Diagnosing wildlife diseases: challenges and opportunities

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World Organisation for Animal Health
Organisation mondiale de la santé animale
Organización Mundial de Sanidad Animal

Since 1924, WOAH is the intergovernmental organisation in charge of improving animal health worldwide.

Transparency/ Disease status	***WAHIS, WAHIS Wild beta***
Expertise network, Disseminate scientific information	***Guidelines, publication***
International solidarity	***Vaccine banks, lab twinning's***
Health safety, emergency preparedness and resilience	***Simulation exercises Contingency plan***
Improve veterinary services	***PVS, capacity for wildlife surveillance***
Safe trade, food safety, animal welfare	***Standards including welfare, WTO/SPS agreements***

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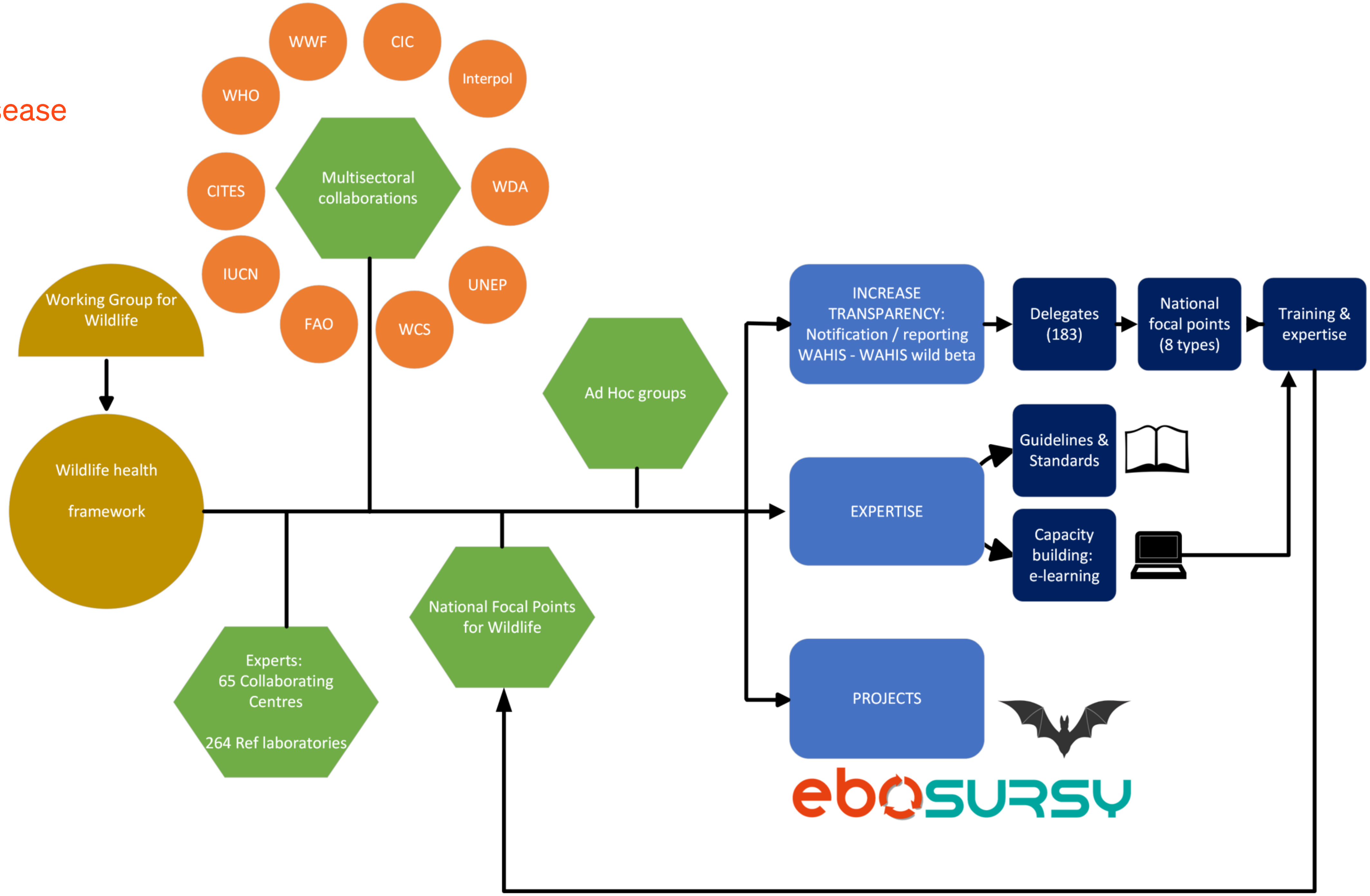
WOAH: Wildlife disease program



Reporting wildlife diseases to WOAH

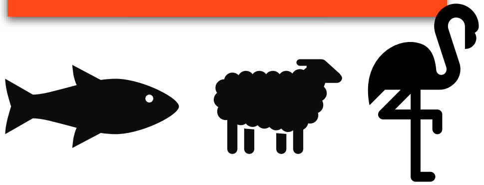
International reporting of animal diseases – WAHIS system

