„Jetzt wird es Wild" – Prävalenzerfassung und Monitoringstudien ausgewählter humanpathogener Erreger in Wildtieren – One Health Aspekte

16.11.2023, BfR-Symposium: Zoonosen und Lebensmittelsicherheit

Martin Richter

Fachgruppe Diagnostik, Erregercharakterisierung und Parasiten in Lebensmitteln, Department of Biological Safety

One Health – Definition

"One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.

It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent."

One Health High Level Expert Panel (OHHLEP) 2021

"Das One Health-Konzept erkennt an, dass die menschliche Gesundheit mit der Gesundheit von Nutz-, Haus-, und Wildtieren und der Umwelt verbunden ist".

CDC, About One Health

Definition of "One Health"
Integrative Management of Health-Related Risks
Simultaneous Consideration of 5 Components

Human Health | Animal Health | Environmental Protection (Soil, Water, Air) | Food Safety | International Trade

International FoodNet Center of the University of Bonn

Wildtiergesundheit und Zoonoseerreger sowie Kontaminanten aus Wildfleisch

Situation um 2018:

Die Datenlage zum tatsächlichen Vorkommen von pathogenen Erregern in Wild in Deutschland ist nicht umfangreich oder unzureichend und es besteht generell Forschungsbedarf.

Das BfR hat deshalb bereits Stellungnahmen und Risikobewertungen im Sinne des Verbraucherschutzes veröffentlicht, in denen auf die geringe Datendichte hingewiesen wird.

Das BfR führt eigene Studien zur Verbesserung dieser Datenlage durch.

Gesundheitliche Bewertung des BfR

DOI 10.17590/20181221-095937-0

**Wildfleisch: Gesundheitliche Bewertung von humanpathogenen Parasiten**

Stellungnahme Nr. 045/2018 des BfR vom 21. Dezember 2018

Fleisch von freilebendem Wild wie Reh, Hirsch und Wildschwein ist nährstoffreich, fettarm und wird nachhaltig gewonnen. Es kann allerdings Parasiten enthalten, die den Menschen auch krank machen können, wenn das Fleisch nicht hygienisch einwandfrei zubereitet wird. Bei der fleischhygienerechtlichen Beurteilung von erlegtem Wild gibt es immer wieder Unklarheiten, etwa in welchen Teilen des Tierkörpers bei befallenen Tieren bestimmte Parasiten vorkommen können. Dies liegt unter anderem daran, dass wenige Daten zu den spezifischen Krankheitserregern in Wildtieren verfügbar sind. Das Bundesinstitut für Risikobewertung (BfR) hat daher ausgewertet, in welchen Wildtierarten und deren Organen die unten genannten Parasiten bisher nachgewiesen wurden und wie häufig solch ein Befall war. Daraus hat das BfR das Risiko einer parasitär bedingten Erkrankung durch den Verzehr von Wildfleisch abgeschätzt und Vorschläge unterbreitet, wie Verbraucherinnen und Verbraucher solche Erkrankungen vermeiden können.

