Ph.D. THESIS

Analysis and prevention of the zoonotic potential between human and non-human primates in West Africa

(SUMMARY OF Ph.D. THESIS)

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ABSTRACT

The natural relationship between people, animals, and the environment is crucial to the development and spread of many infectious diseases (Rahman et al., 2020). Of the 1407 human pathogens identified, 58% are zoonotic, meaning that roughly 60% of emerging diseases in humans are zoonotic (WHO, 1992). Moreover, over 70% of these pathogens originate in wildlife species (Rahman et al., 2020). Due to their close relationships with people, domesticated animals, and the environment, wild animals directly contribute to the spread and maintenance of several infectious illnesses (Rahman et al., 2020).

Africa hosts numerous zoonoses that are either endemic (such as brucellosis, leptospirosis, or tuberculosis), neglected (such as rabies, onchocerciasis, or coenurosis), or emerging (such as COVID-19, anthrax, yellow fever, Ebola, Lassa fever, or yaws) (Otu et al., 2021). Wild primates are threatened by a wide range of factors worldwide, including poaching for bushmeat, illegal commerce, habitat degradation, and the pervasive threat of zoonotic diseases (Koster et al., 2022). Chimpanzees and humans may be at greater risk of contracting diseases from each other because they share at least 98% of their genetic makeup (Wooding, 2006). This also means that many pathogens are shared between humans and chimpanzees (Bell, 1988). The risk of transmission increases with the frequency of encounters between human and chimpanzee populations (Whittier, Nutter, Stoskopf, 2000).

Non-human primates (NHPs) sanctuaries face significant challenges due to the widespread threat of zoonotic illnesses, particularly *Mycobacterium tuberculosis* infections, which likely originate from humans. NHPs also have the potential to transmit this disease to humans as reverse zoonosis (Montali, Mikota, Cheng, 2001). Infections of the skin and mucous membranes can affect both healthy and immunocompromised individuals. Bacteria of the genus Treponema, the agent of yaws disease, can induce the appearance of ulcers on the face and gums and cause facial deformations and dyschromia in both human and NHPs (Vandermeersch, 1990). Other parasites, such as *Taenia serialis*, the agent of coenurosis disease, can infect NHPs and humans as opportunistic intermediate hosts, causing severe cutaneous and subcutaneous lesions.

African sanctuaries must implement strict standards, including quarantine and biosecurity protocols, as well as thorough health checks for recently arrived primates, to handle high-risk, potentially zoonotic diseases. Close interactions significantly heighten the latent risk of disease transmission, encompassing zoonoses and anthropo-zoonoses. It is also crucial to incorporate awareness for all the different categories of humans in contact with captive apes from a One Health standpoint to better prevent and manage any potential zoonotic diseases. Nowadays, conservation activities linked to education and awareness programs are incorporated into complex programs that cross

many disciplines (Bettinger et al., 2021). However, almost no information on the success or failure of these educational activities has been published, and new engagement programs rely only on personal experience.

Despite the numerous studies on NHPs, the knowledge on zoonotic diseases transmitted between NHPs and humans remain insufficient. While the crucial role of sanctuaries in saving endangered primates is unquestionable, the transmission of zoonotic diseases is inevitable even with the best intentions. Therefore, it is hypothesized that identifying, describing, and epidemiologically quantifying the potentially zoonotic diseases of NHPs in sanctuaries, which pose health risks to humans or animals in contact, underscores the importance of raising awareness among local populations around sanctuary areas.

In this framework, the aims of the present thesis were built on several objectives:

- To molecularly prove and validate the role of primates as intermediate hosts in coenurosis, a potentially zoonotic disease.

- To molecularly prove that *Treponema pallidum* subspecies *pertenue* (TPE) could be the cause of syphilis-like lesions in the anogenital area or yaws-like lesions on the face and distal extremities observed in wild chimpanzees from West Africa, and to substantiate the possible zoonotic transmission of this pathogen.

- To highlight the significant zoonotic risks of regular human-chimpanzee interactions in sanctuary settings by demonstrating the possible presence of extrapulmonary lymph node tuberculosis in captive chimpanzees from West Africa.

- To demonstrate the importance of health checks framed in the "One Health" perspective in primate sanctuaries in West Africa as a screening tool for potential zoonotic diseases, particularly latent or asymptomatic diseases that cannot be detected through clear clinical signs in captive ape populations.