Obligatory reporting

Listed Diseases

based on WOAH Animal Health Codes

~120 listed diseases



1. International spread of the pathogenic agent and at least one country has demonstrated freedom

AND

2. Transmission to humans with severe consequences

   OR

   Significant impact on the health of domestic or wild animals

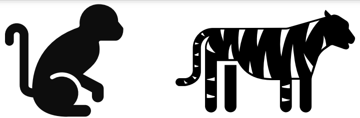
AND

3. Reliable means of detection, diagnosis and precise case definition

Emerging Diseases

Based on standard operating procedure

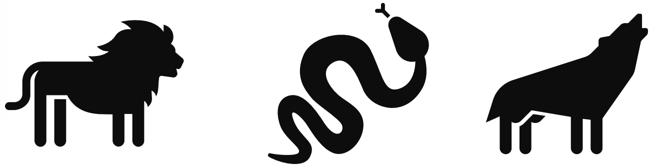
~4 Emerging diseases



Exceptional Epidemiological events

Information available to meet above criteria insufficient

Non-Listed Diseases of Wildlife

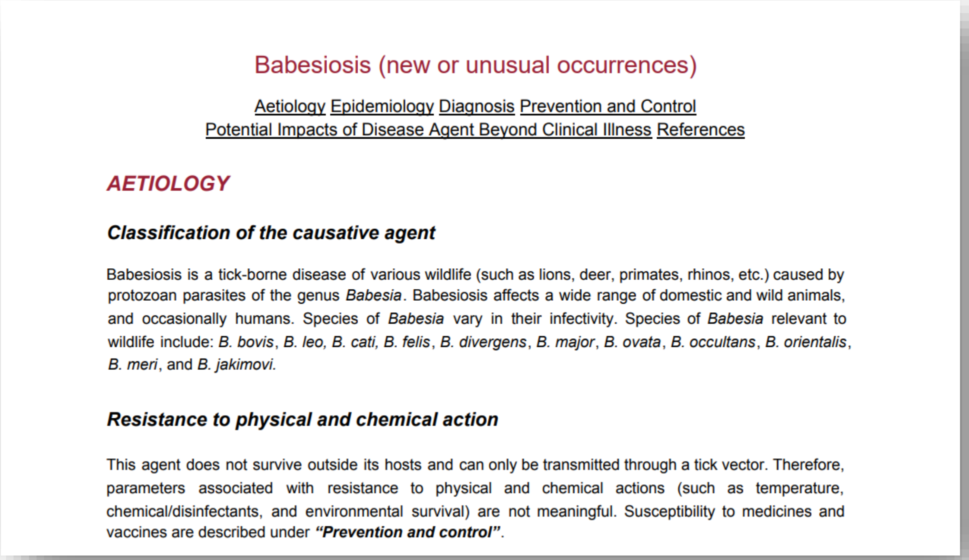


53 non-listed diseases + undiagnosed mortality events

WAHIS wild beta
. . .
System in development

Voluntary reporting, changes in progress

Technical disease cards



Babesiosis (new or unusual occurrences)

Aetiology Epidemiology Diagnosis Prevention and Control
Potential Impacts of Disease Agent Beyond Clinical Illness References

AETIOLOGY

Classification of the causative agent

Babesiosis is a tick-borne disease of various wildlife (such as lions, deer, primates, rhinos, etc.) caused by protozoan parasites of the genus *Babesia*. Babesiosis affects a wide range of domestic and wild animals, and occasionally humans. Species of *Babesia* vary in their infectivity. Species of *Babesia* relevant to wildlife include: *B. bovis*, *B. leo*, *B. cati*, *B. felis*, *B. divergens*, *B. major*, *B. ovata*, *B. occultans*, *B. orientalis*, *B. meri*, and *B. jakimovi*.

Resistance to physical and chemical action

This agent does not survive outside its hosts and can only be transmitted through a tick vector. Therefore, parameters associated with resistance to physical and chemical actions (such as temperature, chemical/disinfectants, and environmental survival) are not meaningful. Susceptibility to medicines and vaccines are described under ***"Prevention and control"***.

Test validation – general considerations

1. Compliance with laboratory standards

- Quality management in veterinary testing laboratories

2. Assay development

- Purpose → determines PV
  - Screening?→ high sensitivity?
  - Confirmation? → high specificity

3. Assay development and experimental studies

- Design and proof of concept
- Standardization and optimization
- Operating range of assay
- Identification of inhibitors in the matrix
- Assay Robustness
- Calibration and normalisation

4. Assay validation

STAGE 1: ANALYTICAL PERFORMANCES

- Repeatability and analytical Se and Sp

→ **Provisional recognition**

STAGE 2: DIAGNOSTIC PERFORMANCE

- Based on reference animal populations
- Diagnostic Se and Sp
- Case definition and cut-off