International FoodNetCenter of the University of Bonn

BfR Studienzentrum für Landnutzungsbezogene Bewertungsverfahren und One Health (LaBeOH) – Seit 2019
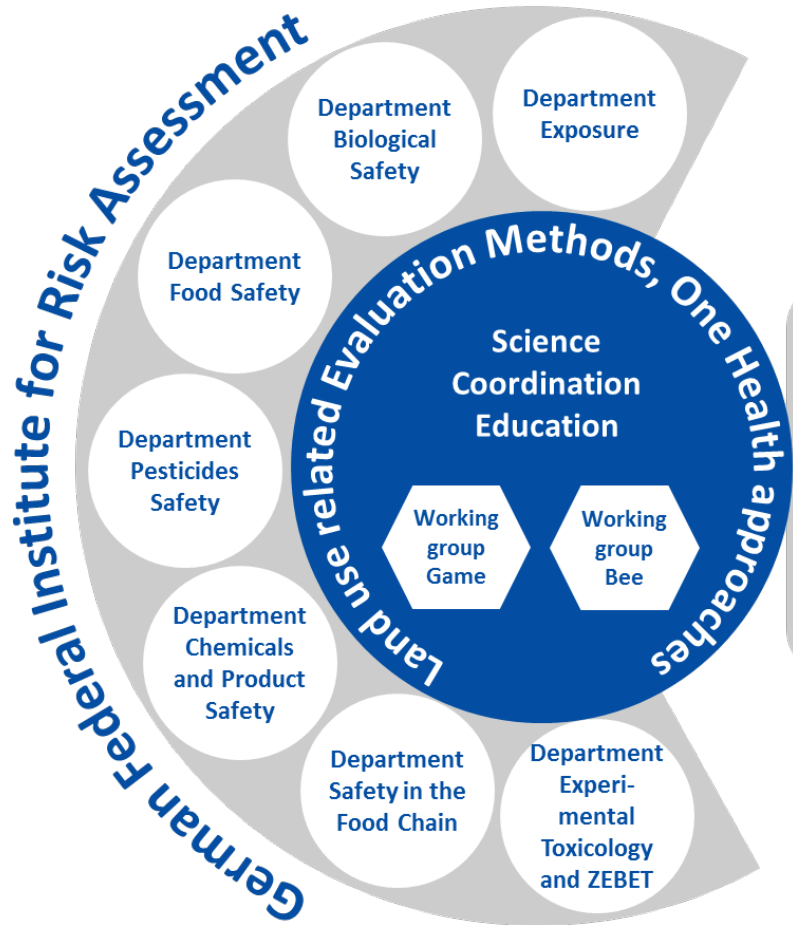
BfR Studienzentrum für Landnutzungsbezogene Bewertungsverfahren und One Health (LaBeOH) – Seit 2019
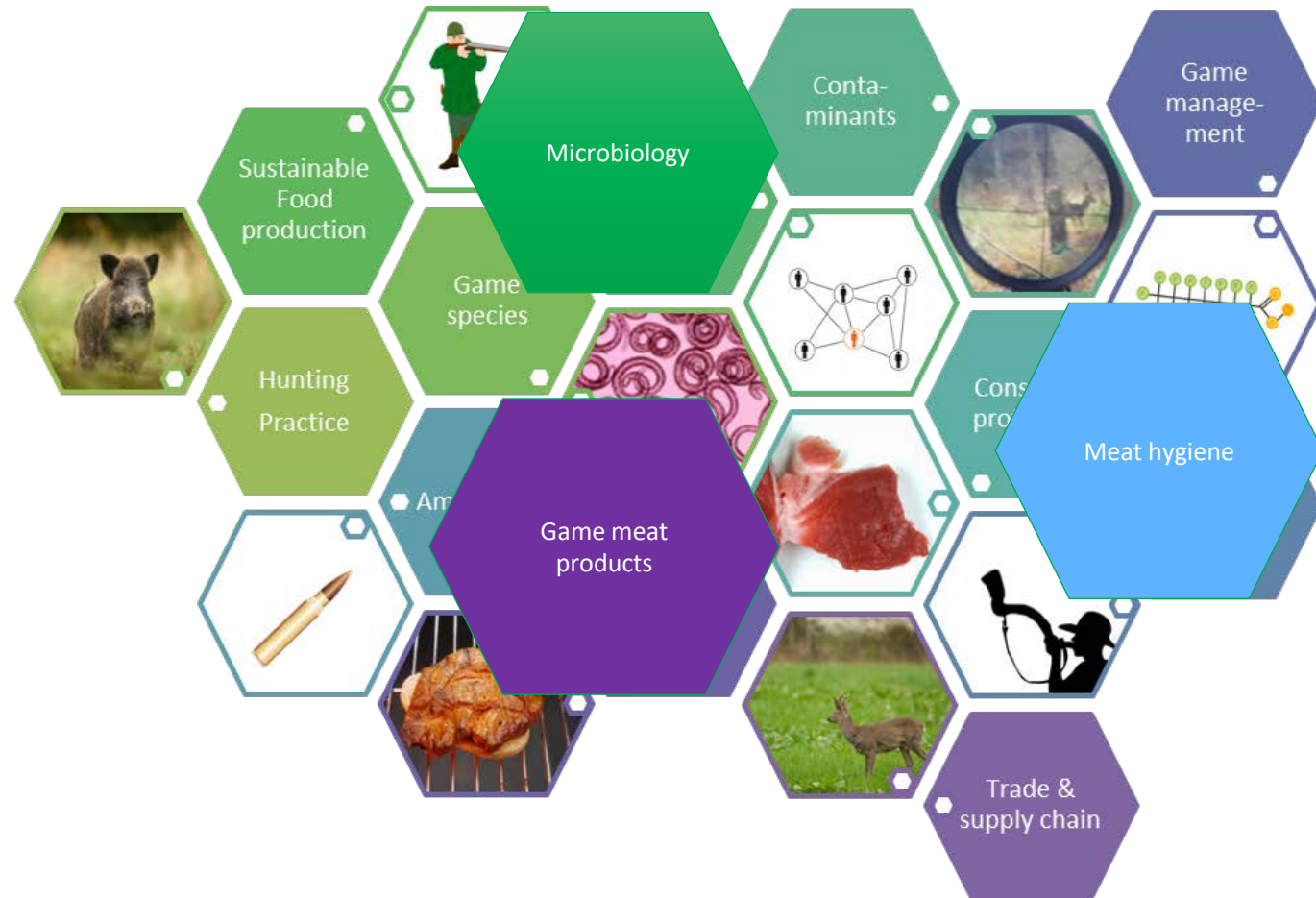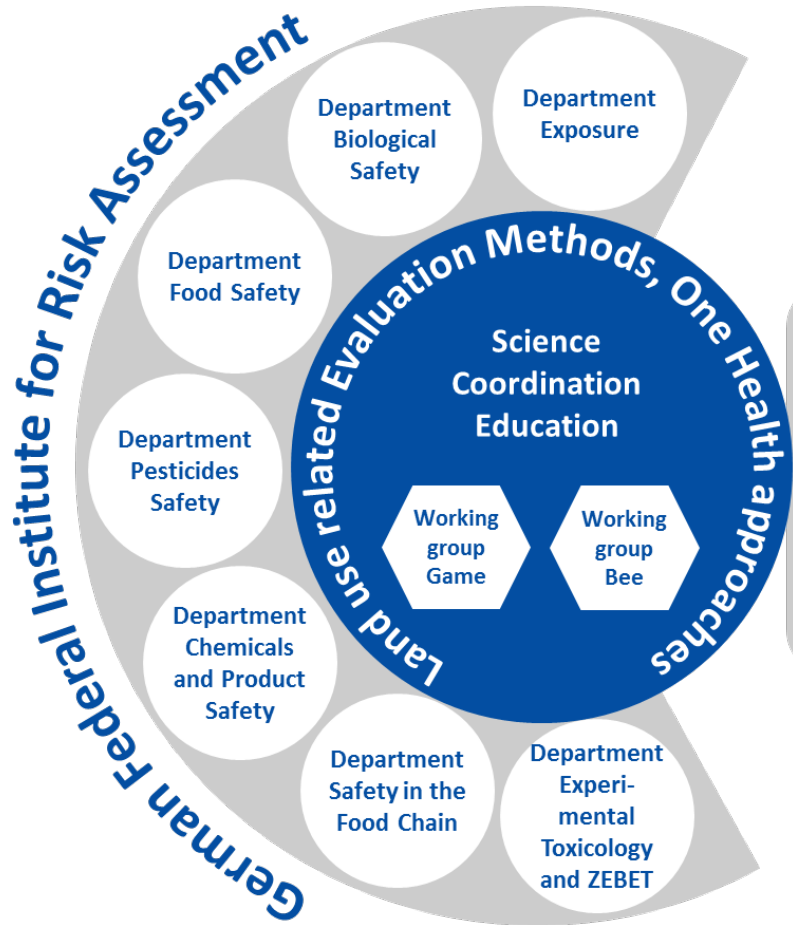
Probenahme