- To emphasize the importance and impact of an awareness program within local schools around the primate sanctuary in Benin to ensure the sustainability of a primate conservation center in West Africa.

- To confirm that the educational program for local keepers at the primate sanctuary in Guinea enhances their understanding of zoonotic diseases and improves their daily practices in the sanctuary, benefiting both the keepers and the NHPs.

- To highlight the importance of a robust awareness program about zoonotic and physical risks for frontline officers and agencies responsible for confiscating and transporting trafficked, potentially sick animals, linking law, environment, conservation, and health of humans and animals within the current One Health concept.

The studies presented in this thesis were conducted during the PhD period (2017-2024) across various African sanctuaries, namely ATO in Benin, the Chimpanzees Conservation Center (CCC) in Guinea, the Jane Goodall Institute (JGI) in Congo, and in collaboration with the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine Cluj-Napoca, Department of Infectious Diseases and Preventive Medicine.

The thesis, entitled "Analysis and Prevention of the Zoonotic Potential Between Human and Non-Human Primates in West Africa," is structured into two main parts: a literature review containing five chapters and a personal contribution containing twelve chapters. The thesis spans 147 pages and includes 16 tables, 92 figures, and 154 references.

The first part (Literature Review) summarizes information from the literature regarding zoonotic diseases as part of the One Health approach, primates from West Africa, threats and causes of their extinction, main diseases present in primates, and prevention of the zoonotic potential between humans and non-human primates. **Chapter 1** encompasses data on zoonotic aspects worldwide, the One Health approach, and zoonotic diseases in Africa. **Chapter 2** includes the classification of primates, the area of distribution of primates of concern, and the descriptions and characteristics of chimpanzees. **Chapter 3** details the various threats to primates leading to their extinction, such as habitat fragmentation, poaching, and diseases, and presents the Pan African Sanctuary Alliance (PASA) as part of the solution. **Chapter 4** describes the main diseases in primates, including digestive diseases, respiratory diseases with a focus on tuberculosis, nervous diseases, and skin and mucous membrane disorders, particularly coenurosis and yaws. **Chapter 5** discusses the prevention of zoonotic potential between humans and non-human primates through biosecurity, quarantine, risk assessment, health checks in sanctuaries, and the importance of awareness programs.

The second part (Personal Contribution) is structured into twelve chapters and includes the work hypothesis of the PhD thesis (**Chapter 6**), followed by the research objectives in **Chapter 7**. **Chapters 8 to 14** (detailed here under) present the original research. The general conclusions and recommendations are included in **Chapter 16**, and the originality and innovative contributions are detailed in **Chapter 17**. The cited references include 154 titles.

- The aim of **Chapter 8** was to identify larval tapeworms found in an olive baboon from Benin (*Papio anubis*) to the species level, characterize the material through molecular means to verify its identity, and evaluate the significance of our discovery in relation to the ecology of the parasite, conservation medicine, and zoonotic potential.

  The zoonotic *Taenia serialis*, which circulates among Canidae, Hyaenidae, lagomorphs, rodents, and primates, is frequently reported (Verster, 1969; Loos-Frank, 2000). Coenurus larvae have been classified into species by various authors using host and/or anatomical location as criteria; however, these methods are inconsistent. Most reports of coenurus-type larvae in different hosts cannot be conclusively linked to species without molecular tools.

  A baboon kept in captivity in a private yard in Benin with stray dogs in the same enclosure developed swellings, mainly subcutaneous. The baboon was transferred to a rescue center, and during a general examination, approximately fifteen partially mobile masses of varying sizes were discovered throughout its body. Following surgery, several cyst-like structures were removed from the intermuscular and subcutaneous tissues. The cysts displayed a characteristic coenurus-like morphology under the microscope (many protoscolices). Genomic DNA was extracted from a single cyst using a commercially available kit (Isolate II Genomic DNA Kit, Bioline, UK). Molecular characterization was carried out by PCR amplification and sequencing (Macrogen Europe) of a segment of the mitochondrial 12S rDNA gene and a region of the nuclear ITS-2 rDNA (Gasser and Chilton, 1995; Von Nickisch-Rosenegk et al., 1999). The sequences were compared to other sequences from the GenBank database using BLAST analysis.

