PREPARE4VBD newsletter

June 2023 Newsletter #2

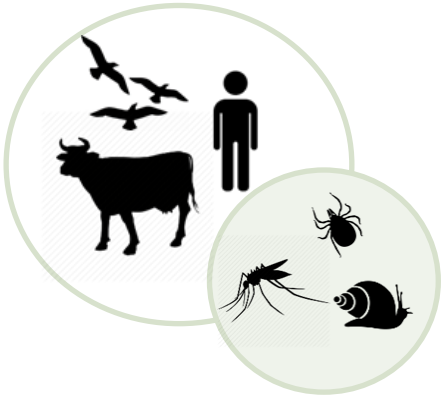
INSIDE THIS ISSUE

News and Views from the Coordinator

The PREPARE4VBD reached an important milestone in February 2023, when we ended the first 18 months and embarked on our first periodic reporting and review process. This was kickstarted with the submission of the first periodic report - followed by an online review meeting with the EU Project Officer, two external reviewers and the PREPARE4VBD Coordinating Team, PIs and WP leaders in June. The meeting was very constructive and a great testimony to all the hard work carried in the past 18 months by the Consortium partners. Thanks to all who contributed! Important milestones and key deliverables in the first 18 months include three project meetings and a succcesfull first PREPARE4VBD "summer-school", an important component of PREPARE4VBD's aim to strengthen VBD research capacity in Africa and Europe, In the first period, many activities has focused on data collation and fieldwork to build baseline knowledge of the target vectors and VBDs in Africa and Europe. Protocols for extraction of survey data from secondary sources has been developed and data compilation is on-going for the target diseases. Standard operating procedures (SOPs) has been developed for several PREPARE4VBD diagnostic tools for early detection, including an environmental DNA (eDNA) sampling SOP to detect snail -borne parasites, which was demonstrated at the annual meeting in South Africa. SOPs & videos for mini-FLOTAC has also been developed and freely available on the web-site and the PREPARE4VBD YouTube channel. Fieldwork to collect ticks, mosquitoes, snails and parasitological data was carried out in several partner countries and exiting preliminary results are emerging. You can read more about these in this issue of the PREPARE4VBD newsletter. We look very much forward to the next 18 months in the PREPARE4VBD project with you all! On behalf of the Coordinating team,

> One of the highlights of PREPARE4VBDs first 18 months include the annual meeting in South Africa in October 2022, attended by 43 delegates from the 8 partner countries, including leading experts and early career researchers in vector-borne disease research in Africa and Europe

Anna-Sofie Stensgaard



About PREPARE4VBD

Vector-borne diseases (VBDs) constitute a major challenge facing African healthcare systems and economies, but also increasingly pose a threat to Europe as spread of vectors and zoonotic VBDs is anticipated more frequently in the future.

The PREPARE4VBD project address this challenge as s a multidisciplinary consortium bringing together **ten university and ministerial partners from five African and three European countries.**

PREPARE4VBD will develop new knowledge, detection tools and surveillance systems to improve preparedness in Africa and Europe for vector-borne diseases transmitted by mosquitoes, ticks and freshwater snails to livestock and humans.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

PREPARE4VBD Research Highlights

Fasciola species and their intermediate hosts: How does agro-climatic condition impact species diversity and geographical distribution?

The team visited 14 locations in seven provinces and inspected 599 livers from cattle slaughtered at commercial abattoirs, and collected flukes from infected livers

