis cases correlate with hunting activity. Similarly armadillos Armadillos (*Dasypus novemcinctus*), frequently consumed as a protein source in rural communities, are known reservoirs of Mycobacterium leprae, the causative agent of leprosy. Surveillance of hunting and trade routes can help identify hotspots of potential zoonotic transmission.

By overlaying satellite imagery and market surveillance data, authorities can pinpoint high-risk zones where wildlife trade is concentrated. Additionally, spatial analysis can help detect illegal trade hubs that bypass formal biosecurity regulations, enabling targeted interventions and monitoring efforts. A study on digital surveillance of the illegal wildlife trade emphasizes the importance of identifying hotspots for emerging zoonoses along commonly utilized illegal wildlife trade routes [19].

#### 2.2.2. Cultural Practices and Zoonotic Risk.

Cultural practices in Bolivia contribute significantly to the risk of zoonotic disease spillover, particularly through human-wildlife interactions in rural and indigenous communities. Certain local cultural practices, such as the consumption of wildlife for food, medicinal purposes, and traditional rituals, enhance the likelihood of zoonotic transmission [8]. For example, some rural Bolivian communities consume wild pumas (*Puma concolor*) and ocelots (*Leopardus pardalis*) for their perceived medicinal benefits. These carnivores can harbor *Trichinella* spp., the parasitic roundworm responsible for trichinellosis, which can be transmitted to humans through the consumption of undercooked meat [9]. Remote sensing and GIS modeling can track hunting activities in these regions, identifying areas where hunting and trichinellosis cases overlap, thus providing valuable information for targeted interventions or active surveillance (World Health Organization [4].

Additionally, reptiles such as turtles (*Podocnemis expansa*) and caimans (*Caiman yacare*), commonly consumed in the Amazon basin, often carry *Salmonella* spp., leading to gastrointestinal infections in humans (World Organisation for Animal Health [10]. By utilizing remote sensing technologies to analyze trade pathways, health authorities can assess areas where these reptiles are frequently harvested, providing insights into regions at heightened risk of contamination [20]. Similarly, primates, such as howler monkeys (*Alouatta* spp.) and spider monkeys (*Ateles*spp.), are hunted in certain parts of Bolivia for traditional ceremonies. These monkeys can be carriers of retroviruses, which possess zoonotic potential [8]. By overlaying GIS data of monkey habitats with known hunting zones, it becomes possible to predict areas where viral spillover risks are greatest [5].

#### 2.2.3. Environmental Changes and Disease Risk Mapping

Environmental changes such as deforestation, urbanization, and agricultural expansion also amplify the risks of zoonotic disease emergence in Bolivia. The loss of forest cover, particularly in areas like Beni and Pando, forces wildlife to encroach upon human settlements, thereby increasing the risk of diseases such as Leishmaniasis and Chagas disease. Leishmaniasis, transmitted by sandflies, and Chagas disease, transmitted by triatomine bugs, thrive in areas of habitat disruption. Remote sensing and GIS can track deforestation patterns and model the movement of wildlife populations, helping to identify regions where human-wildlife conflict and zoonotic disease transmission risks are heightened.

In flood-prone regions such as Trinidad and Riberalta, wetlands create stagnant water bodies that foster the spread of waterborne diseases like Leptospirosis, schistosomiasis, and vector-borne diseases such as Dengue, Zika, and Chikungunya. GIS tools can analyze flood-prone areas and overlay disease outbreak data to improve response strategies and public health interventions. Furthermore, tracking the migration of zoonotic hosts, such as tapirs (*Tapirus terrestris*) and jaguars (*Panthera onca*), across fragmented landscapes using remote sensing can help predict potential disease spillover risks as these animals come into closer contact with human settlements. In these ways, remote sensing and GIS technologies are essential tools for mapping disease risks, enhancing early detection, and informing targeted mitigation efforts in Bolivia's complex ecological and cultural landscape.

## 2.3. Technology and Data Management.

In Bolivia, technology and data management play a crucial role in addressing the risks of zoonotic spillovers associated with wildlife consumption, trade, and cultural practices. With the country's vast biodiversity, especially in regions like the Amazon Basin and Andean highlands, integrating advanced technologies into health surveillance systems is essential to track and manage zoonotic risks.

### 2.3.1. Health Information Systems

To begin, Health Information Systems (HIS), such as the SIIS, need to be strengthened and adapted for the surveillance of zoonotic diseases that are linked to wildlife interactions. In countries like Thailand and Vietnam, the integration of electronic health record systems (EHRs) has allowed for rapid identification and response to zoonotic disease outbreaks. Bolivia could adopt similar practices by digitizing health records across rural and urban hospitals, where healthcare providers can record wildlife exposure histories—such as the consumption of wild meat or handling of wildlife during the participation in traditional rituals. This would allow for real-time reporting of suspected zoonotic cases and generate automated alerts that trigger immediate investigations and interventions, similar to Sistema de Informação de Agravos de Notificação **(**SINAN), an automated surveillance systems used in Brazil, to monitor zoonotic diseases like Leptospirosis in regions with high wildlife consumption. SINAN system in Brazil allows for cross-sectoral collaboration and plays a crucial role in tracking and managing communicable diseases, including zoonoses.

### 2.3.2. Early Warning Systems (EWS)

Bolivia should also focus on the development or integration of Early Warning Systems (EWS) to rapidly detect unusual disease patterns, particularly in high-risk regions. Drawing from examples in Kenya, where early warning systems have been successfully used to detect zoonotic diseases like Ebola and anthrax by analyzing multiple data streams, Bolivia could create a system that integrates veterinary data (from wildlife biologists and veterinarians) with human health reports. For instance, if unusual deaths of capybaras are reported in Beni, the system could cross-reference this with human cases of leptospirosis in the area and trigger a rapid response. The integration of geospatial tools to track wildlife movements, environmental factors, and human-wildlife contact zones could further enhance the accuracy of these alerts, similar to the Integrated Surveillance systems used in Indonesia for avian influenza monitoring, where data from wildlife monitoring and market trade activities is integrated to identify disease hotspots and facilitate early detection of outbreaks.

### 2.3.3. Data Sharing Platforms

The establishment of data sharing platforms is vital to ensure the seamless exchange of information across Bolivia's human health, veterinary, and environmental sectors. A platform could be designed to allow real-time sharing of wildlife disease reports from markets, national parks, and border checkpoints to public health officials. Drawing inspiration from the One Health approach implemented in South Africa, where government agencies and NGOs share zoonotic disease data, Bolivia could ensure cross-sectoral collaboration between institutions like the Ministry of Health, SENASAG and environmental monitoring agencies. These agencies would be able to analyze wildlife trade patterns, environmental stressors, and human exposure to identify emerging zoonotic threats. This integration would enhance early detection of diseases like brucellosis in Jaguars or salmonellosis in turtles, which could be linked to hunting and wildlife trade practices in regions such as Santa Cruz and Pando.

#### 2.3.4. Privacy and Data Governance

To ensure the privacy and protection of sensitive data, Bolivia must implement robust data governance frameworks with clear privacy protocols, much like the General Data Protection Regulation (GDPR) in the European Union. These protocols would secure patient information while allowing for public health and environmental agencies to access the necessary data for rapid outbreak response. Additionally, mobile applications and cloud-based solutionswould help bridge the gap between urban health infrastructure and remote communities, where zoonotic risks are often higher due to traditional wildlife consumption and limited access to medical services. By utilizing offline-capable apps, similar to the “Open Data Kit” used in Madagascar to report zoonotic outbreaks in rural areas, Bolivia can ensure that data reporting is continuous, even in isolated regions like Madidi National Park or the Gran Chaco.

By enhancing the SIIS, developing effective Early Warning Systems, and ensuring robust cross-sectoral data sharing, Bolivia can build a responsive surveillance network that draws inspiration from successful global models and this could be tailored to the country’s unique ecological and cultural landscape.

## SECTION 2: RECOMMENDATIONS ON KEY ZOONOTIC PATHOGENS AND PROTOCOLS FOR THEIR SURVEILLANCE.

From the technical report on the zoonotic risks associated with the trade in wild animals—including pet ownership, meat consumption, and traditional medicine in Bolivia—surveillance is recommended for the following top five zoonotic diseases due to their transmission potential, severity, and prevalence. This section will specifically focus on key recommendations and protocols for passive surveillance of these diseases:

- **Rabies (Lyssavirus spp.)** – A fatal viral disease transmitted through bites from infected animals, with key reservoirs in wildlife, including bats and carnivores such as foxes (*Lycalopex spp.*), raccoons (*Procyon cancrivorus*), and wild dogs (*Canis spp.*).
- **Brucellosis (Brucella spp.)** – Found in jaguars (*Panthera onca*), peccaries (*Tayassu pecari*), and other wild mammals. Transmitted through direct contact with infected tissues or consumption of contaminated meat.
- **Leptospirosis (Leptospira spp.)** – Carried by primates (*Alouatta spp.*, *Ateles spp.*), jaguars, and armadillos (*Dasypus novemcinctus*). Spread via the urine of infected animals and contaminated water or soil.

- **Salmonellosis (Salmonella spp.)** – Associated with turtles (*Podocnemis expansa*), caimans (*Caiman yacare*), and reptiles (*Boa constrictor*). Transmitted through direct contact or ingestion of contaminated food with high exposure risks in wildlife handling.
- **Toxoplasmosis (Toxoplasma gondii)** – Found in felines (*Panthera onca*), deer (*Mazama americana*), and black caimans (*Melanosuchus niger*). Spread through ingestion of undercooked wild meat or contact with feline feces

## 1. Key zoonotic pathogens and passive surveillance protocols.

Passive surveillance plays a critical role in detecting and responding to zoonotic diseases in Bolivia. Passive surveillance relies on routine reporting of cases by healthcare facilities, veterinarians, laboratories, and the public without active case searching. Below are specific recommendations for each disease and protocols to improve passive surveillance.

### 1.1. Passive surveillance for Rabies

Rabies control requires systematic reporting of animal bites and unexplained wildlife mortality. In Bolivia, systematic reporting of rabies from animal bites and unexplained wildlife mortality should be under the responsibility of the Ministerio de Salud y Deportes, the National Center for Tropical Diseases, SENASAG, INLASA, the Ministry of Environment and Water, and the Local Veterinary Clinics and Public Health Centers [21]. Surveillance should include the collection of brain tissue samples from suspected rabid animals for laboratory confirmation using direct fluorescent antibody testing (DFAT) and polymerase chain reaction (PCR) techniques [22]. Data should be integrated into a national rabies surveillance system, with rapid reporting to health authorities. Vaccination campaigns should be prioritized for high-risk wildlife reservoirs, and exposure assessments should be conducted for humans in contact with suspected rabid animals [10].