  The baboon's cyst's ITS-2 sequence matched 96% of an isolate of *T. serialis* from a Canadian rabbit, 88%-98% of other isolates from coyotes in California, USA, and 99% of an isolate from an Ethiopian gelada. The 12S rDNA sequence showed 99% similarity to a *T. serialis* isolate from an Ethiopian gelada, 98% similarity to a *T. serialis* isolate from a Japanese domestic dog, and 96% similarity to an environmental sample from Germany containing *T. serialis* isolates. Our sequence was classified in a clade comprising different *T. serialis* isolates from multiple host species originating in North America, Australia, Asia, and Africa based on the analysis of the 12S rDNA gene. All isolates of *T. multiceps* and *T. serialis* from North America and Europe were included in a second clade. The mean distance between the two clades was 0.01, with a mean of 0.0430 separating them.

This chapter highlights the potential for human infection while reporting a new host (olive baboon) and geographic record (Benin) for the larval form of *T. serialis*. It also validates the role of primates as intermediate hosts. Our research provides additional evidence that *T. serialis* larvae can spread among captive animals in the vicinity of human settlements, in addition to their previously documented presence in wild primate populations. We advise a more thorough parasitological diagnosis for larval cestodes present in humans due to the apparent adaptability in host selection. Human coenurosis is likely more common in Africa than currently thought, as coenurus larvae could be mistakenly identified by medical professionals as other larval cestodes (such as cysticercus type) or because postsurgical identification may not be attempted in most cases.

- The aim of **Chapter 9** was to molecularly prove that *Treponema pallidum* subspecies *pertenue* (TPE) could cause syphilis-like lesions in the anogenital area or yaws-like lesions on the face and distal extremities observed in wild chimpanzees from West Africa in camera traps, and to substantiate the potential zoonotic transmission of this pathogen.

  TPE infects several monkey species in sub-Saharan Africa, typically presenting as syphilis-like lesions or yaws-like lesions. Reports of TPE infection in NHPs from West Africa date back to the 1960s. Despite numerous reports of orofacial and genital lesions in wild African great apes, the cause of these yaws-like lesions has not been identified, and no definitive link between diagnostics and clinical signs had been established.

  In a mining concession in the Sangaredi area of Guinea, we discovered a cachectic wild adult female chimpanzee (*Pan troglodytes verus*) with severe mouth and lip lesions resembling yaws. Due to her suffering and prolonged decline, the chimpanzee was euthanized, and a necropsy was performed. Gross pathology of the skin revealed hypertrophied, edematous lips with noticeable depigmentation, crusts and ulcers on the head, and a large portion of the nose was absent. DNA was extracted from two facial lesion biopsies preserved in RNAlater, and molecular analyses were conducted. High-throughput sequencing analysis covered the TPE genome on average 24 times; >1 read covered 98.6% of the genome, and >3 reads covered 97.6%. Using Bayesian Markov chain Monte Carlo analysis of a genomic alignment that included the reconstructed TPE genome, other available TPE genomes, *T. pallidum* subsp. *Endemicum genomes*, and selected *T. pallidum* subsp. *Pallidum* (TPA, syphilis) genomes from GenBank, the results showed that the chimpanzee-derived genome clustered within the well-supported TPE clade.

This proved that TPE caused the clinical manifestations observed in this specific wild chimpanzee. More specifically, the new chimpanzee-derived genome is part of a clade including TPE strains isolated from NHPs in Senegal, Guinea, Gambia, and Guinea in western Africa, consistent with findings that the genomic diversity of TPE strains infecting NHPs appears geographically structured (Chuma et al., 2019).

This study proved that TPE caused the yaws-like lesions seen in the nearly dead wild chimpanzee found in a mining company in Guinea. By linking yaws-like pathologies in wild chimpanzees in Guinea to the actual detection of TPE in this specific wild chimpanzee, the study demonstrates that some suggestive lesions frequently observed in wild great apes are indeed caused by TPE. These findings add to the growing evidence that TPE infection affects numerous NHP species in sub-Saharan Africa (Knauf et al., 2018).

This discovery may challenge the current global effort to eradicate TPE by 2030 (Dyson et al., 2019). However, information from TPE-infected humans in this region is needed to determine if zoonotic transmission occurs. The severity of the lesions clearly impacts each animal's health. Although the disease's effect on NHP populations is unknown, it could be determined with long-term observation. More research is required to assess the pathogen's implications for conservation.