As part of the ongoing field work in work package 6, the UKZN team led by Professor Mukaratirwa, consisting of Dr Pulane Malatji, Ms Sophy Nukeri, Ms Philile Ngcamphalala and Mr Ignore Nyagura, joined forces with the DALRD personnel, Mr M. Sithole, and Dr D. Tembe (UKZN lecturer) and visited twelve cattle slaughterhouses across six provinces of South Africa following a natural agro-climatic gradient in South Africa to collect liver flukes from cattle livers. This work will provide important clues about the distribution and genetic signature/diversity of the most southerly populations of *Fasciola* liver flukes and intermediate host snails in the PREPARE4VBD project, thus contributing to the objective to determine effects of temperature on the transmission dynamics of fasciolosis across latitudinal and altitudinal gradients. The team visited 14 locations in seven provinces and inspected 599 livers from cattle slaughtered at commercial abattoirs, and collected flukes from infected livers (Figure 1) in five provinces (Figure 2). Liver flukes were preliminarily identified using morphological features followed by molecular using multiple genes including the PEPCK, CO1 and ITS (Figure 2). Survey of freshwater snails was also conducted in water bodies from surrounding areas where the infected animals originated (Figure 3), and these sites will be re-visited for the collection of eDNA water samples as part of WP3 on ring-testing of diagnostic tools for *Fasciola* spp as well as fecal sample to test the sensitivity and specificity of the newly developed mini-FLOTAC technique in the diagnosis of liver flukes in cattle.

(To be continued on page 3)

Fig 3. Ms Sophy Nukeri assessing the sequence quality after Sanger sequencing.

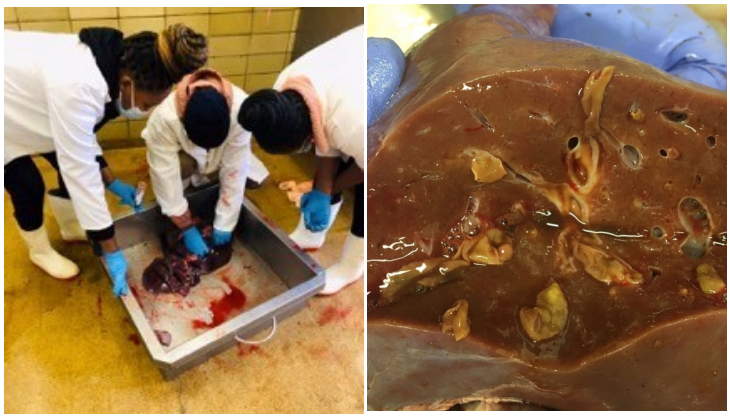


Fig 1. Liver inspection and collection of liver flukes.

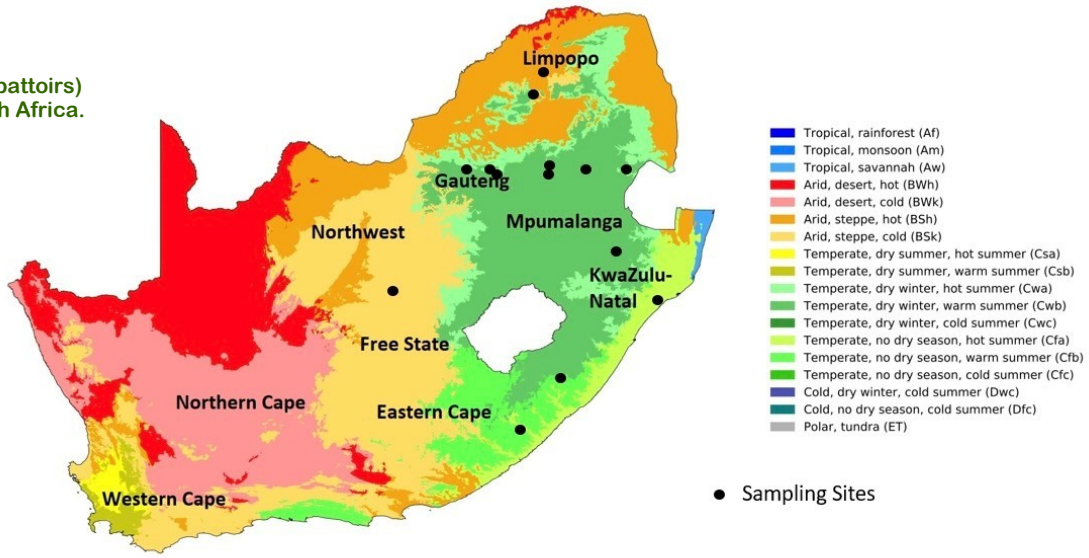


Fig 2. Map showing localities (abattoirs) surveyed for liver flukes in South Africa.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

(continued)

Preliminary results

1) *Fasciola hepatica* was more predominant (Figure 4) and was the only species in five provinces surveyed including in areas of Mpumalanga and KwaZulu-Natal where both species have been previously reported to occur.