#### 1.1.1. General steps involved in proper brain tissue collection.

***A. Protective Measures***

- **Personal Protective Equipment (PPE):** Wear gloves, goggles, face shields, and a lab coat or protective clothing. Use a biosafety cabinet or other containment equipment if available [21].
- **Disinfecting Equipment:** Ensure that all tools used for tissue collection (e.g., scissors, scalpels, forceps) are disinfected or sterilized [21].

***B. Identification of the Animal***

- The animal should be confirmed as potentially rabid based on clinical symptoms (e.g., paralysis, aggression, drooling). Only animals exhibiting signs consistent with rabies or animals that have bitten a human should be tested [22].

***C. Handling and Transporting the Animal***

- Dead Animal Collection: If the animal is deceased, handle it with care and place sample in a sealed, leak-proof container for transport. Sealing the sample in a leak-proof container helps to maintain biosecurity, preventing other people or animals from encountering potentially infected material [22].

- Live Animal: If the animal is alive and needs to be euthanized, ensure euthanasia is performed humanely and according to local regulation [22].
- Transport the animal to a designated rabies testing facility as quickly as possible while maintaining biosecurity [10].

***D. Collection of Brain Tissue***

- Decapitation: The head of the animal should be removed at the base of the skull, ensuring that the brain remains intact [22].
- Postmortem Brain Removal: Place the animal on a clean, sterile surface. Disinfect the surface before beginning any procedure. Use sterilized tools to open the skull and remove the brain. Handle the brain gently to prevent contamination or damage [22].
- The brain should be carefully examined for any obvious lesions or abnormalities before sending it to the lab [21].

***E. Packaging and Transport for Laboratory Testing***

- Place the brain tissue sample into a clean, sterile container (e.g., plastic vial or jar).
- Label the container clearly with the species of the animal, the date of collection, the source of the sample and any other relevant clinical information (e.g., symptoms).
- Transport the sample in a secure, temperature-controlled environment (usually on ice or in a cool box) to a laboratory, such as INLASA and SENASAG, that can perform rabies testing, such as the direct fluorescent antibody (DFA) test or PCR [21].

***F. Laboratory Confirmation***

- Once at the laboratory, the brain tissue will undergo a variety of tests to detect the rabies virus, typically using techniques like the Direct Fluorescent Antibody (DFA) Test (The gold standard for rabies detection) and RT-PCR for more precise viral identification [21].

***G. Disposal of Materials***

- After the sample has been collected and transported, properly disinfect or dispose of any materials used in the collection process to prevent contamination or accidental exposure to rabies [21].

**Key Safety Considerations**

- Rabies is a serious zoonotic disease, so all personnel handling rabid animals or brain tissue must have proper training in handling potentially infected materials.
- Rabies vaccinations for personnel are highly recommended if there is a risk of exposure [21].

### 1.1.2. Recommendations to improve passive surveillance of rabies.

In Bolivia, rabies remains a significant public health concern, particularly in urban areas where stray dog populations are high and in rural regions where livestock and wildlife may serve as reservoirs [22]. Strengthening mandatory reporting of suspected rabies cases in both humans and animals is critical for effective disease control [10]. Healthcare facilities, veterinary authorities, and local communities must collaborate to ensure that all suspected cases are promptly reported and investigated [21]. Laboratory confirmation of rabies should be prioritized using the Direct Fluorescent Antibody (DFA) test or PCR to verify cases accurately [10].Access to diagnostic facilities must be expanded, especially in remote regions where logistical challenges may delay testing and response efforts [21].

Vaccination plays a crucial role in rabies prevention in Bolivia. Mass vaccination campaigns for domestic animals, particularly dogs and cats, should be expanded, with a focus on high-risk urban and peri-urban areas [22]. In rural settings, vaccination programs for livestock that may come into contact with wildlife reservoirs should also be strengthened [10].For humans, Post-Exposure Prophylaxis (PEP) must be readily available at health centers, ensuring that bite victims receive timely and appropriate treatment to prevent the onset of the disease [21]. Additionally, public awareness campaigns should be intensified to educate communities about rabies prevention. These campaigns should emphasize responsible pet ownership, the importance of vaccination, and the risks of contact with potentially rabid wildlife, which could transmit rabies to livestock and humans in certain regions of Bolivia [22].

#### 1.1.3. Passive surveillance protocols for rabies

Passive surveillance is essential for detecting and controlling rabies outbreaks. Health centers should systematically monitor and document animal bite cases, particularly those involving unvaccinated dogs, wild animals, or wild animals, and report them to public health authorities. Veterinary services must also be alert to unusual animal behavior or sudden deaths, as these could indicate rabies infection, and such cases should be immediately investigated. Suspected rabid animals should have their brain samples submitted to diagnostic laboratories for DFA or PCR testing to confirm infection. Strengthening the capacity of Bolivia’s veterinary and public health sectors to conduct timely field investigations, laboratory testing, and coordinated response efforts is critical to reducing rabies transmission [10,21,22].

### 1.2. Passive surveillance for Brucellosis

Passive surveillance for brucellosis involves routine serological testing of at-risk wildlife species and farm animals. Samples should be tested using the Rose Bengal test (RBT), enzyme-linked immunosorbent assay (ELISA), and confirmatory culture methods. Veterinary and public health professionals must be trained to recognize symptoms of brucellosis in humans and animals, ensuring that suspected cases are reported to national health agencies Enhanced traceability of infected animals and proper disposal of contaminated materials are essential for preventing transmission [23].

#### *1.2.1. Sample collection (human and wild animals)*

To test for brucellosis in wild animals such as jaguars (*Panthera onca*) and peccaries (*Tayassu pecari*), as well as in humans, different types of biological samples are required depending on the diagnostic method used [24]. Blood samples are commonly collected for serological tests such as the Rose Bengal Test and ELISA to identify *Brucella*bacteria. Blood cultures are most effective during the acute phase (early infection), while serological tests are preferred in chronic infections due to the persistence of antibodies [25]. For PCR, 5–10 mL of venous blood is collected in Ethylenediaminetetraacetic acid (EDTA) tubes, whereas for serology, blood is collected in plain tubes, allowed to clot, and centrifuged to obtain serum, which can be stored at -20°C for later testing [26].

Bone marrow aspirates are particularly useful for bacterial culture and PCR, especially when blood cultures yield negative results [27] . These aspirates, collected from the sternum under sterile conditions, provide a higher bacterial yield than blood cultures [28]. Similarly, lymph node aspirates, obtained through fine-needle aspiration (FNA), can be used for culture and PCR, particularly in cases with lymphadenopathy. In patients presenting with brucellar arthritis, synovial fluid and joint aspirates are collected to detect *Brucella* through culture and PCR [29].

For chronic brucellosis, urine samples can be used for PCR detection of *Brucella* DNA, requiring a midstream clean-catch urine sample collected in a sterile container [30]. In cases where brucellosis leads to abortion, placental tissues, vaginal swabs, and fetal tissues are collected for culture and PCR to confirm infection, with extreme caution taken to prevent contamination [31].

### 1.2.2. Handling and transport of samples.

Proper handling and transport of samples are critical to maintaining diagnostic accuracy. Blood samples for culture should be stored at 37°C before processing, while serum and urine samples should be refrigerated at 4°C if processed within 24 hours, or frozen at -20°C for extended storage [32]. Tissue samples and aspirates should be transported in sterile, leak-proof containers under cold chain conditions to preserve bacterial viability [33]. By ensuring the correct collection and handling of samples, laboratories can improve the early and accurate diagnosis of brucellosis, leading to better disease management and control strategies [34].

### 1.2.3. Methods for testing brucellosis

- ***The Rose Bengal Test***

The Rose Bengal Test (RBT) is a rapid serological assay used to detect antibodies in serum that respond to *Brucella* antigens [35]. The procedure involves mixing the serum with a prepared *Brucella* antigen, which is typically stained with Rose Bengal dye to facilitate the detection of agglutination (clumping) if antibodies are present. A positive result is indicated by agglutination, observable within 4–10 minutes, while negative samples show no reaction. This test is advantageous due to its quick turnaround, making it ideal for large-scale wildlife screening. It does not require advanced laboratory equipment, enabling its use in field settings. However, it provides only qualitative results and cannot quantify antibody levels [36].

- ***Enzyme-Linked Immunosorbent Assay (ELISA)***

The Enzyme-Linked Immunosorbent Assay (ELISA) is a more specific and quantitative serological test that detects antibodies (IgG, IgM) against *Brucella* species [37]. In this test, *Brucella* antigens are coated on a microplate, and serum is added. If antibodies are present, they bind to the antigen, and an enzyme-linked secondary antibody is added. A substrate reacts with the enzyme, producing a color change proportional to the antibody concentration, which can be quantified by measuring absorbance. ELISA offers higher specificity than RBT, distinguishing between antibodies from active infection and those from vaccination, and can be tailored to detect different types of antibodies or *Brucella* species.

Despite its accuracy, ELISA requires more laboratory resources, time (several hours), and expertise, which may limit its availability in low-resource settings [38, 39].

- ***Confirmatory Culture Methods***

Confirmatory culture methods represent the gold standard for brucellosis diagnosis, involving the direct isolation of *Brucella* bacteria from clinical samples such as tissue, blood, urine, or bone marrow aspirates [40]. The samples are cultured on selective media, such as *Brucella* agar, and incubated in a $CO_2$ enriched environment for several days. After incubation, colonies are examined and subjected to biochemical tests (e.g., oxidase, urease tests) for identification. Molecular techniques like PCR or DNA sequencing can be used for species confirmation. While culture methods are the most reliable for confirming the pathogen, they are time-consuming, often taking several days to weeks, and require specialized equipment and expertise that may not be available in all laboratories. Additionally, the prolonged incubation period can delay diagnosis, particularly in urgent cases [41].

### 1.2.4. Proper disposal of samples to prevent transmission.

Proper disposal of infected animals and contaminated materials is essential for preventing the spread of *Brucella* bacteria [42]. Infected animal carcasses should be either incinerated or buried at a sufficient depth to ensure the bacteria do not survive and spread [43]. Strict disposal protocols should be developed to ensure carcasses are isolated and disposed of promptly and safely, avoiding landfills or open-air disposal [44]. Contaminated materials, such as bedding, manure, feed, and equipment, should be disinfected using appropriate disinfectants, and manure from infected animals should be composted at high temperatures or disposed of in a way that prevents exposure to other animals or humans [45]. Personal protective equipment (PPE) such as gloves, masks, and protective clothing should be worn by individuals handling infected animals, and PPE should be disinfected or safely discarded after use. Furthermore, quarantine facilities should isolate infected animals until they are tested or euthanized and disposed of, and the surrounding environment should be regularly cleaned and disinfected [46].