- The aim of **Chapter 10** was to highlight the significant zoonotic risks associated with regular human-chimpanzee interactions in sanctuary settings by demonstrating the possible presence of extrapulmonary lymph node tuberculosis in captive chimpanzees from West Africa.

  There are few documented cases of tuberculosis (TB) spreading to wild primates; the disease primarily affects humans. However, TB has been frequently observed in captive primates (Wolf et al., 2016). This chapter reports a TB outbreak at a sanctuary which collects and rehabilitates common West African chimpanzees from the Republic of Guinea, victims of poaching or illegal trafficking of living wild animals. There were no previous documented cases of TB at the sanctuary. The episode lasted from January 2019 to April 2020. The first case was diagnosed in January 2019, the second in February 2019, and the remaining three in March 2019. All five affected chimpanzees were part of a group of 14 juveniles that shared the same housing, daily schedule, and caregivers. Following their diagnosis, these five chimpanzees were isolated in a newly constructed quarantine facility. The remaining nine juveniles were also separated and restricted from bush walks until the issue was resolved.

  Chimpanzees at the sanctuary had tested negative for TB in 2017. However, clinical signs started appearing nearly two years later during the outbreak. The diagnostic

tests used included immunological tests (intradermal tuberculin skin test, QuantiFERON®-TB Gold Plus), bacteriological tests (Ziehl-Neelsen method), and molecular diagnostic tests (GeneXpert®). Each case was documented from initial TB suspicion to diagnosis and treatment.

It is suggested that the TB infection causing the lymphadenitis in the young chimpanzees may have originated from humans. The caregiver, diagnosed with pulmonary TB in March 2017, had daily direct contact with the young chimpanzees where all five cases of TB lymphadenitis occurred. This background highlights a significant health issue faced by orphaned or rescued chimpanzees in African sanctuaries, linked to the close physical proximity between the primates and their caregivers, especially in the case of young chimpanzees (Pedersen et al., 2009).

It is hypothesized that a lymph node fistula treated in January 2017 indicated an undetected TB infection transmitted from the caregiver, although chimpanzee C1 was only confirmed TB positive in January 2019. This case series emphasizes the challenges in diagnosing TB in sanctuaries and the inadequacy of relying on a single test, as false positives and negatives can conceal latent diseases in captive populations for years.

These five TB cases, likely resulting from a contaminated caregiver, underscore the necessity for strict quarantine procedures, frequent health examinations, and stringent hygiene measures to maintain the health of sanctuaries and their residents and to significantly reduce the zoonotic risks associated with interactions with great apes. The indispensability of tuberculosis screening as a basic part of routine health examinations in primate sanctuaries, especially in tuberculosis-endemic areas, is highlighted by the difficulties in diagnosing and treating extrapulmonary tuberculosis in primate populations.

- The aim of **Chapter 11** was to demonstrate the importance of regular health checks within the "One Health" framework in West African primate sanctuaries to screen for potential zoonotic diseases. Sanctuaries are essential for ensuring the health of primates during their rehabilitation process. Two major health issues arise from the interactions between NHPs and humans. First, the close physical proximity required for care presents a risk for the transmission of various pathogens, which can affect the health of the primates and pose potential public health concerns. Second, once released back into the wild, chimpanzees may spread pathogens unfamiliar to wild populations, posing additional health risks.

  In Sanctuary 1, nearly 20% of the chimpanzees examined had dental problems (tartar, cavities, broken/blackened teeth, dental abscesses), often occurring in contexts of gingivitis and gingivo-stomatitis, necessitating regular dental monitoring. In Sanctuary 2, less than 15% of the chimpanzees had dental issues.

Cardiac examinations (auscultation, ECG, echocardiography) revealed abnormalities primarily in two symptomatic chimpanzees from Sanctuary 1: one with a large blood clot in the right atrium and another with a slight murmur and endocarditis. In Sanctuary 2, only one chimpanzee exhibited a slight heart murmur (grade 2/6) without associated clinical signs. Reproductive system examinations in Sanctuary 1 identified a probable endometriosis nodule in a 23-year-old female chimpanzee.

Regarding viral diseases, an 11-year-old female chimpanzee in Sanctuary 1 tested positive for HIV, while none did in Sanctuary 2. Hepatitis B virus was confirmed in 6 chimpanzees in Sanctuary 1 and 4 in Sanctuary 2, although none exhibited clinical signs, indicating a low sensitivity to the virus. Rubella and Hepatitis C were absent in both sanctuaries.