- ➤ Möglichkeit der langfristigen Beprobung - Monitoring

- ➤ Begleitung von Jagden

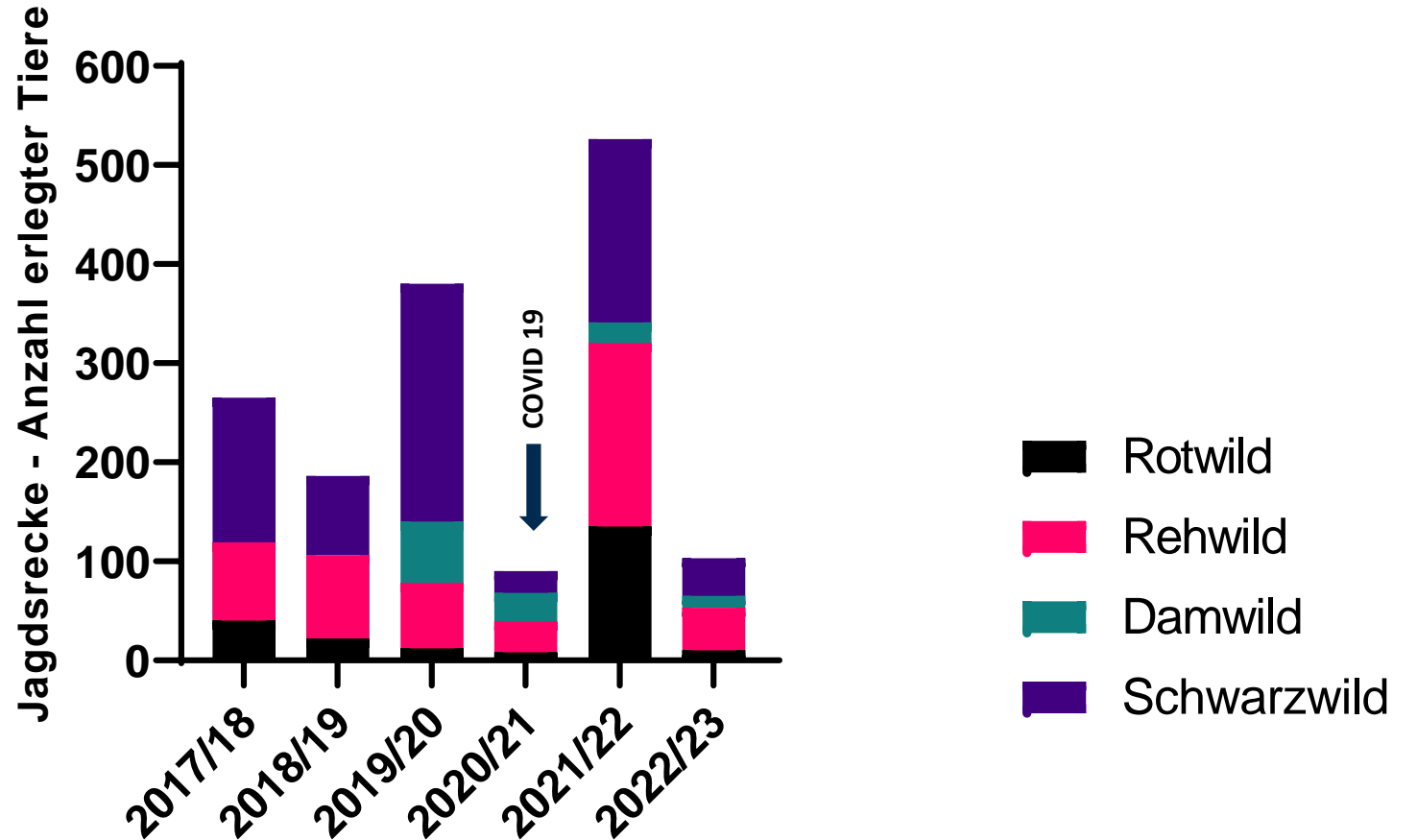- ➤ Unterstützung bei der Probenahme durch Jagdteilnehmer

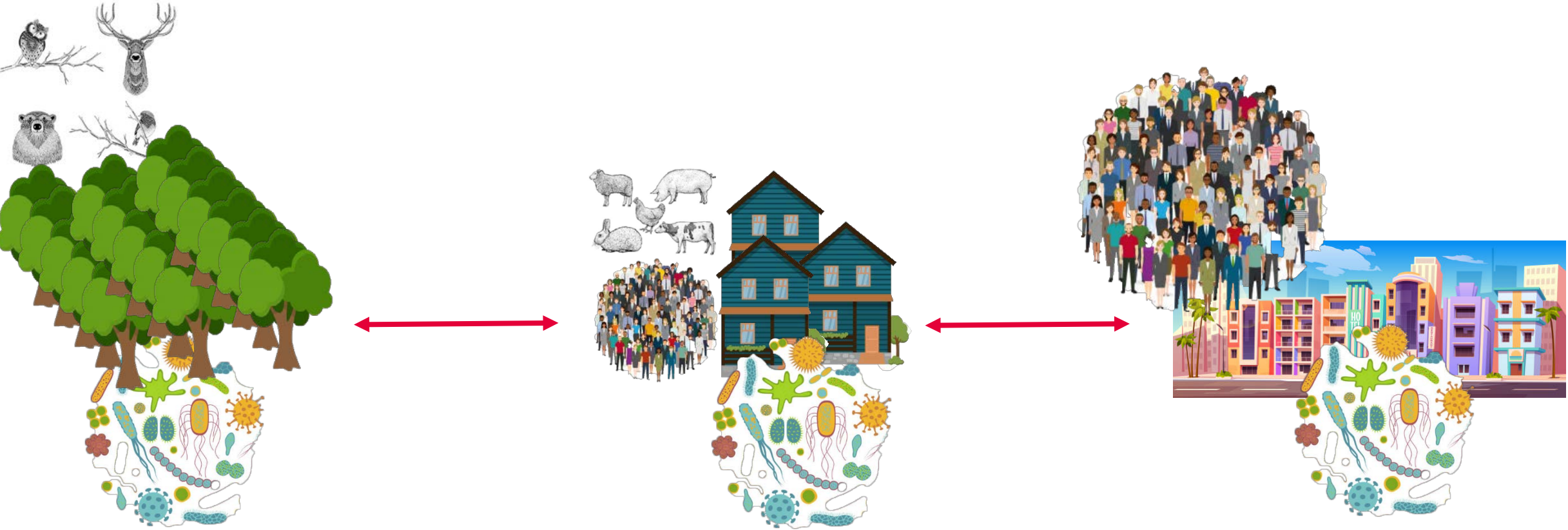- ➤ Bisher– 26 Jagdgebiete mit >100 Jagden

Berlin

animals MDPI

Article

Standardised Sampling Approach for Investigating Pathogens or Environmental Chemicals in Wild Game at Community Hunts

Denny Maaz [1,*], Carl Gremse [1], Kaya C. Stollberg [1], Claudia Jäckel [1], Smita Sutrave [1], Carolyn Kästner [1], Birsen Korkmaz [1], Martin H. Richter [1], Niels Bandick [1], Julia Steinhoff-Wagner [1], Monika Lahrssen-Wiederholt [2] and Anneluise Mader [1]

BfR

Bisher

➤ Wildtiere = 1441

➤ Proben = 5629

➤ Variierende Jagderfolge

Martin Richter | BfR Symposium: Zoonosen und Lebensmittelsicherheit, Berlin | 16.11.2023 |