### 1.2.5. Clinical signs and symptoms

- **Symptoms of brucellosis in humans**

Brucellosis in humans, commonly referred to as "undulant fever," presents with a wide range of non-specific symptoms that can often be mistaken for other illnesses [47]. The disease is characterized by an intermittent fever, which spikes and then subsides, often accompanied by chills and profuse sweating, particularly at night. Patients frequently experience fatigue and malaise, leading to a general sense of weakness and discomfort. Joint and muscle pain, especially in the back, limbs, and larger joints, is a common complaint, along with persistent headaches that may vary in intensity [48]. Gastrointestinal symptoms such as abdominal pain, nausea, and vomiting can also occur, contributing to loss of appetite and subsequent weight loss. In some cases, swelling of the lymph nodes (lymphadenopathy) may be observed, and a subset of patients may exhibit psychological symptoms, including depression, mood swings, and irritability [30]. In chronic or complicated cases, brucellosis can persist for months or even years, with relapsing fever episodes where symptoms temporarily improve before returning [49]. In more severe cases, the infection may spread to vital organs, leading to complications such as liver and spleen enlargement, heart inflammation (endocarditis), and bone infections (osteomyelitis). Though rare, neurological complications, including meningitis or encephalitis, can occur, posing a significant risk to the patient. Due to the broad range of symptoms and the potential for chronicity, early diagnosis and treatment of brucellosis are essential to prevent severe complications and long-term health effects [28].

- **Symptoms of brucellosis in wild animals**

Brucellosis wildlife is often asymptomatic or subclinical, making detection and control challenging [50]. However, in certain cases, the infection can cause significant illness and lead to population-level impacts, particularly in species such as jaguars (*Panthera onca*) and peccaries (*Tayassu pecari*) [51]. One of the most critical manifestations of brucellosis in wildlife is reproductive failure, with abortion in late pregnancy being the most common clinical sign in infected females [52]. Aborted fetuses often contain high concentrations of *Brucella* bacteria, increasing the risk of transmission within a population. Infected males may experience infertility, reduced sperm quality, or testicular swelling (orchitis), further impacting reproductive success [53]. Additional complications such as stillbirths and premature births contribute to population decline, particularly in species with already low reproductive rates. Beyond reproductive issues, infected wild animals may exhibit fever, lethargy, and reduced activity levels, though these symptoms are often subtle and difficult to observe in natural settings [54]. Swelling of the lymph nodes, particularly in the abdominal and thoracic regions, can occur, indicating immune system involvement. Affected animals may also suffer from weight loss or reduced weight gain, likely due to decreased feeding and increased energy expenditure to combat infection [54]. Joint and limb swelling, often resulting in limping or restricted movement, is another key clinical sign and may indicate arthritis or chronic inflammation. In some cases, infected animals display behavioral changes, such as reluctance to move, avoidance of social interactions, or increased aggression, which can disrupt normal group dynamics [35].

Chronic infections in wildlife present a significant ecological concern, as many species may harbor *Brucella* without showing overt symptoms, making them long-term reservoirs for the bacteria [55]. This persistent infection can lead to reproductive failure over multiple generations, ultimately threatening the survival of species, particularly those already under environmental and ecological stress [56]. Given these factors, ongoing surveillance, diagnostic testing, and conservation efforts are essential to monitor and mitigate the impact of brucellosis in wildlife populations [57].

#### 1.2.6. Enhanced Traceability of Infected Animals

Traceability is crucial for tracking the movement of animals, identifying sources of infection, and containing outbreaks of brucellosis. To achieve this, animals should be uniquely identified using microchips, RFID tags, or ear tags, which are linked to centralized databases that store information on vaccination status, test results, movement history, and clinical observations. Additionally, movement permits for animals being transported help prevent the spread of brucellosis from infected regions to non-infected areas, and real-time tracking systems, such as GPS, can monitor animal movements. Surveillance at livestock markets and slaughterhouses ensures that animals are inspected and quarantined if needed. Regular testing and monitoring, including serological tests and PCR for confirmed cases, help to identify infected animals early and manage outbreaks. Furthermore, data sharing between neighboring localities and countries enhances early detection and containment efforts.

#### 1.2.7. Recommendations to improve passive surveillance

In Bolivia, brucellosis remains a significant public health and animal health concern, particularly in livestock-rearing regions (such as Santa Cruz, Cochabamba and La Paz to mention a few) where the disease affects cattle, sheep, goats, and wildlife. To effectively control and prevent its spread, it is essential to implement routine serological screening using tests such as the Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA) for at-risk popultion, livestock and wildlife. These diagnostic tools enable early detection of infection, allowing for timely intervention measures. Additionally, locals, slaughterhouses and veterinary professionals should be required to report reproductive disorders, including abortions, stillbirths, and infertility, as these are key indicators of potential brucellosis infections. Encouraging mandatory reporting will enhance disease monitoring and facilitate a faster response to outbreaks.

Strengthening biosecurity measures in farms and slaughterhouses is critical to minimizing occupational exposure, particularly among farmers, veterinarians, and meat processing workers who are at risk of contracting Brucella through direct contact with infected animals or contaminated materials. Farms should adopt strict hygiene protocols, controlled breeding programs, and vaccination strategies where applicable. Public education campaigns are also necessary to raise awareness about the risks of consuming unpasteurized dairy products, which are a common source of human brucellosis infections in Bolivia. Promoting the consumption of pasteurized milk and properly cooked animal products can significantly reduce transmission risks.

#### 1.2.8. Protocols for Passive surveillance

A robust passive surveillance system should be established to improve the detection and tracking of brucellosis cases across Bolivia. Veterinarians and slaughterhouses must be mandated to report suspected cases, ensuring that all affected animals undergo proper laboratory testing. The collection and analysis of blood, milk, and reproductive tissues from suspected cases will provide crucial data for accurate diagnosis and outbreak control. Additionally, maintaining a national brucellosis database is essential to tracking disease trends, identifying high-risk areas, and implementing targeted control strategies such as quarantine and movement control, vaccination programs and targeted testing.

### 1.3. Passive surveillance for Leptospirosis

Surveillance for leptospirosis should prioritize monitoring clinical cases in both humans and animals, with a focus on individuals involved in wildlife handling for trade, consumption, or cultural practices [58]. Particular attention should be given to species such as primates (*Alouatta spp., Ateles spp.*), jaguars (*Panthera onca*), and armadillos (*Dasypus novemcinctus*), which are potential carriers due to their habitats and exposure to contaminated environments [59].

#### 1.3.1. Sample collection in wildlife and human

Proper collection of blood and urine samples is crucial for the accurate diagnosis of leptospirosis, as it ensures the detection of Leptospira bacteria, DNA, or antibodies [60]. The timing, handling, and storage of these samples significantly impact diagnostic outcomes.

For blood sample collection, the purpose varies depending on the stage of infection. In the acute phase (first 7–10 days of illness), blood samples are used for PCR or culture, as Leptospira is still circulating in the bloodstream. In the convalescent phase (after 10 days), blood is collected for serological tests like the Microscopic Agglutination Test (MAT) or ELISA, which detect antibodies [61]. The collection process begins with preparation, ensuring proper identification and aseptic techniques. A sterile syringe or vacutainer is used to collect 5–10 mL of venous blood from the antecubital vein. For PCR and culture, blood is collected in EDTA (anticoagulant) tubes to prevent clotting, while for serology, a plain (red-top) tube is used to obtain serum. Once collected, samples for PCR or culture should be stored at 4°C and transported within 24 hours, whereas serum for serology should be allowed to clot, centrifuged, and stored at negative 20°C if delayed transport is expected [62]. Proper labeling, leak-proof containers, and documentation are essential to maintain sample integrity [63].

For urine sample collection, the focus is primarily on PCR or culture, particularly in later stages when Leptospira is no longer in the bloodstream but is being excreted in urine [58]. . Leptospira shedding in urine typically begins 7–10 days after symptom onset and can persist for weeks. Patients should be instructed on how to collect a midstream clean-catch urine sample using a sterile screw-cap container, ensuring minimal contamination [62]. If catheterized collection is required, aseptic techniques must be followed. Ideally, 10–20 mL of urine should be collected. Samples should be stored at 4°Cand processed within 24 hours, or frozen at negative 20°C if transport is delayed [59].

#### 1.3.2. Handling of samples

Throughout the collection process, safety precautions must be observed to prevent exposure to Leptospira. Healthcare workers should wear personal protective equipment (PPE), including gloves, masks, and goggles. Accidental needle sticks must be avoided, and all sharps should be disposed of in designated containers. In case of spills, surfaces must be disinfected immediately with a 10% bleach solution. Laboratories handling leptospirosis samples should follow Biosafety Level-2 (BSL-2) protocols to minimize the risk of exposure [62,63].

#### 1.3.3. Methods for testing leptospirosis

- ***Microscopic Agglutination Test (MAT) for Leptospirosis***

The Microscopic Agglutination Test (MAT) is the gold standard for serological diagnosis of leptospirosis. It detects specific antibodies against *Leptospira* in serum by measuring the reaction between antibodies and live *Leptospira* antigens. The test involves mixing serial dilutions of the serum sample with different serovars of *Leptospira*, followed by incubation. After incubation, the mixture is examined under a dark-field microscope to observe agglutination (clumping) of the bacteria, indicating a positive reaction.

MAT is highly specific, as it can differentiate between different *Leptospira* serovars based on antibody-antigen binding. It is commonly used for epidemiological studies, serovar identification, and confirmation of past infections. However, it requires specialized biosafety measures, as it involves handling live *Leptospira* cultures. MAT also has limitations, including low sensitivity in early infection, as antibodies may not be detectable in the first week of illness, necessitating paired samples taken two weeks apart to confirm seroconversion [61].

- ***Polymerase Chain Reaction (PCR) for Leptospirosis***

Polymerase Chain Reaction (PCR) is a molecular diagnostic method that detects Leptospira DNA in clinical samples such as blood, urine, cerebrospinal fluid, or kidney tissue. Unlike MAT, which detects antibodies, PCR directly amplifies specific Leptospira genes (e.g., *lipL32* or *16S rRNA*) to confirm the presence of the pathogen.

PCR methods include conventional PCR, real-time PCR (qPCR), and nested PCR, with qPCR being preferred for its rapid turnaround time, higher sensitivity, and ability to quantify bacterial load. However, PCR requires specialized laboratory infrastructure, trained personnel, and high-quality sample handling, as contamination or degradation of DNA can affect results. Additionally, PCR may yield false-negative results in late-stage infections, where *Leptospira* is no longer present in the bloodstream [60, 61].