For bacterial diseases, *Helicobacter pylori* was detected in 17% of the screened chimpanzees from Sanctuary 1 and in 40% from Sanctuary 2, without any digestive diseases being observed. TB screening revealed a tuberculosis epidemic in Sanctuary 1, with 8% of the chimpanzees testing positive on Genexpert (active TB), 10% showing positive interferon results (possible latent cases), 23% with positive TB skin tests, and 29% suspicious (in contact with the disease). In Sanctuary 2, TB results were more moderate, with 0% positive on Genexpert, 0% mycobacteria detected through ZN staining, 11% positive interferon results, and 5% positive TB skin tests.

For parasites, *Filaria* in blood was present in both sanctuaries: 3% in Sanctuary 1 and 27% in Sanctuary 2. In Sanctuary 1, the most prevalent parasites were Troglodytella (10%), *Strongyloide* (8.5%), *Entamoeba histolytica* (7%), *Balantidium* (7%), and *Chilomastix* (5%). *Troglodytella abrassarti*, a non-pathogenic symbiont of wild apes thought to aid hindgut fermentation (McLennan et al., 2017), was present. Only one case of *Plasmodium* was detected, corresponding to less than 2%. In Sanctuary 2, *Troglodytella* prevalence was higher at 18%, followed by *Entamoeba coli, Entamoeba histolytica, Dientamoeba fragilis, Chilomastix, Balantidium,* and *Strongyloide*, each present at 7%. *Giardia* was insignificant in both sanctuaries.

These results underscore the importance and necessity of regular health examinations (annual or biennial) to screen for potential zoonotic diseases, particularly latent or asymptomatic diseases that cannot be detected through clear clinical signs in ape populations.

- **Chapter 12** aims to highlight the importance and impact of an awareness program implemented in local schools around ATO, the primate sanctuary in Benin, to ensure the sustainability of a primate conservation center in West Africa. Raising awareness

among Beninese children is crucial for addressing current environmental problems and preventing the transmission of diseases.

New educational modules were developed specifically for target classes of 9-12-year-olds. The program, endorsed by the Mayor, ran from November 2017 to December 2018. It involved 10 local schools, with one to two classes per school (18 different classes, averaging 25 to 30 students per class), reaching approximately 500 local children over the year.

The program was divided into two modules conducted at different locations: the first module, lasting two hours, was held at the schools in the morning and included five different activities; the second module, lasting 1.5 hours, took place at the ATO center a week later and featured four outdoor activities. Local keepers and volunteers played a significant role in this collective effort, from the creation and implementation of the program to its ongoing development.

The new awareness modules created in November 2017 had a notably positive impact on the local community. Although pre- and post-test evaluations were not conducted to objectively measure progress, interest, and short- to mid-term impact, the enthusiasm from school directors indicates a strong desire to continue and expand these modules annually.

- **Chapter 13** aimed to confirm that the educational program for local keepers at a primate sanctuary in Guinea enhances their understanding of zoonotic diseases and improves their daily practices, benefiting both themselves and their contact with NHPs. Raising awareness is crucial for the sustainability of a primate conservation center.

  The conservation objective for this subspecies revolves around four key missions: collect, release, raise awareness, and participate. The training program began in February 2020 and concluded in December 2020. An initial multiple-choice questionnaire (MCQ) assessed the keepers' baseline knowledge and needs.

  The training comprised several modules:

  - Module 1: General aspects of diseases
  - Module 2: Modes of disease transmission
  - Module 3: Disease prevention and the importance of hygiene
  - Module 4: Zoonotic diseases (tuberculosis, yaws, intestinal parasites)
  - Module 5: Medical treatments, basic reflexes
  - Module 6: Reproduction and sexually transmitted diseases
  - Module 7: Anaesthesia
  - Module 8: Monitoring an anesthetized chimpanzee

A final MCQ, identical to the initial one, evaluated the knowledge acquired. Both the initial and final MCQs contained 20 questions divided into six categories, with only one correct answer per question. For ease of interpretation, the questions were categorized as follows:

- Questions 1-3: Diseases
- Questions 4-8: Zoonosis, prevention, and transmission
- Questions 9-11: Tuberculosis
- Questions 12-14: Parasites
- Questions 15-17: Malaria
- Questions 18-20: Health care

The results showed significant improvement across categories. The most notable improvement was in the understanding of tuberculosis, with an increase of 11.3 points or 59%. Knowledge of zoonosis, prevention and transmission of diseases, and parasites also improved significantly, with a 7-point or 37% increase in each category. Health care knowledge improved by 6 points or 32%, followed by general disease knowledge with a 5.4-point or 28% improvement. The smallest improvement was in the malaria category, with a 4.3-point or 23% increase, which is understandable given the pre-existing knowledge about malaria in Guinea.