STAGE 3: REPRODUCIBILITY → **Validated for original intended purpose**

STAGE 4: IMPLEMENTATION

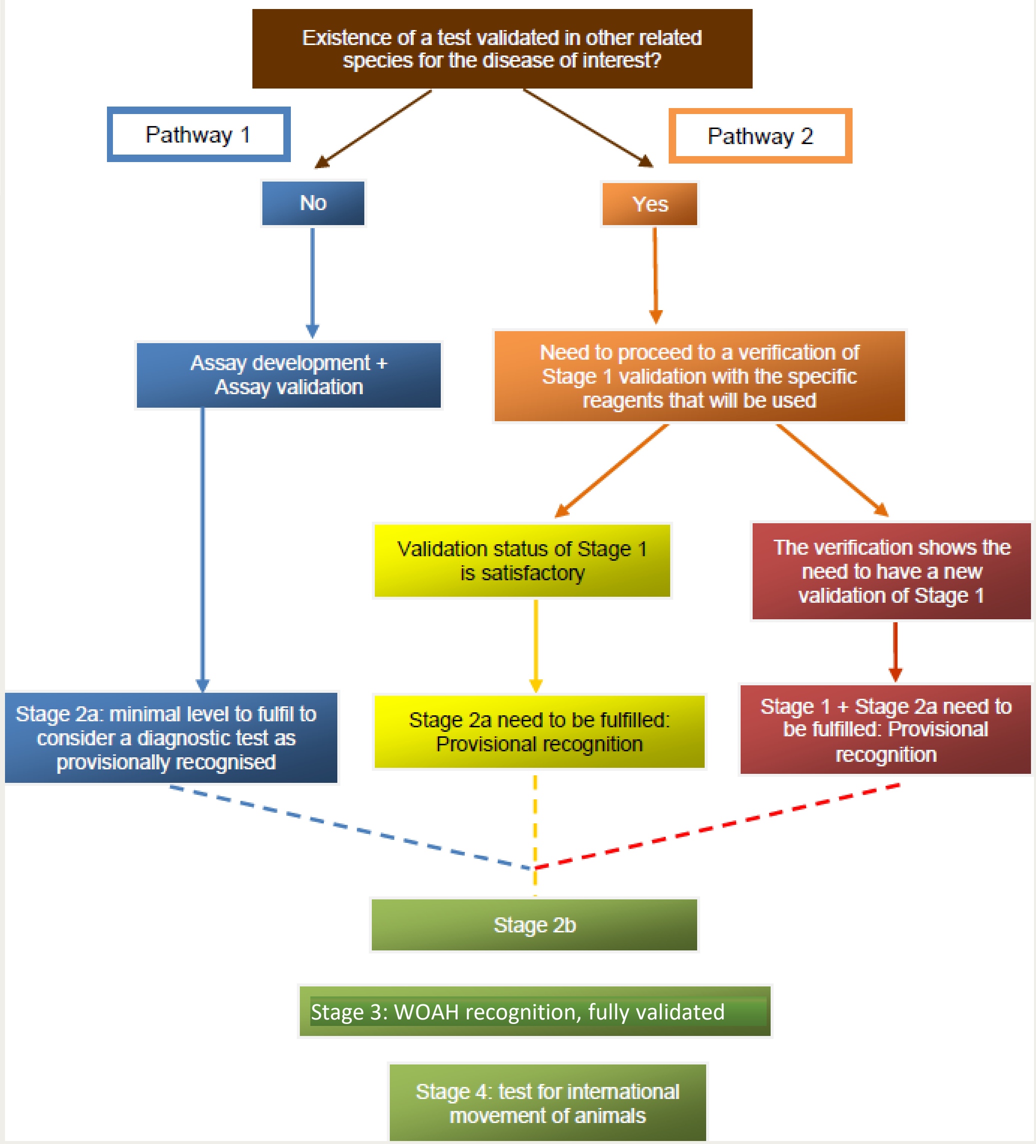
STAGE 5: MONITORING PERFORMANCE

WOAH TERRESTRIAL MANUAL. Chapter 1.1.6. Principles and methods of validation of diagnostic assays for infectious diseases (version adopted in May 2023)

Test validation for diseases in wild species, specific considerations

Acknowledging two potential scenarios:

- **Validated tests in related species exist**
- **Validated test in related species does not exist**



WOAH TERRESTRIAL MANUAL. Chapter 2.2.7. Principles and methods of validation of diagnosis tests for infectious diseases applicable to wildlife (May 2014)

Test validation for diseases in wild species, specific considerations

When possible, follow the general recommendations

Reference samples

- **Use of clinical animals when test will be used on subclinical leads to sur-estimation of sensitivity and specificity**
- **Experimental animals might be the only option but offer limited variation**
- **Reference samples:**
    - Low availability and volume
    - Bank of reference samples with metadata of all characteristics of the sample to be organized, including sample quality
    - Pooling if volume to too low
- **Low quality versus quantity**
- **Latent class model for Se and Sp**

4. Assay validation – specific consideration

STAGE 1: ANALYTICAL PERFORMANCES

- Consider cross-reacting organisms

STAGE 2: DIAGNOSTIC PERFORMANCE

- Based on the similarity of test performance in domestic and targeted wild species
- Might require combining multiple trial from multiple laboratories→ CITES permits might be needed