#### 1.3.4. Proper disposal of samples to prevent transmission.

Proper sample disposal through sterilization, incineration, and controlled burial is essential to prevent environmental contamination and human exposure to *Leptospira spp.* [62].

#### 1.3.5. Clinical signs and symptoms

- ***Symptoms in humans***

In humans, leptospirosis presents with a wide range of symptoms, often resembling other febrile illnesses. Early symptoms include fever, chills, headache, muscle pain (especially in the calves and lower back), and conjunctival redness. In more severe cases, individuals may develop jaundice, kidney failure, hemorrhages, respiratory distress, and, in extreme cases, septic shock. Given that many infections occur through direct with wildlife or indirect contact with contaminated water or soil, people engaged in wildlife handling, hunting, or butchering are at heightened risk and should be closely monitored for early signs of infection [59,61].

- ***Symptoms in Wildlife***

In wildlife, clinical cases of leptospirosis can vary significantly across species. In howler monkeys **(***Alouatta spp.***)** and spider monkeys **(***Ateles spp.***)**, symptoms may include lethargy, anorexia, fever, and jaundice, with some individuals developing neurological signs such as disorientation and muscle tremors. These primates, often hunted for bushmeat, cultural practices or kept as pets, can serve as reservoirs of *Leptospira* and contribute to zoonotic transmission. Jaguars **(***Panthera onca***)** can become infected through their prey, particularly rodents and infected ungulates. While clinical signs are less frequently documented in wild felids, infected jaguars may experience weight loss, kidney dysfunction, fever, and uveitis (eye inflammation), which could compromise their hunting ability and survival. Armadillos **(***Dasypus novemcinctus***)**, commonly consumed in Bolivia, are known carriers of various zoonotic diseases, including leptospirosis. Infected individuals may exhibit weight loss, lethargy, and, in some cases, kidney and liver abnormalities. Since armadillos often burrow in moist environments, they can contribute to the environmental persistence of *Leptospira* and serve as important reservoirs [58, 61].

### 1.3.6. Enhanced traceability of infected wild animals

In Bolivia, enhancing the traceability of wild animals, such as primates (*Alouatta spp., Ateles spp.*), jaguars, and armadillos (*Dasypus novemcinctus*), infected with leptospirosis is crucial for monitoring and controlling the spread of the disease, especially given the high biodiversity in the country and the close interactions between wildlife and human populations. To achieve this, wildlife management programs should incorporate tracking and monitoring systems that can identify and record animals exhibiting symptoms of leptospirosis, such as fever, jaundice, and kidney dysfunction. The use of collars or GPS trackers in certain species can help track their movements and habitat, allowing for better risk assessment of areas where human-wildlife interactions are frequent, particularly in forests, wetlands, and river basins, where rodent populations—the primary reservoirs—are abundant. Additionally, regular health screenings for wildlife in sanctuaries, national parks, and protected areas should be implemented, with blood and urine samples being collected from suspected cases for laboratory testing using Microscopic Agglutination Test (MAT) and PCR. Surveillance programs should also involve local communities and rangers in reporting unusual wildlife behavior or sudden deaths that might indicate leptospirosis infection. Genetic markers and environmental sampling from water sources within wildlife territories can further help trace the presence of Leptospira in the ecosystem.

### 1.3.7. Recommendations to improve passive surveillance

In Bolivia, where wild animals such as primates (*Alouatta spp., Ateles spp*.), jaguars, and armadillos (*Dasypus novemcinctus*) are considered primary reservoirs of Leptospirosis, improving passive surveillance is crucial for early detection and control of this zoonotic disease. Leptospirosis in wild animals can spill over into human populations, especially in rural and jungle areas, where people interact with wildlife either for cultural, trade, or subsistence purposes.

To enhance passive surveillance, it is essential to establish a collaborative reporting system that integrates both public health and wildlife monitoring. Veterinarians, wildlife biologists, and healthcare workers should be trained to recognize and report signs of leptospirosis in animals and humans, including fever, jaundice, muscle pain, and hemorrhagic symptoms. Regular reports from wildlife researchers and local communities who encounter these animals are critical in identifying potential exposure events.

Routine serological testing and PCR-based diagnostic methods should be recommended for monitoring wildlife populations, focusing on the species known to harbor Leptospira, such as primates, jaguars, and armadillos. In areas where these animals are encountered frequently, such as national parks or communities living near wildlife reserves, blood and urine samples should be collected from wild animals during capture and release operations, or from carcasses found in the field. These samples can then be tested for Leptospira using the Microscopic Agglutination Test (MAT) or PCR. For wild primates, fecal samples can also provide valuable insights into their potential role in environmental contamination, as rodent populations often interact with these species, further increasing the risk of transmission.

### 1.3.8. Protocols for Passive surveillance

Passive surveillance can be strengthened by collaborating with local health facilities to report cases of leptospirosis, particularly in rural regions where contact with wildlife may be more common. Public health campaigns should focus on educating local communities about the risks of leptospirosis, especially during seasonal flooding when wildlife and human populations may be more likely to share the same environment. Veterinary clinics working with domestic animals should also be included in surveillance efforts, reporting any unusual illnesses or deaths in animals that may indicate a potential spillover from wildlife. In addition to training personnel and improving sample collection methods, it is essential to implement routine testing of environmental samples from areas where humans and wildlife come into contact. Water sources frequently used by both wildlife and humans should be tested for the presence of Leptospira bacteria to assess contamination risks.

## 1.4. Passive Surveillance for Salmonellosis

Salmonellosis is a zoonotic bacterial infection caused by *Salmonella spp.*, which can spread through contaminated food, direct animal contact, and environmental exposure. Given its prevalence in wild animal species such as turtles (*Podocnemis expansa*), caimans (*Caiman yacare*), and reptiles like the boa constrictor (*Boa constrictor*), effective surveillance protocols are essential to prevent human and animal infections [59, 60].

### 1.4.1. Sample collection

To ensure reliable detection of *Salmonella spp.*, sample collection should be conducted systematically from suspected animals, environmental sources, and human cases. Fecal samples, cloacal swabs, and intestinal contents should be obtained from wild animals, particularly turtles, caimans, and reptiles, as these species are known reservoirs. Environmental samples, including water and soil from wildlife habitats, should also be collected to assess contamination levels. In human cases, stool samples and rectal swabs should be taken from symptomatic individuals, particularly those with recent contact with wildlife or bushmeat. All samples should be collected using sterile techniques, and personal protective equipment (PPE) such as gloves and face masks should be worn to prevent cross-contamination [58, 61, 62,63].

### 1.4.2. Handling and transport of samples

Proper handling and transport of samples are critical to maintaining sample integrity and preventing contamination. After collection, each sample should be placed in a sterile, leak-proof container, properly labeled with the date, location, species, and identification number. Samples must be stored at 4°C and transported in insulated, refrigerated containers to maintain viability. For long-distance transport, samples should be preserved in transport media such as Cary-Blair for fecal samples or buffered peptone water for environmental samples. Chain of custody documentation must be maintained throughout transport, and all samples must be delivered to the designated diagnostic laboratory within 24–48 hours of collection [59, 62].

### *1.4.3. Methods for testing*

Diagnostic confirmation of *Salmonella spp*. in collected samples requires a combination of culture-based and molecular methods. Samples should be inoculated into selective enrichment media such as selenite broth or Rappaport-Vassiliadis broth to enhance Salmonella growth. After incubation at 37°C for 18–24 hours, cultures should be plated onto selective agar media such as Xylose Lysine Deoxycholate (XLD) agar or Hektoen Enteric agar. Suspected Salmonella colonies should be confirmed using biochemical tests, including Triple Sugar Iron (TSI) and urease tests. For molecular confirmation, polymerase chain reaction (PCR) assays targeting the invA gene of *Salmonella spp.* should be performed. Additionally, antimicrobial susceptibility testing should be conducted to identify antibiotic-resistant strains [58, 59, 61, 62, 63].

#### 1.4.4. Proper disposal of samples to prevent transmission

To prevent the environmental spread of *Salmonella spp*., all biological waste, including unused samples, culture plates, and contaminated materials, must be decontaminated before disposal. Solid waste should be autoclaved at 121°C for at least 15 minutes, while liquid waste should be treated with 1% sodium hypochlorite for 30 minutes before disposal in designated biohazard waste containers. Surfaces, workspaces, and equipment used during sample processing must be disinfected using 70% ethanol or 10% bleach solution. Personnel handling Salmonella samples should follow strict biosafety protocols, including frequent handwashing and use of PPE, to minimize occupational exposure [58, 59, 61, 62]

#### 1.4.5. Clinical symptoms

- **Symptoms in human**

Human salmonellosis presents as an acute gastrointestinal illness, with symptoms typically appearing 6 to 72 hours after exposure. Common symptoms include diarrhea (which may be bloody), fever, abdominal cramps, nausea, and vomiting. In severe cases, particularly in immunocompromised individuals, infants, and the elderly, Salmonella infection can lead to septicemia, meningitis, or reactive arthritis. Diagnosis in human patients is confirmed through stool culture and serotyping of Salmonella isolates [60, 62,63].

- **Symptoms in wild animal**

Wild animals often act as asymptomatic carriers of *Salmonella spp.*, intermittently shedding the bacteria through feces without displaying overt signs of illness. However, under conditions of stress or immunosuppression, clinical manifestations may emerge, varying by species. In turtles (*Podocnemis expansa*), signs of infection include lethargy, decreased appetite, and soft or discolored feces, with severe cases leading to shell lesions or skin infections. Caimans (*Caiman yacare*) may experience gastrointestinal distress, weight loss, and reduced feeding behavior, while chronic infections can progress to septicemia and respiratory complications. Similarly, reptiles such as the boa constrictor (*Boa constrictor*) can exhibit regurgitation, oral lesions, and respiratory symptoms, including nasal discharge and wheezing. Given their role as reservoirs of *Salmonella*, regular surveillance of shedding in these species is essential, particularly in high-risk environments such as wildlife markets, breeding centers, and natural reserves. Monitoring efforts should be prioritized to prevent zoonotic transmission and maintain both animal and public health [58, 59, 60, 63].