One Health – Zoonosen
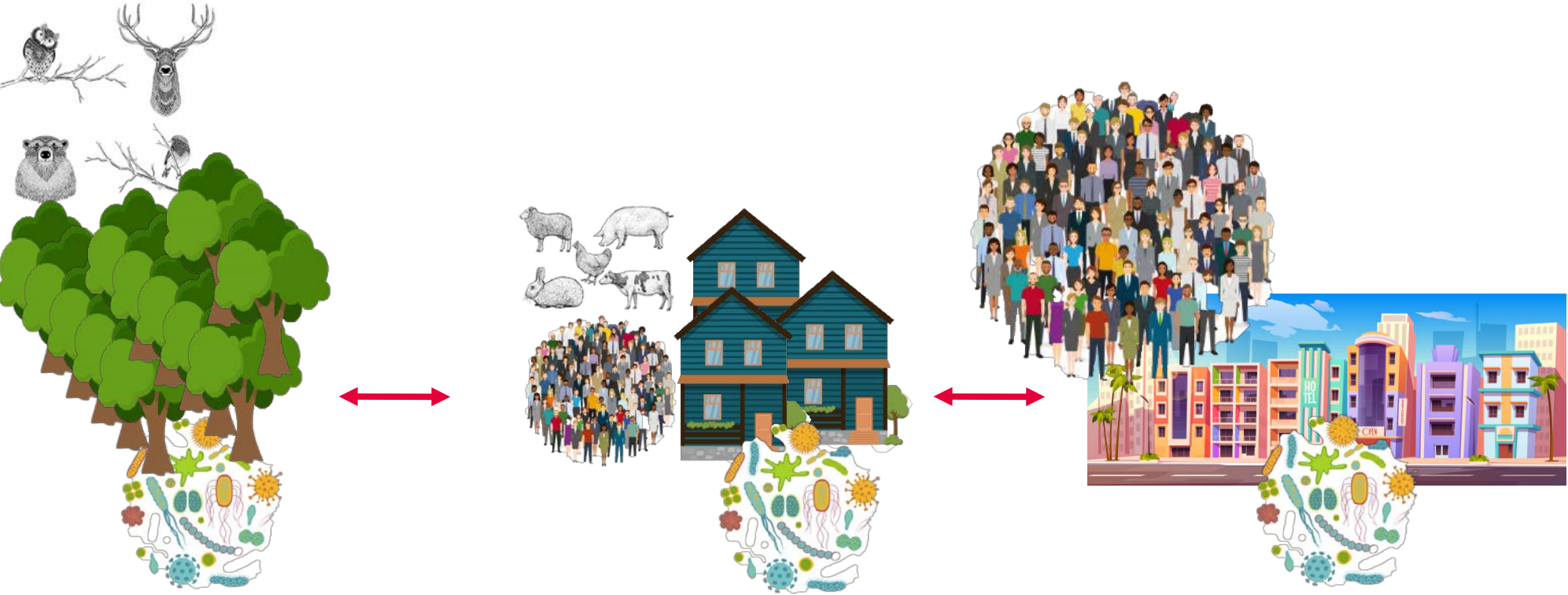## steigender Fokus One Health, warum?
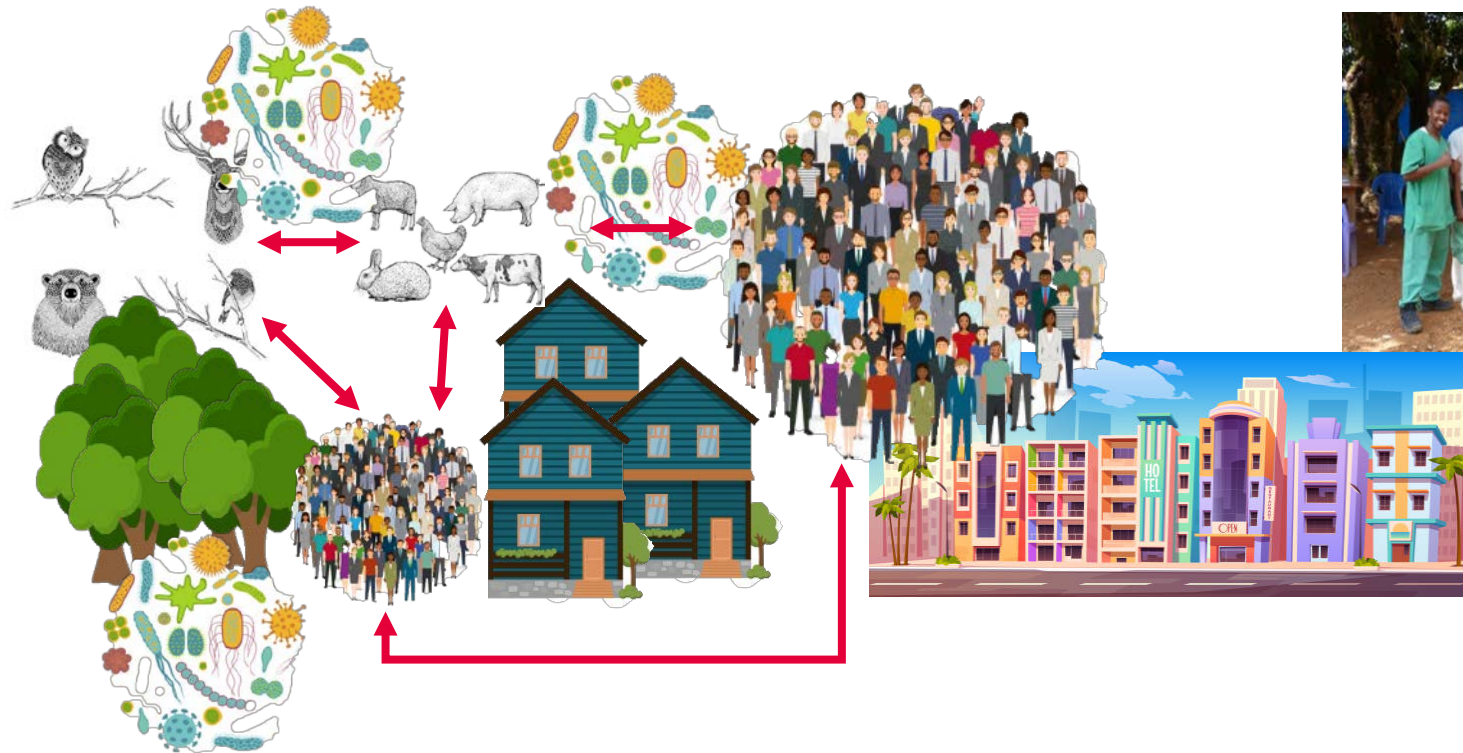
One Health – Zoonosen
steigender Fokus One Health, warum?

One Health – Zoonosen
steigender Fokus One Health, warum?

One Health – Zoonosen
steigender Fokus One Health, warum?

Martin Richter | BfR Symposium: Zoonosen und Lebensmittelsicherheit, Berlin | 16.11.2023 |

One Health – Zoonosen
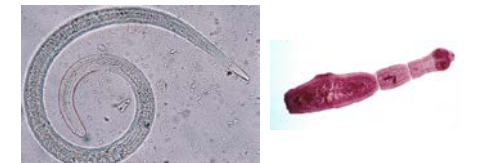## steigender Fokus One Health, warum?