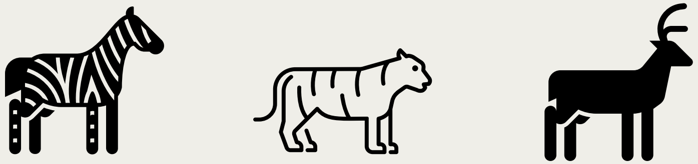
STAGE 3: REPRODUCIBILITY / VALIDATION

- Might require combining multiple trial from multiple laboratories→ CITES permits might be needed

STAGE 4: IMPLEMENTATION

- PV needs information on disease prevalence / often not doable

STAGE 5: MONITORING PERFORMANCE



Diagnosing diseases in wild species: challenges and opportunities

Pathogen detection and disease diagnosis in wildlife: challenges and opportunities. A. Michel, H. van Heerden, B. Crossley, S. Al Dahouk, D. Prasse, V. Rutten. Revue scientifique et technique. 2021

Challenges

Species diversity

Diverse anatomical, physiological, and genetic characteristics complicate the development of diagnostic tests effective across multiple species.

Access to limited individuals

Remote habitats, inaccessible areas, dispersal, tissue loss trough environmental decomposition or scavenging, limited numbers of known positive and negative individuals

Lack of baseline data

Normal parameters unknown in wild species

Lack of commercial interest / ressource

Short-term cost/benefit analysis

Non-specific clinical signs or late observations

Lack of detailed history

Collaborative effort

Sample collection can require to mobilize different sectors

Challenging sample collection

logistical difficulties, safety concerns, and ethical considerations

Development of non-invasive methods and genetic markers needed

remote sensing technologies, collection of faeces, hair, or blow samples

Opportunities

One health approach

Protection of animals' and humans' health

Early detection of potential emerging diseases

Contribute to conservation effort in the era of mass extinction

Contribute to knowledge advancement

New type of test and field kits

For researchers, conservation organizations, government agencies, and veterinary professionals. Direct revenues + technology transfer and licensing

Service provision:

Diagnostic services, consulting and expertise to wildlife researchers, veterinarians, wildlife managers, and conservation organizations.

Collaborative efforts / partnerships

Invent new networks, included with local communities

Partnership with pharmaceutical companies, Start ups, manufacturers.

Research funding and grants:

Development of diagnostic tools can attract research funding from government agencies, foundations, and other grant-giving bodies.

Case study 1: Chytridiomycosis

State of the art:
- Validation using laboratory animals
- Low sample size
- Experimentally infected by high dose
- All animals highly susceptible (naive)
- Same age and physiological status

Sampling of wild animals (unknown status)

- Sterile sampling procedure?
- Skin sampling
- Sample preservation until testing
- Inhibition testing

Testing

qPCR
- DNA extraction
- Positive controls
- Inhibition testing
- Triplicate testing

Histology
- Section
- Staining
- Reading
- Cut off: severity of pathology & development stage of the zoosporangia

Sensitivity and Specificity

Bayesian modelling using latent class analysis on two populations and using two tests

Results

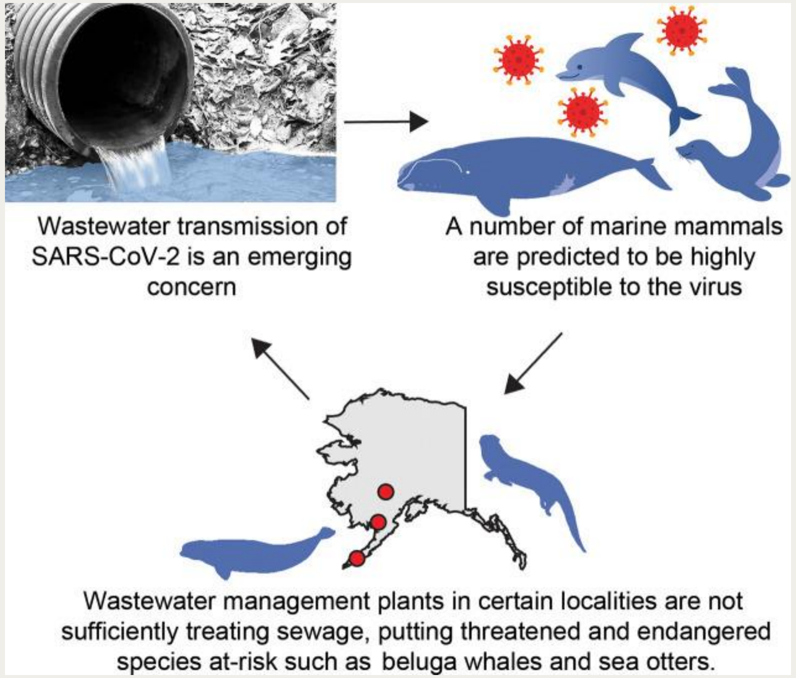
- Cross-contamination on qPCR plate -> reduced qPCR specificity
- qPCR: high sensitivity
- Histology: low sensitivity but high specificity, suggested confirmatory test

Lee F. Skerratt, Diana Mendez, Keith R. McDonald, Stephen Garland, James Livingstone, Lee Berger, Richard Speare "Validation of Diagnostic Tests in Wildlife: The Case of Chytridiomycosis in Wild Amphibians," Journal of Herpetology, 45(4), 444-450, (1 December 2011)

Hunt KE, Moore MJ, Rolland RM, Kellar NM, Hall AJ, Kershaw J, Raverty SA, Davis CE, Yeates LC, Fauquier DA, Rowles TK, Kraus SD. Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. Conserv Physiol. 2013 May 15;1(1):cot006. doi: 10.1093/conphys/cot006

Case study 2: Unusual species

Why?