#### 1.4.6. Enhanced traceability of infected animals

To prevent the spread of *Salmonella* from wildlife to humans and domestic animals, a robust traceability system should be implemented to monitor infected or at-risk animals. Wildlife tracking should incorporate electronic identification methods, such as microchips or identification tags, to facilitate long-term monitoring of infected turtles, caimans, and reptiles. Additionally, a centralized national database should be established to document the capture, trade, and movement of potentially infected animals. This database must be accessible to conservation agencies, health authorities, and law enforcement to ensure coordinated efforts in disease control and wildlife management. Quarantine protocols should be enforced for any wildlife suspected or confirmed to be carrying *Salmonella spp.*, requiring a minimum isolation period of 30 days. During this time, repeated fecal testing should be conducted to assess bacterial shedding status and determine whether an animal can be safely released. Strict movement restrictions must also be applied to prevent the release or sale of infected animals until they test negative for *Salmonella*. Special precautions should be taken when reintroducing rehabilitated wildlife into protected areas to prevent potential environmental contamination and cross-species transmission [60, 61, 62].

#### 1.4.7. Recommendations to improve passive surveillance

The effective surveillance and control of Salmonella in Bolivia require a robust regulatory framework that addresses food safety concerns in both domestic livestock and wild animal species hunted for consumption, trade, and cultural practices. Strengthening food safety regulations at all levels of the meat supply chain—from slaughtering and processing to distribution and consumption—is critical to minimizing Salmonella contamination and reducing the risk of foodborne illness. The following recommendations focus on regulatory enforcement, microbiological surveillance, hygiene practices, policy implementation, and sustainable alternatives to high-risk meat sources.

- **Strengthening Meat Processing Regulations for Domestic Livestock and Wildlife**

To address Salmonella contamination in meat products, Bolivia must implement stricter regulations for the slaughter, processing, and sale of meat from both domestic livestock and wild animals. This includes enforcing proper handling protocols and rigorous hygiene measures throughout all stages of meat processing. The SENASAG should conduct routine microbiological inspections of slaughterhouses, butcher shops, and wild game processing facilities to monitor Salmonella contamination. Additionally, meat processing facilities must adhere to Hazard Analysis and Critical Control Points (HACCP) protocols to identify and mitigate contamination risks at every processing stage. Mandatory hygiene certification programs for all meat handlers, including butchers and slaughterhouse workers, should be implemented before they are allowed to handle raw meat. Meat that fails microbiological safety tests must be removed from the distribution chain immediately, and facilities with recurrent violations should face fines or shutdowns. For wild species such as capybaras (*Hydrochoerus hydrochaeris*), peccaries (*Tayassu pecari*), and primates must undergo veterinary inspections before being sold or consumed, to mitigate the risk of Salmonella transmission. The hunting and processing of wild animals for commercial sale must be confined to authorized slaughtering centers, where veterinary professionals can inspect meat for zoonotic pathogens. To prevent cross-contamination, slaughterhouses and butcheries should designate separate processing areas for domestic livestock and wild game meat. Strict protocols must be established for handling and processing meat, requiring the use of separate knives, cutting boards, and storage containers for different types of meat. Furthermore, the use of chlorine-based disinfectants or organic acids, such as lactic acid sprays, should be mandatory for post-slaughter carcass decontamination in both livestock and wild game processing. Finally, the evisceration process should adhere to stringent guidelines to prevent contamination from intestinal contents during slaughter.

- **Enhancing Microbiological Surveillance and Testing for Salmonella**

A national passive surveillance program for Salmonella should be established to ensure regular testing of meat products, environmental monitoring, and targeted screening of high-risk wildlife species. SENASAG must implement mandatory microbiological testing for poultry, cattle, and wild game meat prior to distribution to markets and restaurants. Additionally, rapid PCR-based detection of *Salmonella* spp. should be mandated in slaughterhouses to facilitate the early identification and removal of contaminated meat. Environmental testing for *Salmonella* should be carried out in processing plants, cold storage facilities, and meat markets, with samples collected from work surfaces, tools, and wastewater. To monitor wildlife in high-risk areas, surveillance teams must collect fecal and tissue samples from wildlife species known to harbor *Salmonella*, such as capybaras, peccaries, primates, and reptiles, particularly in regions where these animals interact with domestic livestock. Local conservation agencies should collaborate with researchers to identify geographic hotspots where *Salmonella* transmission between wildlife and livestock is prevalent. The passive surveillance system should involve a collaborative network of veterinarians, park rangers, and indigenous communities, enabling real-time reporting of disease outbreaks related to bushmeat consumption.

- I**mproving Hygiene Practices in Meat Handling and Trade**

Improving hygiene practices in meat handling and trade is critical to minimizing the risks of Salmonella transmission in meat markets, food establishments, and households. The Bolivian Ministry of Health, in collaboration with NGOs and local authorities, should implement education programs aimed at hunters, butchers, meat vendors, and community leader providing them with clear guidelines on meat safety and proper cooking temperatures. Informational dissemination, including radio programs, posters and leaflets outlining safe meat handling practices, should be prominently displayed in open-air markets, slaughterhouses, and community centers. Training programs must emphasize the importance of personal hygiene practices, such as handwashing after handling raw meat and disinfecting work surfaces. In addition, strict hygiene enforcement should be implemented in markets and restaurants. Open-air meat markets must establish mandatory disinfection routines to ensure that meat stalls and cutting boards are regularly sanitized using approved antimicrobial agents. Vendors should be required to wear protective gloves, masks, and aprons when handling raw meat to minimize pathogen transmission. Furthermore, restaurants and food vendors must be legally obligated to cook meat to temperatures above 75°C to eliminate any potential Salmonella contamination before consumption.

- **Strengthening Legal Frameworks and Policy Implementation**

To strengthen food safety, Bolivia's food safety laws must be updated to incorporate more stringent regulatory frameworks for meat handling and processing. Specifically, species-specific food safety regulations for wild game meat must be established to ensure uniform sanitary standards across all processing sectors. The illicit slaughter and sale of uninspected meat should be subject to criminal penalties, with repeat offenders facing fines and imprisonment. Additionally, enforcement agencies must be adequately resourced and staffed to conduct unannounced inspections of meat markets, restaurants, and hunting operations.

A coordinated multi-sector approach is essential for effective Salmonella surveillance and response. The Ministry of Health, SENASAG, and conservation authorities must collaborate to implement a national strategy for managing Salmonella outbreaks. Rapid detection systems for Salmonella should be integrated with Bolivia's national disease reporting platform, enabling immediate public health alerts upon detection of contamination, thereby facilitating a swift and coordinated response.

#### 1.4.8. Protocols for Passive surveillance

For passive surveillance of salmonellosis it is vital that laboratories and healthcare facilities in Bolivia are required to report gastroenteritis cases potentially linked to Salmonella infections, especially among individuals who handle wild meat or wildl animals as well as those who work in related industries. Fecal samples from suspected cases in both humans and animals should be systematically collected and tested using culture and PCR methods for accurate confirmation of infection. Additionally, trace-back investigations should be implemented to identify sources of outbreaks that may be associated with contaminated food products, particularly in regions where the trade of wild meat is prevalent.

### 1.5. Passive Surveillance for Toxoplasmosis

In Bolivia, where wildlife plays a prominent role in both local diets and cultural practices, the risk of *Toxoplasma* transmission from wildlife to humans is particularly relevant. Many indigenous communities and local populations rely on wild animals, such as deer (*Mazama americana*), black caimans (*Melanosuchus niger*) and other mammals, as part of their traditional cuisine, and these species may serve as reservoirs for *Toxoplasma* infections. Given the lack of robust surveillance systems, the potential for contamination of wild game meat through hunting, processing, and consumption remains a significant public health threat [64, 65].

#### 1.5.1. Sample collection

Biological specimens are collected from both suspected infected wild animals and humans in high-risk areas. For wildlife, blood, tissue (brain, muscle, or organs), and fecal samples should be obtained from felines (*Panthera onca*), deer (*Mazama americana*), and black caimans (*Melanosuchus niger*). For human surveillance, blood samples should be collected from individuals with suspected infections, particularly in communities involved in wildlife trade, bushmeat consumption, or close interaction with wild animals. All samples should be labeled correctly with essential metadata, including species, geographic location, date, and clinical observations [66,67].

#### 1.5.2. Handling and transport of samples

Proper handling and transport of toxoplasmosis samples are crucial to maintaining sample integrity and preventing cross-contamination. Blood samples should be collected in anticoagulant-treated tubes (EDTA or heparin) and stored at 4°C until processing. Tissue samples should be preserved in sterile containers with physiological saline or stored in 10% buffered formalin for histopathological examination. Fecal samples must be refrigerated at 4°C and processed within 48 hours or stored in a fixative for prolonged transport. Samples should be transported using biohazard-labeled containers with adequate insulation to maintain temperature stability. All sample transfers must comply with Bolivia's biosafety regulations and international guidelines for zoonotic pathogen transportation [68,69,70].

#### 1.5.3. Methods for testing

Toxoplasmosis diagnosis in both humans and animals is primarily achieved through serological, molecular, and histopathological methods, each offering specific advantages for detecting Toxoplasma gondii infections.

- **Serological Testing**

In the case of serological testing, the *Indirect Fluorescent Antibody Test* (IFAT) and the *Enzyme-Linked Immunosorbent Assay* (ELISA) are commonly used to detect antibodies against *T. gondii* in blood samples. The IFAT utilizes fluorescently labeled secondary antibodies that bind to the host's antibodies, allowing the identification of *T. gondii*-specific antibodies under a microscope. This method is highly sensitive and can detect both acute and chronic infections, but it does not provide a definitive indication of active infection [68]. The ELISA test, on the other hand, uses antigen-coated plates to capture antibodies present in the serum. A color change in the assay indicates a positive result, helping in the screening of large numbers of samples, and is especially useful for large-scale surveillance efforts in both human and animal populations [69].

- **Molecular Diagnostics**

Molecular diagnostic methods, particularly *Polymerase Chain Reaction* (PCR), are essential for detecting *T. gondii* DNA, offering the highest level of specificity for confirming infections [71]. PCR can be used to amplify *T. gondii* DNA from blood, tissues, or feces, enabling detection even in the early stages of infection or when low parasite loads are present. In wildlife surveillance, PCR of tissue samples is the gold standard for diagnosing toxoplasmosis, as it can pinpoint the presence of the parasite in various tissues, such as lymph nodes, liver, and brain, which may harbor *T. gondii* cysts or tachyzoites. This method provides definitive evidence of infection, as it identifies the genetic material of the parasite, distinguishing it from other similar pathogens [67].

- **Histopathological Examination**

Histopathological examination, using *Hematoxylin and Eosin* (H&E) staining, is another crucial method for diagnosing toxoplasmosis, particularly in tissue samples from both humans and animals. This technique involves preparing tissue sections, staining them with H&E, and examining them under a microscope to identify the characteristic cysts or tachyzoites of *T. gondii*. The tissue samples typically come from organs where the parasite is likely to persist, such as the brain, liver, or muscles. In wildlife surveillance, this method allows for the detection of tissue cysts or active tachyzoites in infected animals, confirming the presence of the parasite. Histopathological examination provides visual confirmation of infection, but it requires well-preserved tissue samples and expertise in identifying parasite forms [69, 72].