Invasive Spezies, Erreger re-emergence, urbane Anpassung

lokal, endemisch, Aufrechterhaltung von Erregerzyklen

Invasive Spezies, Erreger re-emergence

lokal, endemisch, urbane Anpassung

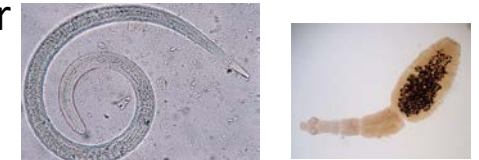# One Health – Zoonosen
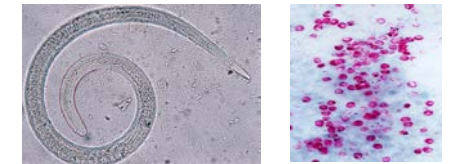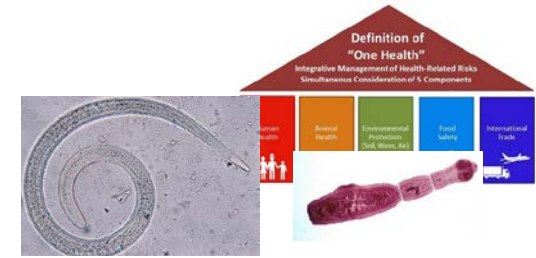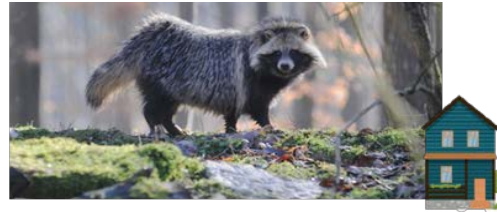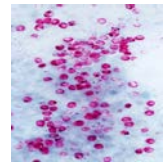## steigender Fokus One Health, warum?

Invasive Spezies, Erreger re-emergence, urbane Anpassung

lokal, endemisch, Aufrechterhaltung von Erregerzyklen

Invasive Spezies, Erreger re-emergence

lokal, endemisch, urbane Anpassung

Erreger

Parasites

Cryptosporidium spp.

Background:
- protozoan parasite with a low infectious dose (10-100 oocysts) that causes gastroenteritis in numerous hosts

Methods:
- wild boar, roe deer, and red deer feces were investigated using nested PCR protocol targeting three loci (COWP, 18S rRNA and GP60)

Alaria alata

Background:
- mesocercariae have been detected in wild boar as incidental finding during Trichinella examination
- wild boars are considered to be paratenic hosts

Methods:
- tongue and layrnx of wild boars are investigated for *Alaria alata* via *Alaria* spp. mesocercariae migration technique (*Riehn et al.*, 2010).

Preliminary results:
- mesocercariae were found in 26% (16/61, 95% CI:15,8-39%) of the samples
- 422 mesocercarie were collected
- Protein extracted from the collected mesocercarie will be used to develop a novel detection and identification method for *Alaria alata* using MALDI-TOF-MS

Toxoplasma gondii

Background:
- protozoan parasite causing toxoplasmosis
- raw or undercooked game is a potential source for infection

Methods:
- meat juice of heart muscle and serum was tested by a commercially available ELISA assay (ID Screen® Toxoplasmosis Indirect Multi-species by IDVet)
- where available 5g of heart muscle were analyzed by qPCR targeting the 529 bp-repeated element
- where available 50 g of heart muscle were analyzed using pepsin digestion combined with qPCR and/or a mouse bioassay

Perspective:
- comparison of methods for *T. gondii* tissue cysts in game meat
- use of serological as well as molecular detection methods to assess whether there is correlation between seropositivity and actual presence of *T. gondii* tissue cysts in game

Erreger

Bacteria

Campylobacter spp.

Background:
- the most common reported cause of bacterial diarrhoea in Germany
- most infections caused by consumption of raw undercooked meat or through intake of contaminated foodstuffs or water
- contamination of food and water primarily occurs through feces from animals that are infected with or are colonised with *Campylobacter* spp.

Methods:
- feces of roe deer and wild boar underwent a cultural examination for *Campylobacter* spp..

Yersinia spp.

Background:
- although symptoms may vary with age fever, abdominal pain, and diarrhorea are common signs of yersiniosis
- wild boar as well as domestic pigs are considered to be the most important animal reservoir for *Y. enterocolitica*
- *Y. pseudotuberculosis* is also commonly isolated from wild animals

Methods:
- tonsils and feces underwent cultural enrichment steps followed by PCR targeting the ail-gene and wzz-gene
- all samples positive for the ail-gene where later plated onto CIN-agar-plates
- Spot assays were used to screen the samples for Yersinia-specific phages

Preliminary results:
- only three samples of wild boar tonsils tested positive for the ail-gene by PCR
- through spot testing on eight *Y. enterocolitica*- and 14 *Y. pseudotuberculosis*-strains, 5 phages were detected and isolated.

Atypical Brucella spp.

Background
- highly contagious, one of the most ubiquitious zoonoses in the world
- More than 500.000 cases per year
- large host spectrum
- recently discovered virulent new species – atypical brucella with broad host spectrum
- investigation of potential reservoirs

Methods:
- NGS, qPCR of spleen, submandibular lymph nodes and liver

Shiga toxin producing Escherichia coli

Background
- recently also discovered in game meat samples (>40% in deer feces)
- possible source of infection for free roaming cattle and contamination for crop-fields, possible connection between wildlife and STEC in flower
- STEC, EPEC and ETEC screen

Methods:
- NGS, sero-typing PCR, qPCR of fecal roe-, red-deer and wild boar samples

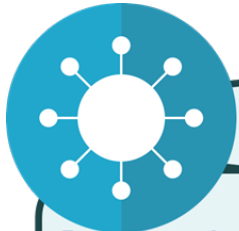### Methicillin resistant Staphylococcus spp.

Background
- potentielle Quelle für humane Infektionen, Resistenzfaktoren, **die auf human-adaptierte Bakterienpopulationen übertragen werden können**

Methods:
- Anreicherung und Isolation präsumtiver Methicillin-resistenter *Staphylococcus* spp.
- MALDI-ToF, real-time PCR, NGS

Erreger

Viruses

rotavirus

Background:
- the most common cause of diarrhoea in children worldwide
- very low infectious dose of 10 virus particles, risk for infection is high, especially for children

Methods:
- fecal samples of wild boar (*Sus scrofa*) were tested for rotavirus using qPCR targeting a 80-nucleotide sequence within NSP3

hepatitis-E-virus

Background:
- in 2017, 2943 cases were reported in Germany and the number of cases has been increasing continously
- domestic pigs are an important reservoir for hepatitis-E-virus and it is assumed that wild boar is an important reservoir as well

Methods:
- liver of wild boar (*Sus scrofa*) was examined for hepatitis-E-virus using qPCR targeting a 70-nucleotide sequence within ORF 3

small circular DNAs (SPHINX, BMMF)

Background
- has been hypothesized to be connected to intestinal cancers
- epidemiological situation in the population unclear
- large host spectrum
- recently discovered broad host spectrum
- investigation of potential reservoirs

Methods:
- NGS, serum, blood, buffy coats

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Ergebnisse - Auswahl

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Parasites

Cryptosporidium

Kann Menschen und Tiere gleichermaßen infizieren und zeigt hohe Tenazität in der Umwelt – One Health Erreger

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Cryptosporidium – ein One Health Erreger

Cryptosporidium DNA – Vorkommen [%]