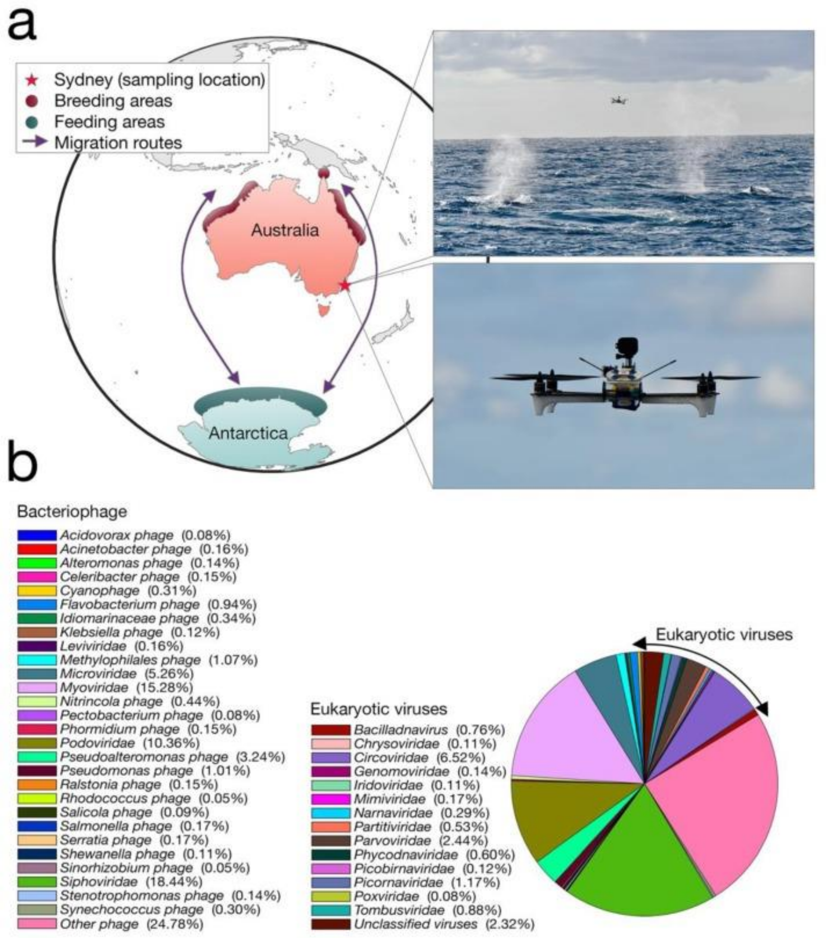
How?

fecal sample	exposure to toxins, pollutants, and parasites + physiology
respiratory samples ('blow’)	respiratory microbes + physiology (respiratory metabolites)
biopsy darts	Contaminants (+ physiology)
photographs	skin condition, ectoparasite load, traumatology

Conservation + Mathavarajah S, Stoddart AK, Gagnon GA, Dellaire G (2021) Pandemic danger to the deep: the risk of marine mammals contracting SARS-CoV-2 from wastewater. Sci Total Environ 760: 143346. - PMC - PubMed

Case study 3: Unusual species and matrix

- Eastern Australian humpback whale (*Megaptera novaeangliae*)
- Drone sampling of blow collected on a Petri dish opened at the last minute remotely
- Controls: seawater, nonblow flight
- Viromics → virome diversity
- Consistent core microbiome → relevance health monitoring



Geoghegan JL, Pirotta V, Harvey E, Smith A, Buchmann JP, Ostrowski M, Eden JS, Harcourt R, Holmes EC (2018) Virological sampling of inaccessible wildlife with drones. Viruses 10: 300. Apprill, A.; Miller, C.A.; Moore, M.J.; Durban, J.W.; Fearnbach, H.; Barrett-Lennard, L.G. Extensive core microbiome in drone-captured whale blow supports a framework for health monitoring. mSystems 2017, 2, e00119-17

Case study 4: predator strategy: *Dorylus* ants

Ant **sampling** (forest edges), liquid nitrogen storing

Pooled in library and **sequencing**, negative controls (buffers) and bioinformatics

Viral **metagenomics**: targets DNA and RNA virus.