2) *Fasciola gigantica* was reported only in Limpopo province and as a predominant species (Figure 5). This is the first report of this species in this province in the last 6 decades whilst *F. hepatica* seem to have dominated in the provinces which have been historically known to have *F. gigantica*.

3) The results reiterated the predominance of *F. hepatica* in South Africa (Figure 5), and further showed rapid spread of *F. hepatica* as it was found as the only species in areas and abattoirs with previous record of *F. gigantica*. Our next step is to determine if there is correlation between these results with the presence of invasive snail *P. columella* and agro-climatic temperature gradients of the locations.

4) Preliminary results showed that microsatellites markers used in this study were highly polymorphic, with 9 – 23 alleles identified. Considering abattoir fluke collections from provinces as individual populations, *F. hepatica* populations shared more than 50% similarity in alleles with three loci across populations. However, other loci contrariwise showed more than 50% differences in alleles across populations. *Fasciola gigantica* showed seven unique alleles with one microsatellite locus which might be of use as markers when correlated with temperature when further analysis is done. We presume that the analysis of all samples will show a trend of variable populations of *F. hepatica,* separated by different agro-climatic gradient in South Africa.

Fig 6. UKZN team surveying freshwater snails in Free State.

5) Twelve freshwater snail species were collected (Figure 6) and identified. Amongst these were *Radix natalensis* and *Pseudosuccinea columella*, which were co-endemic in Limpopo and Mpumalanga, and both *Fasciola* spp. were currently identified in Limpopo province.

6) *Pseudosuccinea columella* was the only lymnaeid species occurring in Gauteng, KwaZulu-Natal and Eastern Cape province, with exception to Free State where *Bulinus truncatus* were predominant, which might be the predominant snail fuelling the transmission of *F. hepatica* in this province.

In conclusion, the fieldtrips allowed us to identify suitable study sites that both falls into our temperature gradient areas and are suitable for testing of WP3 tools, collection of snails for breeding for experimental temperature studies and *Fasciola* specimens from cattle abattoirs following the temperature gradient in the country. Furthermore, important areas of co-occurrence of *F. hepatica and F. gigantica* and thus potential hybridization-zones were identified.

The team visited 14 locations in seven provinces and inspected 599 livers from cattle slaughtered at commercial abattoirs, and collected flukes from infected livers

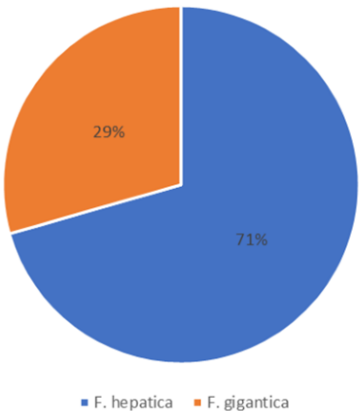


Fig 4. Proportions of *Fasciola hepatica* to *F. gigantica* across six provinces.

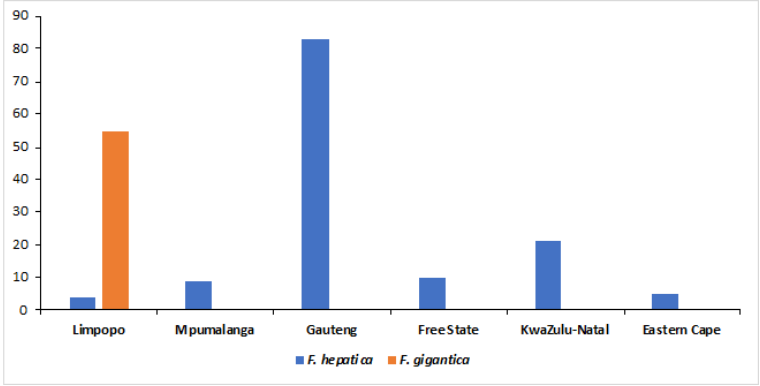


Fig 5. Distribution and number of specimens included in the molecular characterisation of *Fasciola* spp. in South Africa.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