Overall, the educational program for the keepers at the CCC sanctuary in Guinea was a resounding success, with a global improvement of about 36% between pre- and post-tests. The keepers effectively understood and integrated key concepts, especially regarding tuberculosis, intestinal parasites, and zoonotic diseases, including their transmission and prevention. These concepts are crucial in their daily work, as tuberculosis and parasites are among the primary zoonoses of concern in a primate sanctuary. Initially unaware of the importance of zoonotic diseases in their routine, the keepers found the training highly valuable and expressed gratitude for the opportunity.

- **Chapter 14** emphasizes the critical need for a robust awareness program about zoonotic and physical risks for frontline officers and agencies responsible for confiscating and transporting trafficked, potentially sick animals in Congo. This program links the law, environment, conservation, and the health of humans and animals within the One Health concept. This program was implemented at The Tchimpounga Chimpanzee Rehabilitation Center (TCRC), established by The Jane Goodall Institute (JGI) in Congo, the largest wildlife sanctuary in Africa. It is home to 140 rescued chimpanzees as well as mandrills, African grey parrots, pangolins, and Cercopithecus monkeys.

Funded by the U.S. Department of State's International Narcotics & Law Enforcement (INL), the awareness program was conducted by the Jane Goodall Institute in Congo. Its primary goal is to reduce the mortality of animals from illegal trade and train personnel on the potential zoonotic risks associated with the conservation of these animals by promoting good practices in the management of live animal confiscations. It is important to note that many animals involved in illegal trade are either fully protected or endangered, highlighting the need for additional measures to combat potential zoonotic and physical risks.

The training sessions were conducted over three days at various locations: Tchimpounga Nature Reserve (TNR), Conkouati Douli National Park (CDNP), Dimonika Biosphere Reserve (DBR) for ecoguards, and the cities of Pointe-Noire (PN) and Brazzaville (BZV) for government officials (Departmental direction and Ministry).

Day 1:

- Introduction about the importance of formation and awareness
- Pre-training survey
- Identification of the 10 most trafficked species in Congo
- Presentation of the confiscation kits
- Legislation in Congo regarding animals
- Zoonotic risks and their transmission
- Prevention of zoonotic risks
- Presentation and prevention of other risks
- Handling monkeys

Day 2:

- Disease transmission through sick and injured animals
- Animal triage and emergency response
- Unit transport
- Capture, handling, and placement of animals to avoid zoonotic and physical risks
- Animal welfare

Day 3:

- Transport of live animals
- Biosecurity rules
- Collection of evidence
- ArcGIS Survey 123
- Post-training survey
- Satisfaction survey
- Certificates distribution

To evaluate progress, pre- and post-test scores were analyzed at each location. Among the 77 participants who completed the surveys, 33 were frontline officers, 26 were agency officials, and 18 were NGO partners. Notably, 96% of participants improved their scores in the post-test, with group scores increasing by an average of 17% (from 72% in the pre-test to 89% in the post-test). In the post-test, 16 out of 31 questions were correctly answered by over 90% of participants, compared to only 2 in the pre-test.

Participants were selected based on their roles. Frontline officers, primarily ecoguards, are directly involved with animals, rescuing, confiscating, and transporting them. They need to understand potential diseases, transmission patterns, zoonotic and physical risks, preventive measures, as well as health care and welfare notions. Question 10, which addressed potential zoonotic risks, showed significant improvement, with correct responses increasing from 41.6% pre-training to 64.7% post-training. Regarding prevention, participants already had some awareness of the importance of facial masks to prevent airborne diseases, as seen by the slight improvement in Question 9 (from 70.5% to 71.3% correct answers). However, theoretical knowledge of zoonosis and emerging diseases remained unclear, as indicated by no improvement in Question 6.

Government agency participants also played a crucial role, as it is essential for the government to be aware of potential risks to their agents and to implement strict biosecurity rules to protect humans and animals. These agencies serve as a bridge between law, environment, conservation, and health within the One Health framework.

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