1. extraction and discard of contaminant non-encapsulated nucleic acid

2. amplification

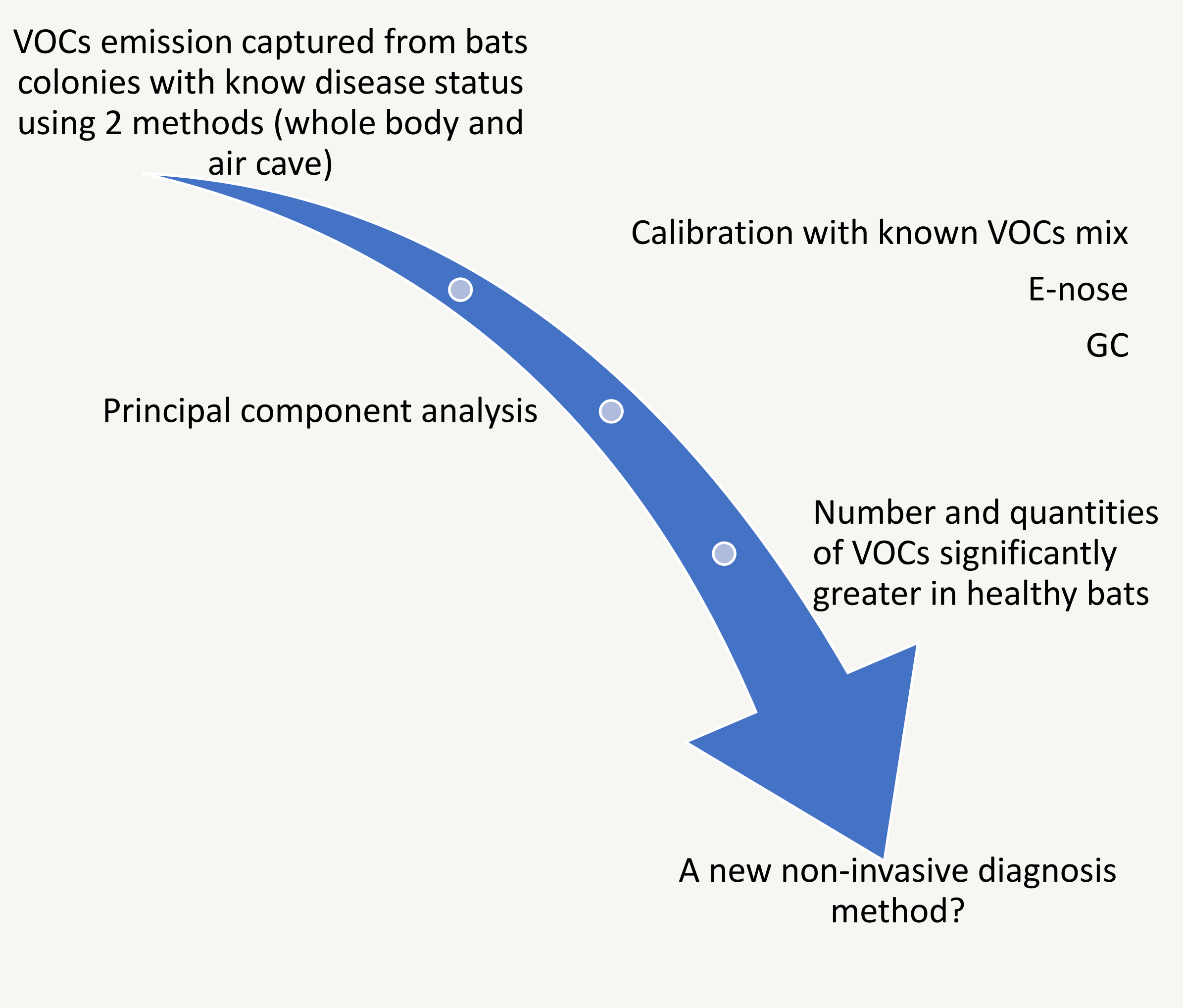
Ant species **identification**, **phylogenetic** characterization of viral sequences

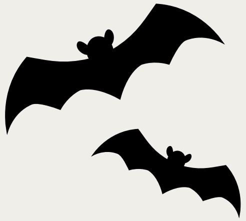
- Predator-enabled metagenomic strategy
- Applied to remote areas difficult to sample
- virion-associated nucleic acid-based metagenomics
- bacterial, plant, invertebrate and vertebrate viral sequences (56 viral families) were accumulated by army ants when compared with the leaf-foraging ants → access to virome of the preys?