PREPARE4VBD Research Highlights

Disease susceptibility of European and African cattle breeds

Our working hypothesis is that different breed susceptibilities to VBD are rooted in genetics and differential immune responses, which can be identified using a blood-based ex vivo platform

A team at the Department of Infectious Diseases and Pathobiology of the University of Bern is a member of the international PREPARE4VBD research consortium. The team, which includes Dr. Jörg Jores, Dr. Thomas Démoulins, Dr. Fabien Labroussaa and Dr. Hatice Akarsu, focusses its effort on the better understanding of bovine immune responses against pathogens. Indeed, although cattle are the mammalian species with most planetary biomass associated with a huge impact on the planet, their immune system remains - compared to humans or mice - poorly understood. Nevertheless, it is well accepted that different livestock breeds show different susceptibilities to pathogens of vector-borne diseases (VBD) such as East Coast Fever or Trypanosomiasis.

Our working hypothesis is that different breed susceptibilities to VBD are rooted in genetics and differential immune responses, which can be identified using a blood-based *ex vivo* platform (Figure 1). We developed the platform employing fresh bovine blood (Figure 2), from which we isolate primary cells that comprises most of immune cell subsets. The comparison of different bovine breeds takes advantage of *Bos taurus*-(European) cattle, iconic in Switzerland and *Bos indicus*-type (African) cattle of which >200 heads are scattered around Switzerland. Moreover, our *ex vivo* platform (Figure 1) promotes animal welfare under the umbrella of 3R (replacement, reduction and refinement).

We make use of the latest biologicals to tag different bovine surface markers, chemokines and cytokines and equipment for the analysis of bovine immune responses. Firstly, a novel 12-14 color multiparameter flow cytometry assay was developed enabling the measurement of maturation (modulation of cell surface marker expression) and activation (intracellular cytokine detection) of the major immune cell subsets. Secondly, a multiplex immunoassay was employed to monitore the secretion of chemokines and cytokines (crucial soluble factors in the orchestration and orientation of the immune response) by bovine cells following interaction with pathogens.

(To be continued)

Fig 2. We are taking blood samples in the Agroscope animal facilities (Posieux, Switzerland), by jugular vein puncture. This procedure is associated with minimal distress for the animals and blood is collected into vacutainer EDTA tubes.

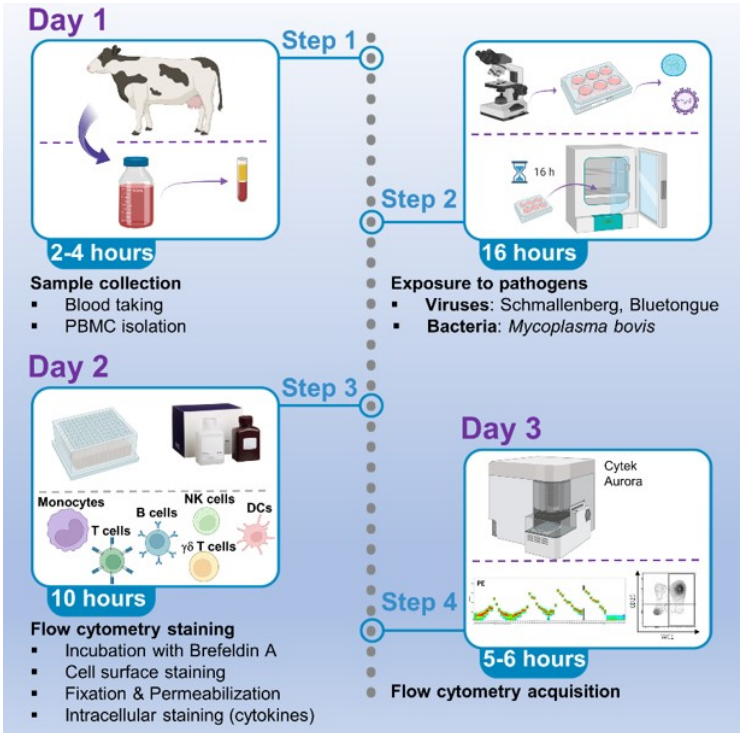


Fig. 1. summarizes a typical experimental schedule employing of *ex* vivo platform, requiring three full days from bovine blood collection to the acquisition of flow cytometry data. Day 1: blood collection, cell isolation and immune cell stimulation by pathogens; Day 2: antibody staining of immune cell subsets; Day 3: flow cytometry data acquisition.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