18S- und COWP Nested-PCRs

Jahr	Schwarzwild	Rehwild	Rotwild	Damwild	unbekannt	Total
2017/18	24,11	42,37	10,53	-		26,64
2018/19	11,11	1,85	0	-	25	5,22
2019/20	30,85	8,74	16,67	34	-	24,54
2020/21	31,6	16,1	16,7	50	10	30,95
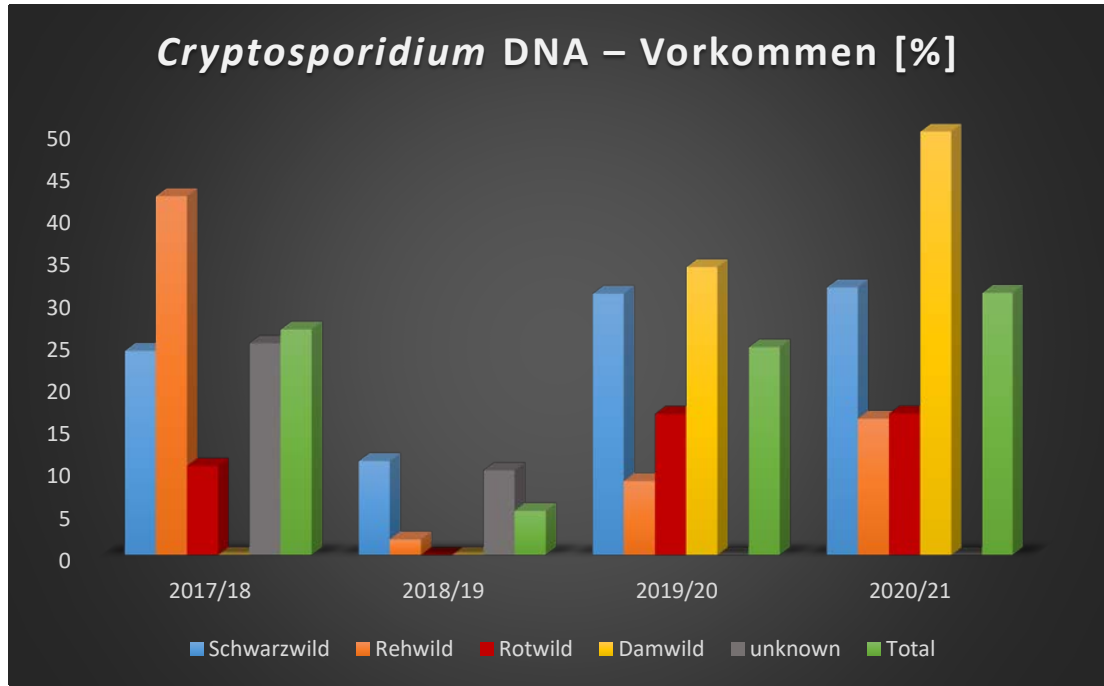
- Starke Schwankung (gleiche Reviere) - Warum?
- Höheres Vorkommen bei jüngeren Tieren

- **Schwarzwild**: *C. scrofarum, C. suis, C. ubiquitum, C. parvum, C. sp. deer genotype*
- **Rot-/Rehwild**: *C. sp. deer genotype, C. parvum*
- **Damwild**: *C. sp. deer genotype, C. ubiquitum*

BfR

Cryptosporidium – ein One Health Erreger

Cryptosporidium DNA - Vorkommen [%]

Jahr	Schwarzwild	Rehwild	Rotwild	Damwild	unbekannt	Total
2017/18	24,11	42,37	10,53	-	-	26,64
2018/19	11,11	1,85	0	-	-	5,22
2019/20	30,85	8,74	16,67	34	25	24,54
2020/21	31,6	16,1	16,7	50	10	30,95

Chart legend: Schwarzwild, Rehwild, Rotwild, Damwild, unbekannt, Total
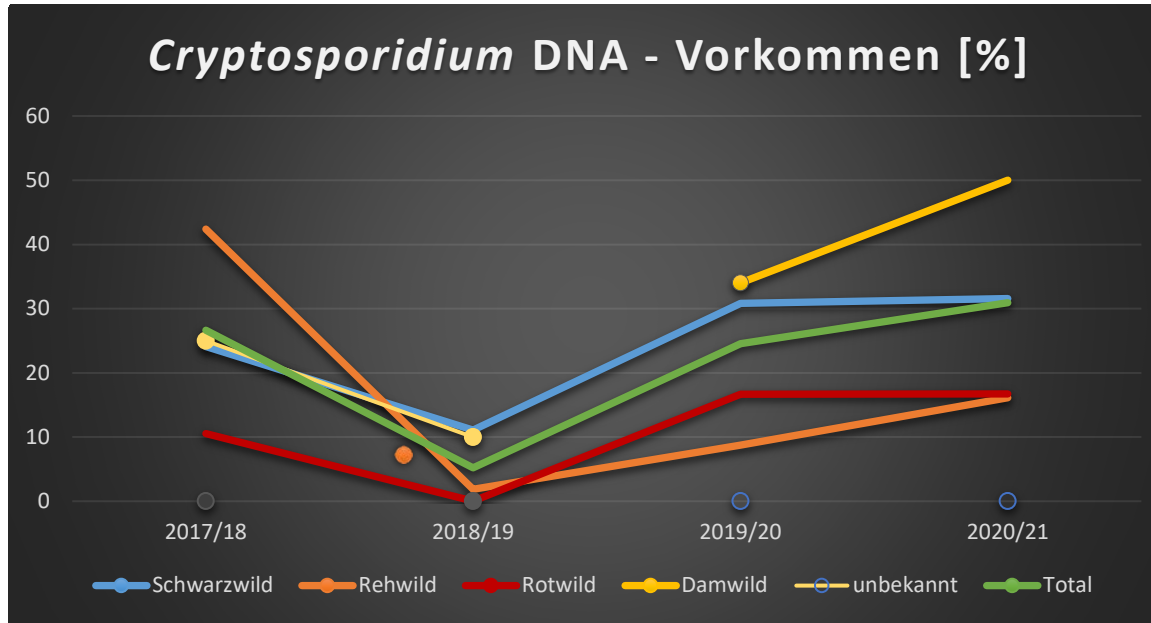
year	temperature	precipitation
summer 2017	18.3 (mean) +0.3 (dev. long term mean)	**241%** (resp. long term mean)
summer 2018	20.4 (mean) +2.4 (dev. long term mean)	**49%** (resp. long term mean)
summer 2019	20.5 (mean +2.5 (dev. Long term mean)	**125%** (resp. long term mean)
summer 2020	19.6 (mean) +1.0 (dev. Long term mean)	**75%** (resp. long term mean)