#### 1.5.4. Proper disposal of samples to prevent transmission

Proper disposal of biological samples is essential to prevent environmental contamination and secondary transmission of *T. gondii*. All biological waste, including blood, tissue, and fecal samples, should be treated as biohazardous material. Samples must be incinerated or autoclaved at 121°C for 30 minutes before disposal. Laboratory equipment, including pipettes and work surfaces, should be disinfected using a 10% bleach solution or ethanol. Personal protective equipment (PPE), such as gloves and masks, must be discarded in designated biohazard containers. In field settings, carcasses of infected animals must be burned or buried at least 1.5 meters deep to prevent scavenger access [69,70, 72].

### 1.5.5. Clinical symptoms

- **Symptoms in human**

Infected humans may exhibit flu-like symptoms, including fever, muscle aches, fatigue, and swollen lymph nodes. In immunocompromised individuals, such as those with HIV/AIDS, toxoplasmosis can lead to severe complications, including encephalitis, seizures, confusion, and respiratory distress. Congenital toxoplasmosis, resulting from maternal transmission during pregnancy, can cause severe birth defects, including hydrocephalus, blindness, and developmental delays [65,73, 74].

- **Symptoms in wild animal**

Jaguars may develop neurological signs such as incoordination, tremors, and weakness. Infected individuals may also exhibit weight loss, lethargy, and ocular lesions. Some jaguars may act as asymptomatic carriers, shedding *T. gondii* oocysts into the environment through feces. Infected deer often show respiratory distress, anorexia, and muscle weakness. Neurological symptoms, including circling and ataxia, have been observed in advanced cases. Mortality rates are higher in juvenile deer and pregnant females. Reptiles such as black caimans rarely show overt symptoms, but chronic infections can lead to lethargy, reduced feeding behavior, and localized granulomatous lesions in tissues. Histopathological analysis is required to confirm *T. gondii* infection in this species [65,66, 68,72].

### 1.5.6. Enhanced traceability of infected animals

To monitor and control the spread of toxoplasmosis, traceability systems must be implemented for both domestic and wild animal populations. Infected or suspected animals should be microchipped or tagged with unique identifiers for tracking purposes. Geographic information system (GIS) mapping can be used to monitor outbreak locations and identify high-risk transmission zones. Data collection should include the movement of infected animals, interaction with other species, and potential environmental contamination. Surveillance teams must collaborate with local conservation agencies and veterinary authorities to ensure coordinated monitoring efforts [67,72].

### 1.5.7. Recommendations to improve passive surveillance

To improve passive surveillance and mitigate the risks associated with *Toxoplasma gondii* infections, particularly from the consumption of undercooked wild animal meat, several key recommendations are proposed. First, targeted educational campaigns should be implemented for pregnant women, immunocompromised individuals, and other vulnerable groups, focusing on the risks of handling and consuming undercooked or raw wild animal meat known to be potential carriers of *Toxoplasma gondii*. These campaigns should highlight the potential impact of toxoplasmosis on maternal health and fetal development, emphasizing the importance of safe meat handling and proper cooking temperatures, ideally above 75°C, to ensure parasite destruction. Local radio, television, and social media platforms can be utilized to reach rural areas where wild meat consumption is prevalent, and community workshops can be organized in collaboration with healthcare providers to offer practical advice on meat safety.

In addition to education, public health initiatives should focus on routine toxoplasmosis screening for at-risk individuals, including pregnant women and immunocompromised patients. Regular toxoplasmosis serology testing should be incorporated into prenatal care, particularly in regions with high consumption and handling of wild game meat and wild animals, to enable early detection and prevent congenital toxoplasmosis. This early identification can allow for timely treatment to protect the fetus. Likewise, routine screening for immunocompromised individuals, such as those with HIV/AIDS or organ transplant recipients, should be implemented to detect infections early and prevent severe complications. Healthcare providers, including obstetricians, gynecologists, and primary care physicians, should be trained to recommend testing and inform patients about the risks of wild meat consumption, along with safe cooking practices as well as handling of wild animals.

To support these efforts, a national passive surveillance system for toxoplasmosis should be established, linking healthcare facilities, veterinarians, and local health authorities to monitor infection rates in both humans and animals. This system would allow for the tracking of incidence trends and inform public health policies. Collaboration with wildlife health authorities is also essential to monitor *T. gondii* prevalence in wild animal populations as potential carriers. Such data would help identify high-risk areas for transmission and enable more targeted educational outreach. Finally, community outreach programs should be implemented in rural and remote areas to educate local populations on the safe hunting, handling, and cooking of wild game meat. These programs should emphasize hygiene practices such as proper handwashing after handling raw meat and sanitizing cutting surfaces to reduce *T. gondii* transmission. Additionally, incentivizing local markets and meat vendors to adopt safe practices, such as using temperature-controlled storage and providing clear labeling of inspected and processed meat, can further reduce the risk of infection.

#### 1.5.8. Protocols for Passive surveillance

In terms of passive surveillance, it is vital to report human cases of congenital toxoplasmosis or severe infections to health authorities, ensuring timely intervention. Surveillance should also include serum sample collection from at-risk populations such as hunters, butchers, and those involved in trade, cultural practices or consumption of wild meat. Testing these samples with IgG/IgM ELISA can provide information about active or past infection. Additionally, monitoring wild animals for *Toxoplasma gondii* is necessary to understand the prevalence of the parasite in the local ecosystem. Surveillance should focus on rodents, jaguars, deers and reptiles, which are known to be natural reservoirs. Testing these species through serology and PCR-based methods can help map out hotspots of Toxoplasma in wildlife populations, providing valuable information to prevent human exposure from wildlife-related sources.

## SECTION 3: RISK ANALYSIS AND MANAGEMENT STRATEGY THAT IDENTIFIES THE MOST EXPOSED INSTANCES, THE PROCESSES WITH THE GREATEST RISK, AND STRATEGIES TO MITIGATE THESE RISKS.

### 1. Identification of the most exposed Instances for Zoonotic Disease Transmission in Bolivia.

The trade and use of wildlife in Bolivia present significant public health risks due to the potential transmission of zoonotic diseases. Three primary pathways—pet ownership, bushmeat consumption, and traditional medicine—serve as the most critical points of exposure, each carrying distinct risks due to direct and indirect contact with infected animals or their byproducts. Addressing these risks requires a multi-sectoral approach, including stronger regulations, enhanced public awareness, and improved health surveillance. The following paragraphs identify the high risks instances for zoonotic spillovers which was analyzed from the technical report that focuses on the analysis of zoonotic risks associated with wild animals in Bolivia.

- **Pet Ownership**

The practice of keeping wild animals as pets is prevalent in Bolivia, particularly involving primates (*Alouatta spp., Ateles spp*.), psittacine birds, and small carnivores. These animals act as reservoirs for several zoonotic pathogens, including rabies, psittacosis, salmonellosis, and simian foamy virus (SFV). Transmission occurs through bites, scratches, contaminated feces, saliva, and urine. Additionally, escaped or abandoned wild pets can become invasive species, disrupting ecosystems and facilitating further disease spread. Birds, particularly parrots and macaws, are a significant concern, as they frequently harbor *Chlamydia psittaci*, the causative agent of psittacosis, which can lead to severe respiratory infections in humans.

- **Bushmeat Consumption**

The hunting, butchering, and consumption of wild meat are widely practiced, both for subsistence and as a luxury market item. Species commonly consumed include armadillos (*Dasypus novemcinctus*), deer, peccaries, and capybaras, all of which have been identified as carriers of pathogens such as *Brucella spp., Leptospira spp.,* and *Salmonella spp*.. During hunting and slaughter, individuals are at risk of exposure to bloodborne diseases such as brucellosis and leptospirosis, which can be contracted through direct contact with contaminated tissues. Additionally, the consumption of undercooked or improperly stored meat increases the risk of parasitic infections, including toxoplasmosis, trichinosis, and hydatidosis, with hydatidosis being particularly severe due to the formation of cysts that require surgical intervention. The lack of sanitary conditions during meat processing and transport exacerbates the risk of foodborne illnesses such as campylobacteriosis and colibacillosis.

- **Traditional Medicine**

The use of animal parts for medicinal purposes is a culturally significant but high-risk practice in Bolivia. Wild animal products such as blood, amphibian extracts, and mammalian organs are commonly sold in traditional markets like the Witches' Market in La Paz and rural fairs. Among the most concerning practices is the consumption of liquefied amphibians, such as the Titicaca water frog (*Telmatobius culeus*), which poses a high risk for *Aeromonas spp.* and

*Vibrio spp*. infections, leading to severe gastrointestinal illness. Handling animal-derived materials without protective measures increases exposure to Venezuelan encephalitis, trypanosomiasis (Chagas disease), and leptospirosis, all of which have serious health implications. Additionally, bats and wild rodents used in folk remedies can harbor emerging viral pathogens such as coronaviruses, lyssaviruses (rabies-related), and hantaviruses.

## 2. High-Risk Processes and Factors for Zoonotic Disease Emergence in Bolivia

The probability of zoonotic disease emergence in Bolivia is closely linked to specific high-risk practices involving human-animal interactions, food consumption, and cultural traditions. These risks are particularly evident in pet ownership of wild animals, bushmeat consumption, and the use of animal products in traditional medicine. The informal nature of these activities, combined with weak regulatory enforcement and limited public awareness, increases the potential for zoonotic disease transmission. The following section present high risk processes and factors influencing zoonotic spillovers in Bolivia.

- **Pet Ownership**

The practice of keeping wild animals as pets in Bolivia poses a significant risk for zoonotic disease transmission. Many individuals, particularly in rural and indigenous communities, capture and raise wild animals such as monkeys, parrots, and felines as exotic pets. This increases human exposure to pathogens carried by these animals, particularly through direct contact, bites, scratches, and exposure to bodily fluids. Diseases such as rabies, leptospirosis, and psittacosiscan be transmitted through saliva, urine, or respiratory secretions. In addition, feces and urine from infected animals can contaminate household environments, increasing the likelihood of disease spread to family members. The illegal wildlife trade exacerbates this problem, as many wild animals are captured, transported, and sold under unsanitary conditions, making disease control and monitoring extremely difficult. The lack of veterinary oversight and proper quarantine measures further increases the risk of introducing zoonotic pathogens into human populations.