PREPARE4VBD Research Highlights

Main findings

We identified new key players of immune cells whose role was so far largely neglected

(Continued)

After one year, the objective to set up an *ex vivo* platform to be subsequently probed with different VBD is now achieved and a manuscript describing this platform is ready for submission. Briefly, we tested our platform on a major bovine pathogen, namely *Mycoplasmopsis bovis* (*M. bovis*) (Figure 3). Besides reaffirming the tight cooperation of the different primary blood cells, we identified new key players of immune cells whose role was so far largely neglected. Additionally, we proved that high fever, a clinical feature associated with many infectious diseases, can attenuate the capacity of most immune cell subsets to respond to this specific pathogen (Figure 4). The proven powerfulness of our experimental approach can now be applied to host-pathogen interaction studies involving a large spectrum of pathogens causing VBD. In the long term, we envisage to make predictions about susceptabilities of different breeds using this platform. Moreover, a better understanding of host-pathogen interactions fosters the development of rationale vaccines.

As a logical follow-up in the context of PREPARE4VBD, we are currently investigating the impact of bacterial and viral coinfections with two distinct vector-borne viruses: Bluetongue and Schmallenberg viruses, both transmitted through the bite of infected mosquitoes and midges, and for which we received clearance to work in biosafety level two (BSL-2) conditions.

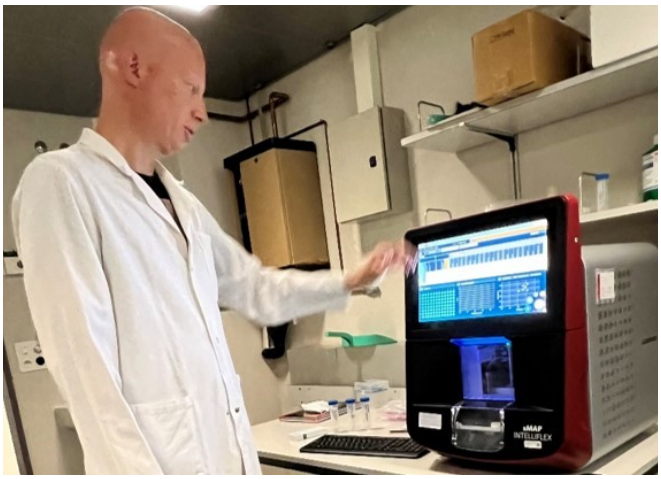


Fig. 3. we are conducting a multiplex immunoassay with a new generation MAGPIX® instrument (Luminex system), to evaluate the production of cytokines by primary blood cells in response to bovine pathogens.

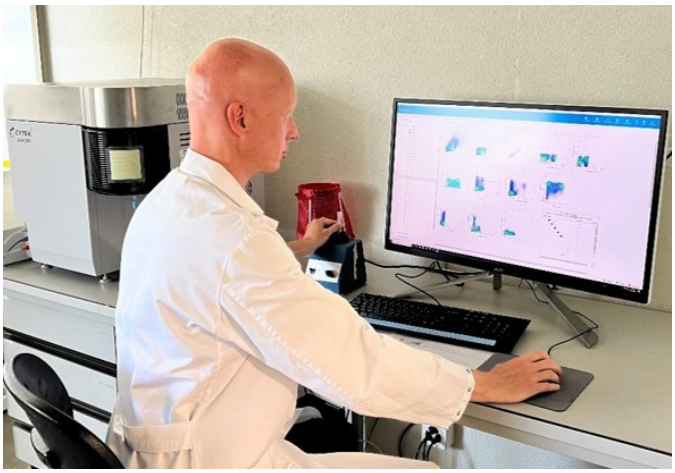


Fig 4. we are acquiring the data with a new generation Flow Cytometer (Cytek® Aurora), to evaluate the specific response of monocytes, conventional dendritic cells, plasmacytoid dendritic cells, natural killer cells, $\delta\gamma$ T cells, B and T cells.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

Introducing the PREPARE4VBD Fellows

In this edition of the PREPARE4VBD newsletter, we asked Agrippa Dube (PhD@UKZN), Rua Khogali (PhD@*icipe*), Philile (PhD@UKZN) and Godlisten (PhD@NIMR) to tell us a bit about themselves.