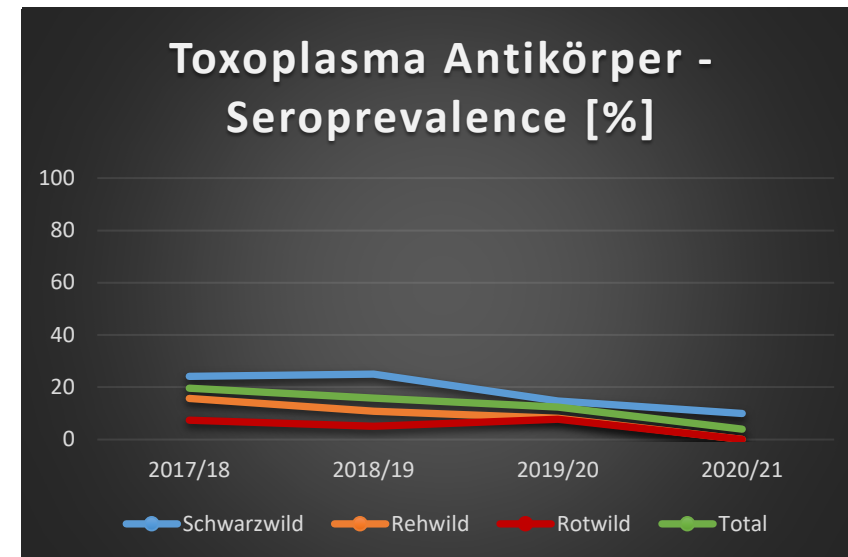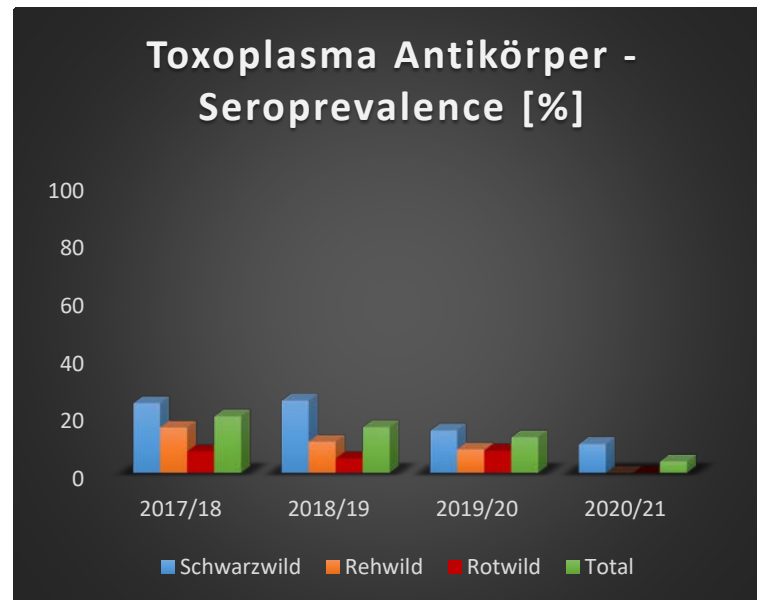# *Toxoplasma gondii* – qPCR

DNA-Isolation aus 5g Herzmuskel

Vorkommen %
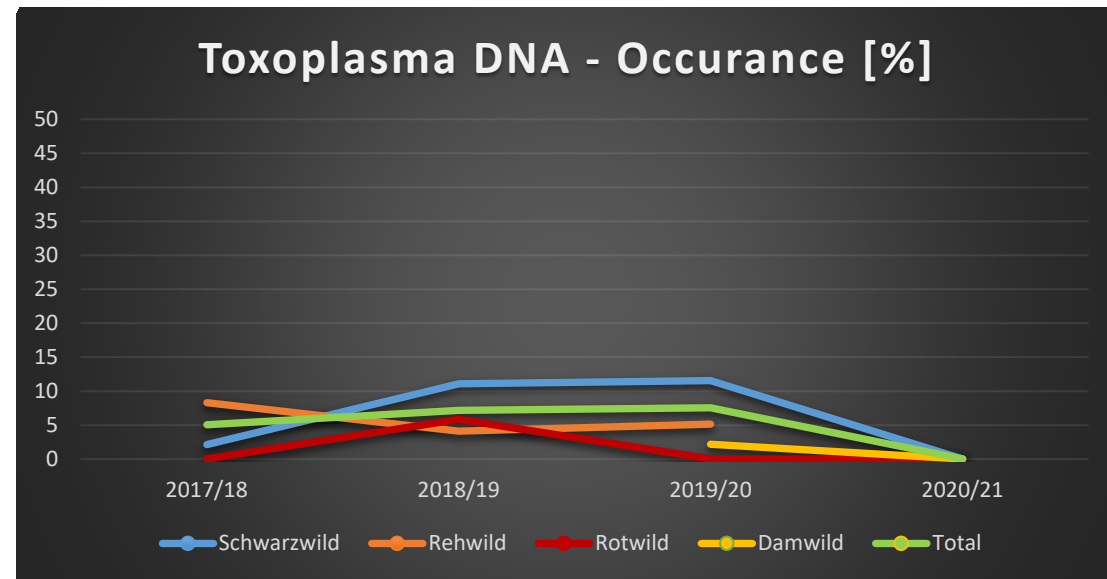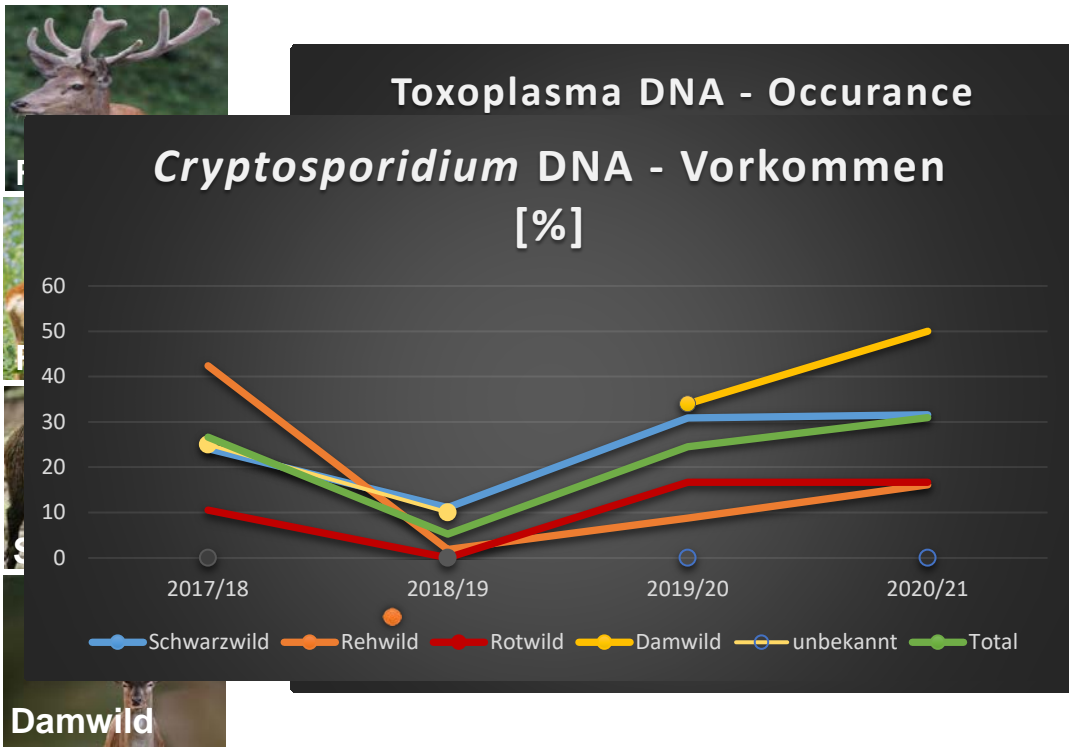
32% (16/50; 19.5-46.7%) **of sero-positive animals were also qPCR positive.**