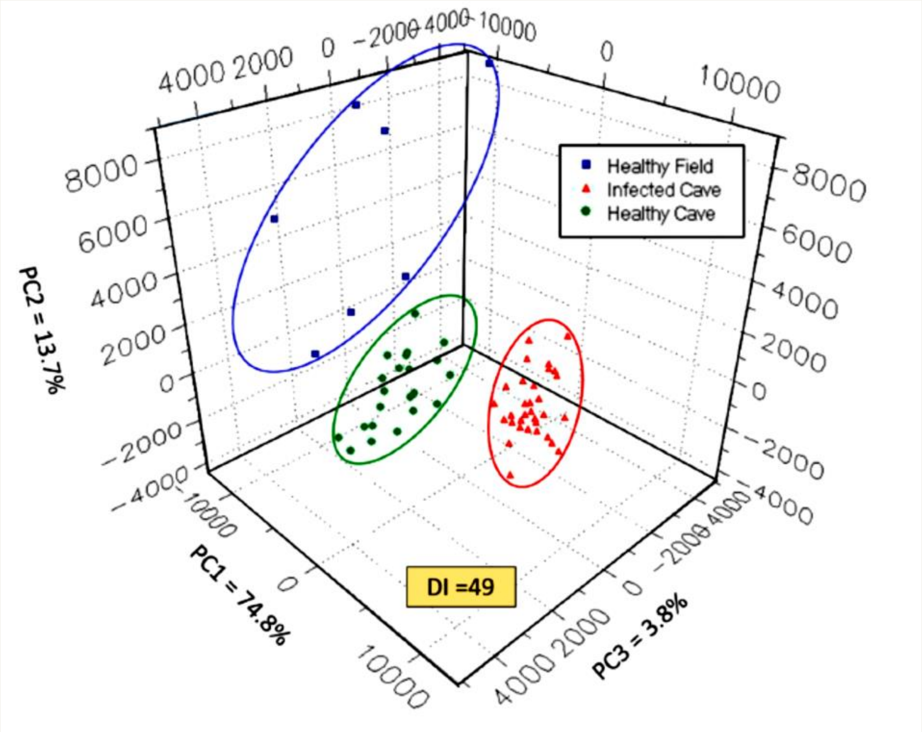
"Using predators and scavengers such as army ants to sample tropical forest viromes can shed light on the composition and the structure of viral populations of these complex and inaccessible ecosystems."?

Fritz, Matthieuet al. Roumagnac, Philippe. African army ants at the forefront of virome surveillance in a remote tropical forest . Peer Community Journal, Volume 3 (2023), article no. e24. doi : 10.24072/pcjournal.249. https://peercommunityjournal.org/articles/10.24072/pcjournal.249/

Case study 5: Volatile Organic Compounds for white-nose syndrome in bats







"Results suggest that GC/E-nose dual-technologies based on VOC-detection and analyses of physiological states, provide noninvasive alternative means for early assessments of Pd-infection, WNS-disease status, and other physiological states"

Doty, A. C., Dan Wilson, A., Forse, L. B. & Risch, T. S. Biomarker Metabolites Discriminate between Physiological States of Field, Cave and White-Nose Syndrome Diseased Bats. Sensors ***22****, 1–27 (2022).*

Case study 7: Point-of-care tests

Point-of-care tests (POCTs) "a fully or partially automated table-top, portable or disposable device able to be operated in a non-laboratory environment by non-technical staff to deliver a same-day, on-site, clinically relevant, diagnostic test result"

1. Interest in rural communities in LMICs
2. Rapid diagnosis
3. Non laboratory setting
4. Some have good performances

But:

1. Target only a few diseases
2. No evaluation in field conditions
3. Varying quality of tests on the market, challenge for customers
4. Consequences of false positive / false negative

"Further research is needed and technical, regulatory frame must be implemented"