Agrippa Dube is a PhD Student at UKZN working on the effect of temperature on fascioliasis transmission. He will combine the data from laboratory experiments on temperature effects on life history traits of *Lymnaea* snails and field work to produce a mechanistic model for predicting future effects of environmental changes on fascioliasis transmission.

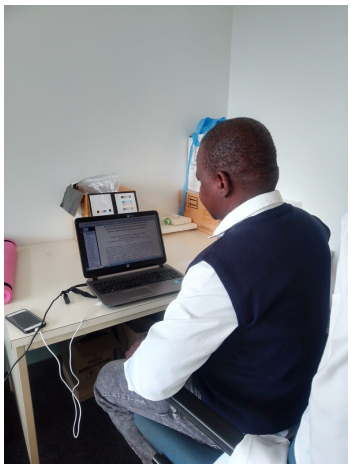
What do you like most about doing research? Will empower me with new knowledge and skills in ecology and biology of intermediates host snails and trematode parasites and modelling of the disease outbreak and spread in the near future. Research work allows me to link with other researchers in the study of neglected tropical disease worldwide.

What excites you about your work? Is that my work will contribute much to the society, and it's supposed outputs will help the community, public health, veterinary departments by enlighten them about the impact of climate change on fascioliasis transmission.

What is your favorite food? Rice and chicken.

What is your favorite celebration? My favorite celebration is Easter. This event provides an opportunity for churchgoers from around Zimbabwe's Apostolic Church to unite, worship, and pray as they remember Jesus Christ's resurrection.

What do you like to do outside work? Going to church and watching movies.



In the picture, I am sitting at my desk with a computer working on a research review paper.

Introducing the PREPARE4VBD Fellows

Rua Khogali is a PhD student at *icipe* and the University of Pretoria working on her thesis: "Using metagenomics to unravel how camel tick microbiomes affect pathogen prevalence and transmission risk".

What do you like most about doing research? My research allows me to expand my capacities and explore my potential. I benefit greatly from working closely with mentors, being a part of a team, and diverse networking and collaboration opportunities. In an environment that encourage learning and growth, the resulting knowledge and idea exchanges guide me in my scientific thinking and promote professional relationships that can lead to new prospects and collaborations.

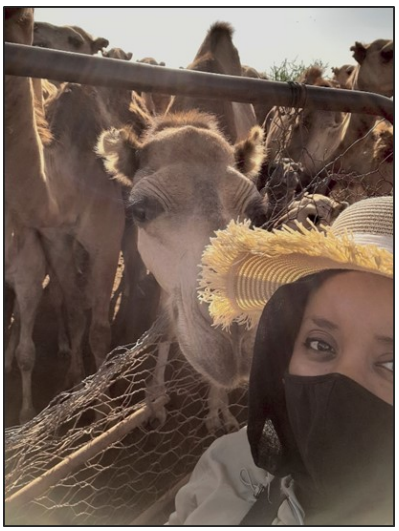
What excites you about your work? I particularly enjoyed developing protocols for collecting saliva, hemolymph, salivary glands, and midgut from individual ticks. It has allowed me to screen for pathogens and conduct metagenomic analysis within these tissues for the first time, despite my initial knowledge gaps. Moreover, I love going to the field, where I can interact with pastoralist communities that provide me with valuable opportunities to observe and learn from these key stakeholders in my work.

What is your favorite food? A traditional Sudanese dish, "Tagalia", which is prepared using dry or minced meat, okra powder, tomato, and onions.

What is your favorite celebration? I have deep appreciation for Eid celebrations, which have religious significance and are occasions to enjoy the company of my family and friends as we take part in joyful festivities.