Toxoplasma gondii - results – ELISA (ID Vet)

Seroprevalence %

Metadatenabgleich – Meteorologie, Geografie, Wildbesatz und -ausbreitung

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Bacteria
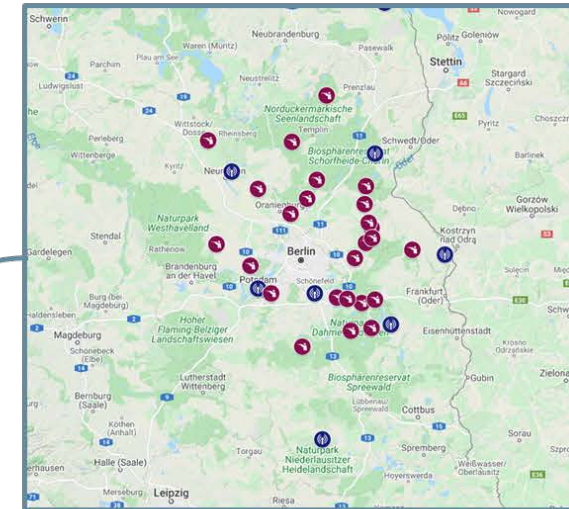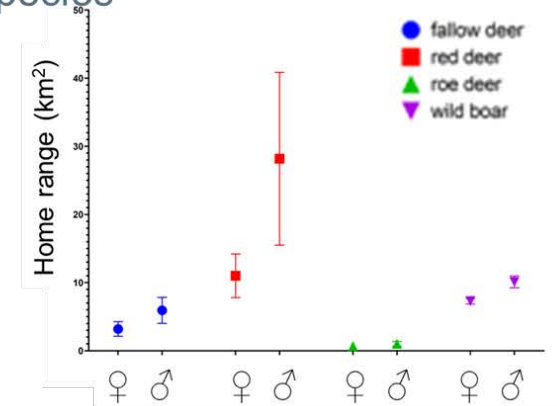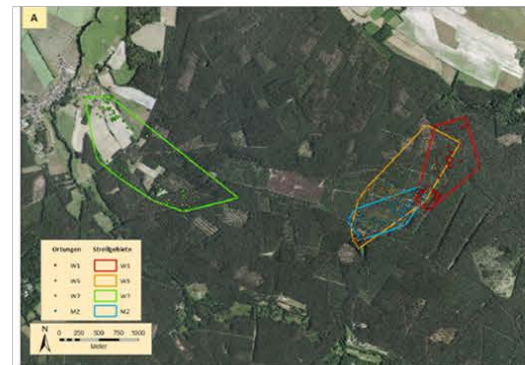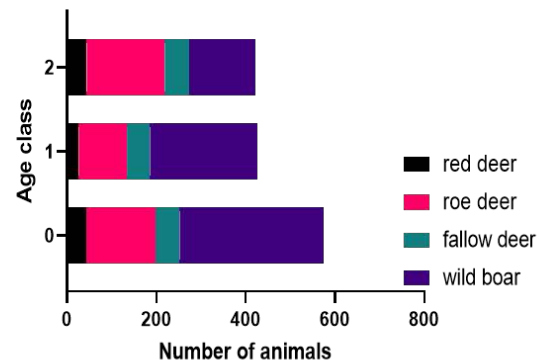
Yersinia – Tonsillen, Schwarzwild

PCR

Species	2018/19 PCR (*ail & wzz*)		2019/20 RT-PCR (*ail*)	
	n	positive (%)	n	positive (%)
Y. enterocolitica	40	3 (10%)	150	47 (31 %)
Y. pseudotuberculosis	40	0	150	5 (2 %)

Culture / isolation

Species	2018/19		2019/20	
	n	positive (%)	n	positive (%)
Yersinia spp. culture	40	1 (2.5 %)	150	52 (34.6 %)
Yersinia spp. isolation	40	0	150	34 (22.7 %)

Zusammenfassung und Ausblick

- Erfasste Daten wichtig für künftige Risikobewertungen

- Erkenntnisgewinn zur Verbreitung von Zoonosen in Wildtieren in Deutschland

- Langzeitstudien ermöglichen den Einbezug von Langzeitdaten, wie Wettereinflüsse und klimatische Veränderungen und deren mögliche Auswirkungen auf die Erregerökologie

- Identifikation von „Hot Spots"

- Probenahme soll weiter ausgeweitet werden und auch andere Tierarten einbeziehen

- Grenzflächenanalyse (Nutztiere / Wildtiere)

- Metagenomanalysen

Zoonoses AND PUBLIC HEALTH

Received: 5 November 2019 | Revised: 6 February 2020 | Accepted: 25 February 2020

DOI: 10.1111/zph.12702

SHORT COMMUNICATION

WILEY

Seroprevalence of *Toxoplasma gondii* in wild boar and deer in Brandenburg, Germany

Nadja Seyhan Bier[1] | Kaya Stollberg[2] | Anne Mayer-Scholl | Annette Johne[1] | Karsten Nöckler[1] | Martin Richter[1]

[1]Department for Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany

Abstract

Consumption of game in Germany has increased during the past 10 years. Wild boar

microorganisms — MDPI

Communication

Serological Evidence That SARS-CoV-2 Has Not Emerged in Deer in Germany or Austria during the COVID-19 Pandemic

Andres Moreira-Soto [1], Christian Walzer [2,3], Gábor Á. Czirják [4], Martin H. Richter [5], Stephen F. Marino [5], Annika Posautz [3], Pau De Yebra Rodo [4], Gayle K. McEwen [4], Jan Felix Drexler [1,*,†] and Alex D. Greenwood [4,6,*,†]

Article

Comparison of Direct and Indirect *Toxoplasma gondii* Detection and Genotyping in Game: Relationship and Challenges

Kaya C. Stollberg [1], Gereon Schares [2], Anne Mayer-Scholl [3], Iryna Hrushetska [3], Susanne Diescher [3], Annette Johne [3], Martin H. Richter [3] and Nadja S. Bier [3,*]

Article

Development of a Novel Method for Identification of *Alaria alata* Mesocercariae by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Carolyn Kästner [1], Peter Bahn [1], Ralph Schönfelder [2], Zanda Ozoliņa [3], Laura Alksne [3], Martin Heinrich Richter [1], Gunita Deksne [3,4], Anne Mayer-Scholl [1] and Annette Johne [1,*]

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