- **Bushmeat Consumption**

The hunting and consumption of bushmeat remain widespread in Bolivia, particularly among indigenous and rural communities where wild animals provide an important source of protein. However, this practice presents significant health risks due to the potential for zoonotic disease transmission. Processing and handling risks arise during the butchering of wild animals, as direct contact with blood, organs, and tissues can expose hunters and meat processors to pathogens such as brucellosis, salmonellosis, and tuberculosis. Improper hygiene and the use of contaminated knives, cutting boards, and work surfaces further increase cross-contamination risks. Undercooked meat consumption is another major concern, as diseases such as trichinosis and toxoplasmosis are commonly transmitted through improperly cooked or raw meat. Many zoonotic parasites and bacteria remain viable if the meat is not heated to sufficient temperatures, posing a serious risk of foodborne illness. Additionally, the lack of health regulations in informal wildlife meat markets makes disease control difficult, as bushmeat is often sold without veterinary inspections or adherence to food safety standards. This uncontrolled trade increases the likelihood of introducing novel pathogens into human populations, similar to past zoonotic spillover events.

- **Traditional Medicine**

The use of animal parts in traditional medicine is another high-risk practice contributing to zoonotic disease transmission in Bolivia. Various species, including jaguars, snakes, birds, and amphibians, are harvested for their bones, organs, and secretions, which are believed to have medicinal properties. However, direct handling and ingestion of these raw animal products can transfer zoonotic agents such as leptospirosis and brucellosis to healers and consumers. Many of these products are processed and consumed without boiling, cooking, or sterilization, increasing the likelihood of infection.

Furthermore, protective measures such as gloves, masks, and proper sanitation are rarely used, exposing practitioners and patients to potentially dangerous pathogens. Despite the health risks, these practices remain deeply ingrained in Bolivia's cultural and indigenous traditions, making behavioral change difficult. Many communities view these treatments as essential for spiritual healing and disease prevention, creating resistance to public health interventions aimed at reducing zoonotic risks. Addressing these challenges requires targeted education, engagement with traditional healers, and the promotion of safer alternatives to high-risk animal-based remedies.

## 3. Risk Management Strategies for Zoonotic Disease Prevention in Bolivia

Bolivia faces significant challenges in mitigating the risks associated with zoonotic disease transmission, particularly due to the wildlife trade, bushmeat consumption, traditional medicine practices, and pet ownership of wild animals. To effectively address these risks, a multi-level approach integrating policy enforcement, surveillance, education, and behavioral change is required. The following risk management strategies focus on regulatory enforcement, improved disease surveillance, public awareness, and enhanced handling practices to reduce zoonotic spillover and protect both human and wildlife populations.

### 3.1. Regulatory and Policy Measures

Bolivia's regulatory framework must be strengthened to control illegal wildlife trade, improve sanitary standards in bushmeat markets, and prohibit high-risk animal sales to reduce zoonotic disease transmission.

- **Stronger Law Enforcement Against Illegal Wildlife Trade**

Illegal wildlife trade remains a major contributor to zoonotic disease transmission in Bolivia, as many species are captured, transported, and sold under unsanitary conditions that facilitate pathogen spread. The Ministry of Environment and Water and the National Service of Protected Areas (SERNAP) must enhance regulations governing wildlife trade by implementing stricter licensing requirements for traders and imposing harsher penalties on those caught trafficking high-risk species. Enforcement capacity must be increased for the Forestry and Environmental Preservation Police, which plays a crucial role in intercepting illegal wildlife shipments. For example, monitoring efforts should be intensified in regions where wildlife trafficking is most prevalent, such as the Amazonian and Chiquitano forests, where species such as primates, jaguars, and wild birds are frequently captured for trade.

- **Sanitary Standards for Wildlife Trade**

Poor hygiene practices in wildlife markets and bushmeat processing sites significantly increase the risk of zoonotic disease transmission. To address this, Bolivia must establish mandatory disease screening protocols for legally sold wildlife products, ensuring that all animal carcasses and meat sold in markets are inspected for Salmonella, Brucella, and other important zoonotic pathogens. Additionally, bushmeat markets should be regulated and inspected regularly, requiring vendors to implement proper hygiene measures such as wearing gloves, using clean butchering equipment, and maintaining separate processing areas for different species. Similar to food safety standards applied to domestic livestock, regulatory agencies should develop specific sanitary guidelines for wildlife meat handling to prevent cross-contamination and pathogen spread.

- **Ban on High-Risk Animal Trade for Pets**

Many zoonotic pathogens originate from wild animals that are kept as pets. Species such as primates and birds are commonly captured and sold in Bolivia's illegal pet trade, posing a significant risk for pathogen spillover to humans. To mitigate this, Bolivia should prohibit the sale of species known to carry zoonotic diseases, particularly primates (which carry herpes B and tuberculosis) and wild birds (which can spread avian influenza and psittacosis). Additionally, the government should implement public awareness campaigns highlighting the dangers of keeping wild animals as pets, particularly in urban areas where exotic pet ownership is growing.

## 3.2. Surveillance and Monitoring

An effective zoonotic disease surveillance system is crucial for early detection and rapid response to emerging health threats. Strengthening Bolivia's national passive surveillance system will allow authorities to track disease outbreaks and implement timely mitigation measures.

- **Zoonotic Disease Surveillance Network**

Bolivia must adopt a One Health approach, integrating the Ministry of Health, SENASAG, and conservation agencies into a unified zoonotic disease surveillance network. This will allow authorities to share real-time data on emerging pathogens and develop coordinated outbreak response plans. A passive surveillance system should be implemented to monitor zoonotic pathogens in domestic animals, wildlife, and human populations, with routine testing of high-risk species such as rodents, bats, and non-human primates in areas where human-wildlife interaction is high.

- **Early Warning System for Zoonotic Disease Outbreaks**

Rapid detection of zoonotic outbreaks is essential to prevent large-scale epidemics. Bolivia should establish rapid response teams trained to investigate suspected zoonotic disease cases in humans and animals. This requires equipping laboratories with advanced diagnostics, including PCR-based testing for pathogens such as leptospirosis, brucellosis, and toxoplasmosis. Additionally, mobile diagnostic units should be deployed in remote regions with high bushmeat consumption and illegal wildlife trade to improve early detection capabilities.

## 3.3. Public Awareness and Behavioral Change

A key aspect of zoonotic disease prevention is changing public behaviors through education, awareness campaigns, and economic alternatives to high-risk practices.

- **Educational Campaigns on Zoonotic Disease Risks**

Many rural communities in Bolivia lack knowledge about the dangers of zoonotic disease transmission from wildlife. The Ministry of Health and conservation NGOs should develop educational programs that inform communities about the risks of keeping wild pets, consuming bushmeat, and handling raw animal products in traditional medicine. Hygiene promotion should be a priority, encouraging people to wear gloves when handling wild animals, properly cook meat, and avoid direct contact with high-risk species.

- **Alternative Livelihoods for Wildlife Traders**

To reduce dependence on the illegal wildlife trade and bushmeat consumption, Bolivia must promote sustainable economic alternatives that provide viable income sources while minimizing zoonotic disease risks. Ecotourism, sustainable livestock farming, and beekeeping can serve as financially viable alternatives for communities engaged in wildlife hunting and bushmeat sales. The government should support ex-hunters and traders through microfinance programs and training initiatives, helping them transition to legal, disease-free livelihoods.

Additionally, developing alternative protein sources is crucial for reducing reliance on bushmeat, particularly in rural and indigenous communities. The Bolivian government should invest in community-led poultry and livestock farming projects, ensuring access to safe and regulated protein sources. Expanding aquaculture programs in riverine communities can provide sustainable fish protein, reducing the need for hunting wild species.

Furthermore, economic incentives should be offered to indigenous groups that transition from subsistence hunting to eco-friendly livestock farming, promoting both food security and biodiversity conservation.

## 4. Risk Mitigation Strategies

Bolivia must implement stricter penalties against illegal wildlife trade and unsanitary bushmeat markets to deter activities that contribute to zoonotic spillover. The Ministry of Environment and Water and the National Service of Protected Areas must enforce wildlife protection laws, such as Law No. 1333 on the Environment, which regulates the conservation and sustainable use of biodiversity. Additionally, the Forestry and Environmental Preservation Police should increase inspections and improve monitoring systems to detect and dismantle wildlife trafficking networks. To ensure food safety, Bolivia must require mandatory health certification for all legally traded wildlife products, similar to the sanitary standards enforced by SENASAG for domestic livestock. Implementing systematic disease screening and hygiene protocols in bushmeat markets will help prevent contamination and pathogen transmission. Furthermore, the use of high-risk species in traditional medicine—such as primates, and wild birds known to carry zoonotic pathogens—should be restricted through updated legal frameworks, with alternative plant-based remedies promoted to reduce dependency on wildlife-derived treatments.

Educational outreach in high-risk regions is essential to changing behaviors that contribute to zoonotic disease emergence. The Ministry of Health, in collaboration with non-governmental organizations (NGOs) and local governments, should conduct workshops and awareness campaigns on the dangers of handling wild animals, consuming bushmeat, and poor sanitation practices in wildlife markets. These initiatives should particularly target Amazonian and Chaco regions, where bushmeat consumption and wildlife trade are most prevalent. Promoting alternative protein sources such as sustainable poultry, livestock farming, and aquaculture programs can help reduce dependence on bushmeat, mitigating disease exposure risks. Additionally, training local health workers in disease surveillance, pathogen identification, and rapid outbreak response will enhance early detection and containment capabilities in remote communities.

Bolivia must increase investments in veterinary and public health training programs to strengthen zoonotic disease monitoring and response capabilities. Providing specialized training for veterinarians, biologists, and public health officials will ensure that professionals can identify, diagnose, and manage emerging zoonotic threats. Strengthening partnerships between government agencies, conservation organizations, and research institutions—such as the National Institute of Health Laboratories —is necessary to facilitate data sharing and coordinated response efforts. Additionally, upgrading national laboratory infrastructure by equipping regional diagnostic centerswith PCR-based pathogen detection technologies will improve the speed and accuracy of zoonotic disease identification, reducing response times and minimizing outbreak risks.