What do you like to do outside work? I enjoy the gym and Zumba and aerobic dancing classes, which not only keep me fit, but have the power to alleviate stress and uplift the spirit. In general, I

"My research allows me to expand my capacities and explore my potential."



In the picture (selfie with a smiley camel), I am collecting ticks and blood from camels in Mpala, Laikipia County.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

Introducing the PREPARE4VBD Fellows

Philile Ngcamphalala is a PhD Student at UKZN working on developing and validating protocols for detection of eDNA of *Fasciola gigantica* and its intermediate hosts in selected provinces of South Africa.

What do you like most about doing research? Learning something new – new techniques and making new findings i.e. parasites are forever evolving and adapting, therefore, there will always be new information to learn about them. Furthermore, being a researcher keeps me updated on new developments in my field and other relevant fields.

What excites you about your work? I work with parasites that are part of the neglected tropical diseases group and that makes me feel like I/ we are doing important work in shedding light on these parasites or diseases that are not well known by the public, yet they affect many lives globally.

What is your favorite food? Braaied meat

What is your favorite celebration? I love celebrating birthdays.

What do you like to do outside work? I enjoy being indoors, reading novels.

In the picture, I am filtering water to obtain eDNA from a dam in the Free state province, South Africa.

Introducing the PREPARE4VBD Fellows

Godlisten Shedrack Materu is a PhD Student at National Institute for Medical Research and Sokoine university of Agriculture in Tanzania working on the ecology and diversity of freshwater snails and their role in the transmission of snail borne parasites of humans and animals (zoonotic) in different ecological zones of Tanzania.

"I love research and I love to stay in research because it has "no rules"

What do you like most about doing research? I love research and I love to stay in research because it has "no rules". Research is competitive, challenging, stretches my mind and introduces me to such interesting people around the globe.

What excites you about your work? I am working on area which has not much explored in Tanzania. The findings from this work will provide avenue for more research and will help to formulate policy for better surveillance and control of snail borne parasites in human and domesticated ruminants.

What is your favorite food? Pork with vegetables served with Ugali, banana with meat (chicken/beef) served with varieties of fruits i.e avocado

What is your favorite celebration? Christmas celebrations because we always get together with family member and friends after a long period of not meeting each other.

What do you like to do outside work? I love jogging, hanging out with friends and watching football. My favorite club is Manchester City.

In the picture I am packing snails collected in the field.

The fellows are an integrated and important component of PREPARE4VBD

By providing a cross-country supporting network and mentoring of a cohort of >15 early career scientist, PREPARE4VBD will enhance the individual and institutional capacity and help foster a new generation of experts in vector biology, VBD surveillance and control in both Africa and Europe. Capacity building in PREPARE4VBD not only include developing scientific and technical knowledge, but also broader competencies, such as writing, disseminating research and other generic skills for early career researchers. Such skills are essential to promote long-term career success for the fellows, but are also key in driving the implementation, impact and success of PREPARE4VBD.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

News and Updates from the Project Management

Open Research Europe

We would like to make all partners aware of the possibility for rapid, peer review and open access publication for research stemming from Horizon 2020, Horizon Europe and Euratom funding across all subject areas:

https://open-research-europe.ec.europa.eu/

Next annual meeting in the PREPARE4VBD consortium

The next annual meeting in the PREPARE4VBD will be hosted by *icipe* in Kenya 26. February- 1.March 2024. Prior to the meeting, a summer school on diagnostic methods and molecular tools for the project fellows will take place 23.-25-February 2024.

Important deadlines:

- 31 Aug 2023 – Deliverable 3.1 (*icipe*)
- 31 Aug 2023 – Deliverable 4.2 (UCPH)
- 31 Aug 2023 – Deliverable 5.1 (UBERN)
- 31 Aug 2023 – Deliverable 5.2 (UBERN)
- 31 Aug 2023 – Deliverable 6.2 (UKZN)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

Newsletter editorial board:

Anna-Sofie Stensgaard

(asstensgaard@sund.ku.dk)

Mita Eva Sengupta

(msen@sund.ku.dk)

Katrine Mohr

(katrine.mohr@sund.ku.dk)

University of Copenhagen

Denmark

The PREPARE4VBD newsletter series are biannual and will provide updates on project progress incl. research activities and capacity building, important deadlines in the project and upcoming events.