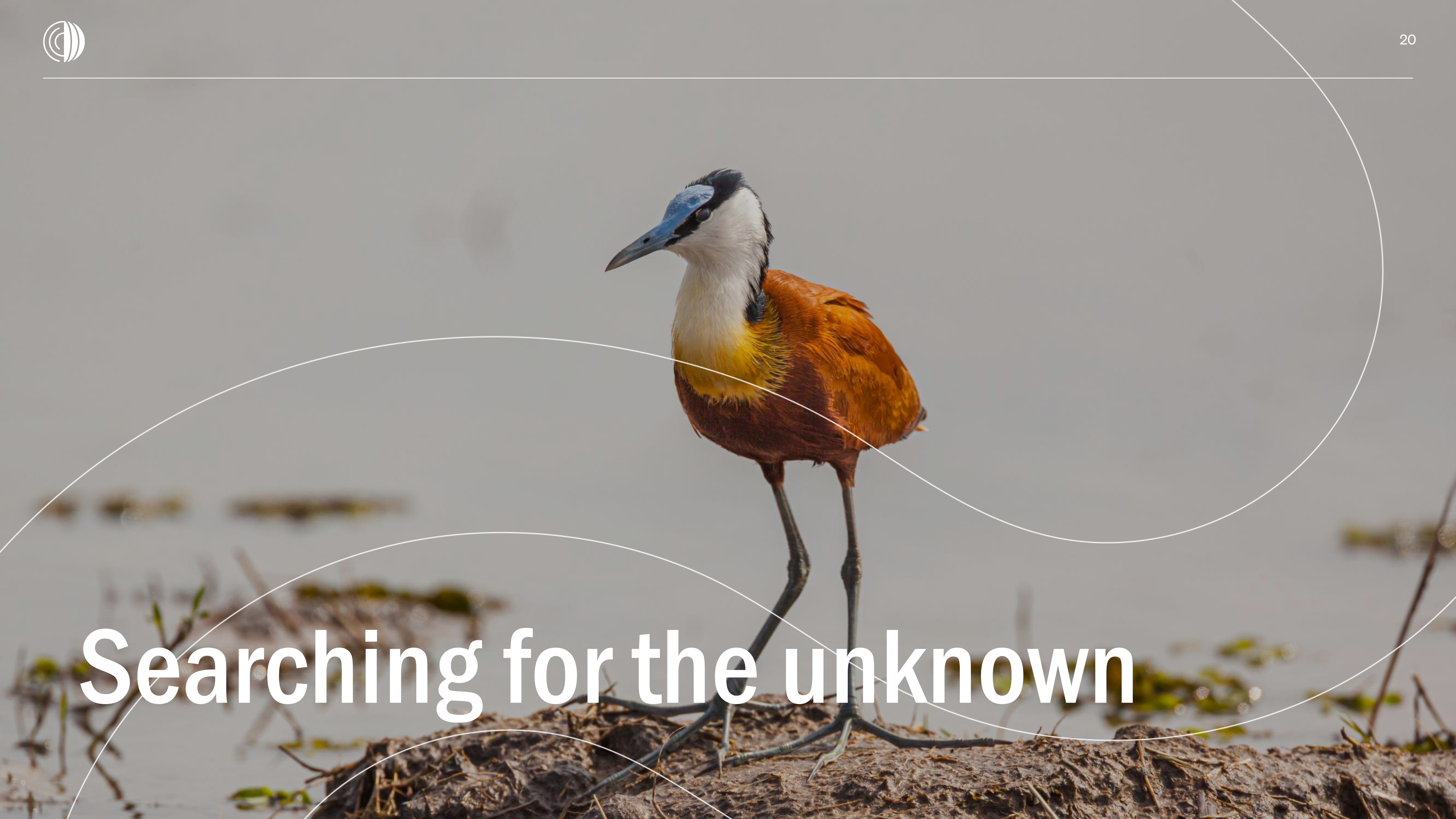
Hobbs, E. C., Colling, A., Gurung, R. B. & Allen, J. The potential of diagnostic point-of-care tests (POCTs) for infectious and zoonotic animal diseases in developing countries: Technical, regulatory and sociocultural considerations. Transbound. Emerg. Dis. 68, 1835–1849 (2021).

Case study 7: Point-of-care tests

- Used in the field under varying environmental conditions, on a range of sample types collected in non-sterile settings, by operators with a diverse range of experience, training and proficiency.
- Variable field-testing conditions (temperature, humidity, water and reagent quality, inadequate cold chain, operator ability, non-existent quality assurance systems) can alter test accuracies

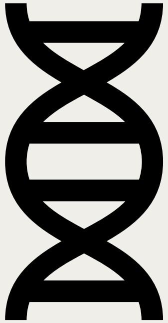
1. WOAH acknowledges the pressing need to develop validation guidelines and standards for the rapidly increasing use of point-of-care tests.
2. Need for confirmatory testing and reporting by an accredited laboratory in particular when performing a test for an exotic disease.
3. POCT-specific standards and recommendations, such as the point-of-care key evidence tool (POCKET) checklist for multi-dimensional evidence reporting; scorecards and guidelines for POCT evaluation; and guidelines on quality practices in non-instrumented POCTs to be developed
4. POCT accreditation with organisations such as the WOAH and national testing authorities is encouraged but not mandatory.

WOAH TERRESTRIAL MANUAL. Chapter 1.1.6. Principles and methods of validation of diagnostic assays for infectious diseases (version adopted in May 2023)


Searching for the unknown

Molecular methods for emerging diseases

- 16S ribosomial RNA
- Multi-locus sequence enzyme typing
- Variable number tandem repeat
- Next-Generation sequencing (single nucleotide polymorphism)



1. Pathogen phylogenies → understanding of the rate of new strain emergence, understanding selection pressure that has led to the emergence of a new strain
2. Characterized viral swarm
3. Identify transmission routes
4. Identify multiple reservoirs (when strain diversity is too high to be only the cause of mutation)
5. Inform the development of strain-adapted vaccines
6. Monitor the effectiveness of vaccine strategy (escape mutants = strain mutants that are affected by vaccination and grow when vaccination strategy is deployed)

Benton, C.H., Delahay, R.J., Trewby, H. et al. What has molecular epidemiology ever done for wildlife disease research? Past contributions and future directions. Eur J Wildl Res 61, 1–16 (2015). https://doi.org/10.1007/s10344-014-0882-4/Van Borm, S. et al. (2015). Next-Generation Sequencing in Veterinary Medicine: How Can the Massive Amount of Information Arising from High-Throughput Technologies Improve Diagnosis, Control, and Management of Infectious Diseases?. In: Cunha, M., Inácio, J. (eds) Veterinary Infection Biology: Molecular Diagnostics and High-Throughput Strategies. Methods in Molecular Biology, vol 1247. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-2004-4_30

Disease diagnosis in wild species

- Needs for non-invasive sampling methods compatible with welfare and species diversity
- Need to explore new sampling strategies and matrix
- Needs for validation on new matrix, new species
- Needs to take into account field conditions in validation protocols
- Control and validation of Point-Of-Care tests in the field
- Testing for early warning

Thank you!

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