## 5. Implementation Plan and Stakeholder Roles

| Strategy | Implementing Agencies | Short-Term Actions | Long-Term Actions |
|---|---|---|---|
| **Wildlife trade enforcement** | POFOMA, SENASAG, MMAyA | Increase wildlife market inspections | Strengthen penalties for illegal trade |
| **Zoonotic disease surveillance** | Ministry of Health, SENASAG | Passive surveillance in pet markets | Establish wildlife disease monitoring labs |
| **Public education** | NGOs, local governments | Awareness campaigns in high-risk areas | Integrate zoonotic education in school curricula |
| **Alternative livelihoods** | Ministry of Rural Development | Training for ex-wildlife traders | Sustainable ecotourism programs, livestock farming and aquaculture. |
| **Safe handling practices** | Health and veterinary agencies | Provide protective gear to hunters | Enforce sanitation laws for bushmeat markets |

## 6. Recommended Monitoring and Evaluation Framework for Zoonotic Disease Prevention in Bolivia.

To assess the effectiveness of Bolivia's zoonotic disease prevention strategies, a structured monitoring and evaluation system must be established. This includes Key Performance Indicators (KPIs), annual risk assessments, and community engagement metrics to measure progress in reducing illegal wildlife trade, improving public health outcomes, and strengthening compliance with food safety regulations. These indicators will help government agencies, conservation organizations, and public health authorities adjust policies and interventions based on data-driven insights.

### 6.1. Key Performance Indicators (KPIs)

To track progress in zoonotic disease mitigation, Bolivia must establish quantifiable benchmarks across law enforcement, public health, and market compliance. The following KPIs will serve as measurable targets to ensure that policy enforcement, public education, and surveillance efforts produce tangible results:

- **Reduction in Illegal Pet Trade Cases by 30% Within Five Years**

The illegal wildlife pet trade is a major driver of zoonotic disease transmission, as exotic pets such as primates, parrots, and reptiles can harbor pathogens that spill over to humans. To monitor the effectiveness of law enforcement efforts, Bolivia should aim for a 30% decrease in reported illegal pet trade cases within five years. This will be measured by:

- Increased arrests and fines imposed on illegal wildlife traders through POFOMA-led operations.
- Stronger enforcement of wildlife protection laws, including Law No. 1333 (Environmental Law) and CITES (Convention on International Trade in Endangered Species) regulations.
- Improved surveillance at key trafficking hotspots, such as markets in La Paz, Cochabamba, and Santa Cruz, and along border regions with Brazil and Peru.
- Expanded public education campaigns to discourage demand for exotic pets and inform citizens about legal consequences.

- **Decrease in Reported Zoonotic Infections Linked to Wildlife by 20%**

Reducing zoonotic disease cases associated with wildlife exposure is critical for public health. Bolivia should aim for a 20% reduction in confirmed zoonotic infections (e.g., leptospirosis, toxoplasmosis, brucellosis, avian influenza, and rabies) linked to bushmeat consumption, wildlife handling, and traditional medicine practices. Progress will be tracked by:

- Data from the Ministry of Health and SENASAG's passive surveillance system, monitoring hospital and veterinary clinic reports of zoonotic disease cases.
- Increased laboratory testing capacity, enabling faster diagnosis and response to emerging outbreaks.
- Public health interventions, including vaccination campaigns for high-risk populations and expanded hygiene education programs in rural communities.
- Monitoring wildlife populations for zoonotic pathogens through environmental sampling and necropsies in protected areas.

- **Increase in Safe Handling Compliance in Bushmeat Markets by 50%**

Bushmeat markets present a high risk for pathogen transmission due to poor hygiene, improper meat storage, and lack of veterinary oversight. Bolivia should aim for a 50% increase in compliance with food safety standards in wild game markets by:

- Mandatory disease screening of bushmeat sold in markets, ensuring compliance with SENASAG regulations.
- Regular health inspections of bushmeat vendors to verify proper handling, storage, and processing practices.

- Training programs for butchers, hunters, and vendors, focusing on safe slaughtering techniques, cross-contamination prevention, and proper meat preparation.
- Stronger enforcement of market sanitation protocols, including the use of protective gear (gloves, masks, and aprons) to prevent direct exposure to animal fluids.

### 6.2. Annual Risk Assessments

To ensure ongoing effectiveness, Bolivia must implement annual risk assessments to evaluate zoonotic disease trends and policy effectiveness. These assessments should include:

- **Analysis of Surveillance Data**
  - Review zoonotic pathogen reports from public hospitals, veterinary clinics, and wildlife monitoring programs.
  - Identify emerging disease threats linked to human-wildlife interactions.
  - Adjust public health interventions and market regulations based on infection trends.

- **Legislative Updates**
  - Assess compliance with national and international wildlife trade laws.
  - Strengthen legal frameworks, such as introducing stricter penalties for illegal wildlife trade if enforcement gaps are identified.
  - Update sanitary regulations for bushmeat trade and wildlife markets based on annual inspection findings.

- **Geospatial Risk Mapping**
  - Use GIS technology to map disease hotspots based on confirmed cases of zoonotic infections.
  - Prioritize high-risk regions (Amazon basin, Chiquitano forests, and Andean valleys) for targeted interventions.

### 6.3.Community Engagement Metrics

Community engagement is crucial for long-term success, as changing cultural behaviors and trade practices requires education, participation, and alternative economic solutions. Bolivia must monitor awareness levels, behavioral change, and local compliance through:

- **Surveys on Public Knowledge and Attitudes Toward Zoonotic Diseases**
  - Conduct pre- and post-education campaign surveys to measure changes in awareness about zoonotic disease risks, hygiene practices, and wildlife trade impacts.
  - Target key populations, including indigenous groups, bushmeat vendors, and traditional medicine practitioners.

- **Tracking Adoption of Alternative Livelihoods**
  - Monitor participation rates in sustainable livelihood programs, such as ecotourism, poultry farming, and aquaculture.
  - Assess economic shifts away from wildlife trade, ensuring sustainable income sources for former hunters and traders.

- **Measuring Compliance with Hygiene Standards in Markets**
  - Conduct randomized inspections to evaluate vendor adherence to safe meat handling protocols.
  - Assess handwashing station use, protective gear compliance, and sanitation practices in high-risk markets.

## SECTION 4: MONITORING AND EVALUATION

Monitoring and evaluation (M&E) are essential components of passive surveillance systems for zoonotic diseases in Bolivia, ensuring continuous assessment and adaptive management to respond effectively to emerging public health threats. Given the country's complex human-wildlife interface, extensive bushmeat trade, and cultural use of wild animal products, a robust surveillance framework is necessary for early detection, risk mitigation, and policy refinement. Continuous assessment allows health authorities to systematically analyze epidemiological trends, case reports, and sentinel species data, facilitating the early identification of zoonotic disease outbreaks such as leptospirosis, brucellosis, and rabies. Without routine evaluation, critical signals indicating increased pathogen circulation within high-risk areas, such as wildlife markets or bushmeat trade hubs, may be overlooked, delaying intervention efforts.

The dynamic nature of Bolivia’s ecosystems and socio-environmental factors requires an adaptive management approach to ensure surveillance strategies remain responsive to new epidemiological patterns. Continuous monitoring enables real-time adjustments to surveillance protocols, allowing authorities to reallocate resources based on emerging risks. For instance, an observed increase in primate trafficking linked to simian foamy virus (SFV) or yellow fever cases would necessitate targeted interventions in specific wildlife markets and trade routes. Similarly, climatic variations influencing vector-borne disease transmission, such as changes in mosquito populations affecting arboviral outbreaks, highlight the need for flexible surveillance measures. Integrating M&E into Bolivia’s One Health framework strengthens cross-sector collaboration between public health, veterinary, and environmental agencies, ensuring that zoonotic disease risks are assessed holistically. Surveillance efforts must incorporate data from wildlife reservoirs. By continuously evaluating these factors, Bolivia can refine wildlife trade regulations, meat safety protocols, and public health outreach programs to minimize disease transmission.

## SECTION 5: KEY CHALLENGES AND CONSIDERATIONS

Passive surveillance plays a critical role in monitoring zoonotic diseases in Bolivia, where the intersection of wildlife trade, bushmeat consumption, and traditional medicine creates multiple pathways for disease transmission. However, the effectiveness of passive surveillance is influenced by several key challenges and considerations that must be addressed to ensure an efficient and sustainable system. The following paragraphs describes three main challenges and considerations.

### 1. Geographic Diversity and Regional Adaptation

Bolivia’s highly diverse geography, ranging from the Andean highlands to the Amazon basin and Chaco lowlands, presents a major challenge for passive surveillance of zoonotic diseases.

Each ecological region harbors distinct wildlife species and zoonotic risks, requiring region-specific surveillance approaches. For instance, in the Amazon and lowland regions, where bushmeat consumption and traditional medicine practices are widespread, passive surveillance must prioritize the monitoring of species such as primates (Alouatta spp., Ateles spp.), armadillos (Dasypus novemcinctus), and rodents, which are known reservoirs for rabies, leptospirosis, and hantaviruses. In contrast, the Andean highlands, characterized by camelid farming and poultry trade, require a focus on pathogens like avian influenza, brucellosis, and echinococcosis. The diverse climates and ecological conditions further influence vector-borne disease patterns, such as the distribution of mosquito-borne viruses like yellow fever and Venezuelan equine encephalitis, necessitating adaptive surveillance strategies tailored to regional disease dynamics.

### 2. Data Reliability and Community Engagement

Ensuring accurate and timely data collection is a major hurdle in passive surveillance, particularly in remote, indigenous, and underserved areas where health infrastructure and reporting mechanisms are limited. Many rural communities rely on traditional healers and informal markets, making it difficult to track zoonotic disease cases through conventional health reporting systems. Additionally, limited access to diagnostic facilities results in underreporting and misclassification of zoonotic infections, as many cases remain undiagnosed or are mistaken for other endemic diseases such as dengue, malaria, or tuberculosis. Addressing these data gaps requires stronger community engagement and participatory surveillance programs, where local hunters, market vendors, livestock owners, and indigenous leaders are trained to recognize early signs of zoonotic diseases and report unusual animal mortality or human symptoms to health authorities. Mobile health technologies, such as SMS-based reporting systems or community-led data collection platforms, could enhance passive surveillance in isolated regions.

### 3. Sustainability and Institutional Strengthening

Long-term financial support, capacity building, and policy integration are essential for maintaining an effective passive surveillance system. Bolivia faces challenges in securing sustained funding for zoonotic disease monitoring, as resources are often reallocated to urgent outbreaks rather than long-term surveillance efforts. Establishing multi-sectoral collaboration between the Ministry of Health (MS), the National Service for Agricultural Health and Food Safety (SENASAG), and environmental agencies is crucial for integrating zoonotic surveillance into broader public health and conservation strategies. Additionally, ongoing training of healthcare providers, veterinarians, and field researchers ensures that passive surveillance remains resilient and adaptable to emerging threats. Investing in low-cost diagnostic tools and cross-border disease monitoring networks would further strengthen Bolivia's ability to detect and respond to zoonotic risks in a sustainable manner.

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