This PREPARE4VBD newsletter can be shared with relevant or interested institutions and stakeholders.

Find the PREPARE4VBD newsletters here:

prepare4vbd.eu/newsletter

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365

The PREPARE4VBD Consortium

PREPARE4VBD Contact information

E-mail:
info@prepare4vbd.eu

Project Coordinator:
Anna-Sofie Stensgaard, UCPH
asstensgaard@sund.ku.dk

Project Manager:
Katrine Mohr, UCPH
katrine.mohr@sund.ku.dk

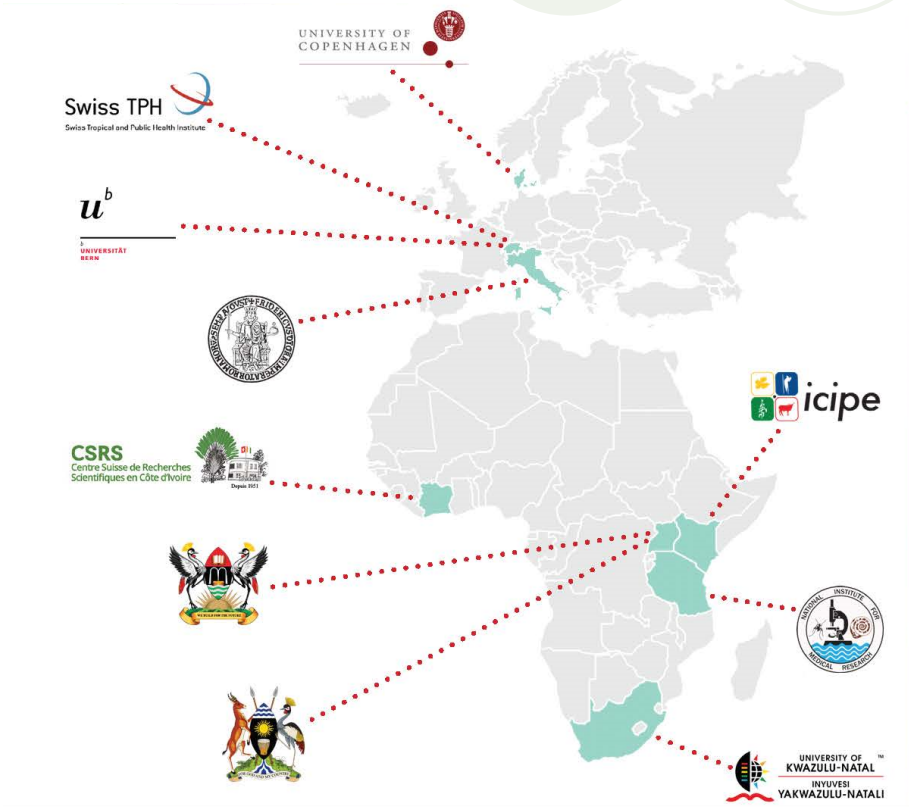
We are on the web!

Follow the project results, news and subscribe to our newsletter on:

www.prepare4vbd.eu

https://twitter.com/prepare4vbd

https://www.linkedin.com/company/76113199



UNIVERSITY OF COPENHAGEN	UNIVERSITY OF COPENHAGEN
icipe	THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
	MAKERERE UNIVERSITY
Swiss TPH	THE SWISS TROPICAL AND PUBLIC HEALTH INSTITUTE
	UNIVERSITY OF BERN
UNIVERSITY OF KWAZULU-NATAL	UNIVERSITY OF KWAZULU-NATAL
	UNIVERSITA DEGLI STUDI DI NAPOLI FEDERICO II
	NATIONAL INSTITUTE FOR MEDICAL RESEARCH
CSRS	CENTRE SUISSE DE RECHERCHES SCIENTIFIQUES EN CÔTE D'IVOIRE
	VECTOR CONTROL DIVISION MINISTRY OF HEALTH

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000365