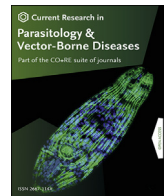


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## Xenosurveillance proof-of-principle: Detection of *Toxoplasma gondii* and SARS-CoV-2 antibodies in mosquito blood meals by (pan)-specific ELISAs

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### ABSTRACT

Blood-fed insects can be used to analyse the host blood for circulating vertebrate pathogens or antibodies directed against them. We tested whether naturally acquired antibodies in different host species can be detected by host-specific and pan-specific ELISAs in mosquito blood meals. Cat- and alpaca-specific ELISAs could detect antibodies against *Toxoplasma gondii* or SARS-CoV-2 in blood meals of *Aedes japonicus* for 48 and at least 24 h, respectively. In the pan-specific ELISA, a conjugated protein A/G and anti-IgY were used to detect antibodies of mammalian and bird hosts. Thus, *Toxoplasma* antibodies could be detected in mosquitoes fed on blood from humans, chicken, pig, and sheep up to 72 h after the blood meal. The results, however, demonstrated differences in sensitivities between different host species, and the assay requires further evaluation. Xenosurveillance with antibody detection in mosquito blood meals can be an additional surveillance tool that would especially be helpful when it is difficult to sample the potential animal reservoirs.

### 1. Introduction

Monitoring of vertebrate pathogens circulating in the environment is challenging. Sentinel animals or wildlife or excretions thereof can be analysed, but this can be difficult, costly, and laborious. Analysis of the host blood in hematophagous arthropods for pathogens can be an alternative. The use of blood-feeding insects as a “flying syringe” is often called xenosurveillance, xenodiagnosics or vector-enabled metagenomics. The technique has been tested for the detection of viruses, bacteria and parasites in a range of arthropods that fed on different vertebrate hosts (reviewed in [Brinkmann et al., 2016](#)). In Gabon, for example, blood-engorged flies were captured, and their blood meals analysed by PCR/sequencing ([Bitome-Essono et al., 2017](#)). Blood meals were identified from 20 vertebrate species, and known haemosporidian parasites but also unknown parasite lineages, were detected.

However, many parasites are sequestered in the host and not present at all or in very low numbers in blood and, therefore, the detection of antibodies directed against them is preferred. Indeed, antibodies against pathogens could be detected in mosquito blood meals by standard ELISA

([Lackie & Gavin, 1989](#); [Barbazan et al., 2009](#); [Leighton et al., 2014](#); [Komar et al., 2015](#); [Pauvolid-Corrêa & Komar, 2017](#)). In these studies, antibodies against vector-borne pathogens were detected in naturally fed insects or in vectors artificially fed on blood spiked with antibodies. In the present proof-of-concept study, we tested whether acquired antibodies can be detected by ELISA in blood meals of mosquitoes from different hosts.

To test this methodological concept, blood from different hosts with antibodies against the same pathogen was investigated. *Toxoplasma gondii* can infect a wide range of hosts including humans, other mammals and birds ([Boothroyd, 2009](#)) and was therefore selected for this study as a proof-of-concept. Mosquitoes were fed on cat, human, pig, sheep and chicken blood or serum with *Toxoplasma* antibodies, and their abdomens were analysed at several time intervals after feeding. Similarly, it was tested whether antibodies against the SARS-CoV-2 spike protein can be detected in mosquito blood meals from immunized alpacas. Typically, an ELISA depends on a host-specific detection antibody which makes the method unsuitable for monitoring multiple host species. Therefore, we developed a pan-specific ELISA by using a conjugated protein A/G to detect antibodies from mammalian hosts and anti-IgY for bird hosts.

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## 2. Materials and methods

### 2.1. Mosquito rearing and feeding

*Aedes japonicus* mosquitoes were reared from eggs collected around the Institute of Parasitology (IPZ), Zürich, as described (Verhulst et al., 2020). They were 7–12 days-old when fed blood on Hemotek feeders (Hemotek Ltd., Lancashire, UK) filled with 2 ml blood and covered with a parafilm membrane. To increase the feeding rate, mosquitoes were starved by replacing the glucose solution by water 24 h before the blood meal. Groups of up to 80 mosquitoes were blown into 500 ml plastic bottles whose lids were replaced by a nylon sock (15 Denier, Migros, Switzerland) worn for 12 h as a feeding stimulus. On the other side of the 500 ml plastic bottle, the bottom was removed and replaced with a piece of foam which was moved slowly toward the other side of the bottle until all mosquitoes were within 2–3 cm from the feeding membrane.

After 1.5 h of feeding, the mosquitoes were released into a 17.5 × 17.5 × 17.5 cm cage (BugDorm, MegaView Science, Taichung, Taiwan). All non-blood-fed or partially fed mosquitoes were removed by aspiration, and the remaining blood-fed females were provided with a fresh 5% glucose solution every day. Groups of 4–7 blood-fed mosquitoes were then removed from the cage at different time points and stored at –20 °C until analysed individually in the ELISAs. All samples from an experiment were tested under the same conditions in the same ELISA run.

### 2.2. Blood samples

Anonymized human EDTA blood samples from voluntary blood donors were provided by the Blood Donation Center Zürich (ZHBSD) and tested for *Toxoplasma* antibodies as described below. Whole EDTA blood samples from cat blood were from routine screenings (Vetsuisse Faculty, University of Zürich), and bird blood serum samples from screenings of birds from the Zoo Zürich (kindly provided by Small Animal Clinic and Sections of Veterinary Bacteriology and Poultry and Rabbit Diseases, Vetsuisse Faculty, University of Zürich). Sheep and pig blood serum samples were from previous studies at the institute (Basso et al., 2013; Glor et al., 2013). Whole EDTA blood with SARS-CoV-2 antibodies was obtained from alpacas that were challenged with recombinant SARS-CoV-2 spike protein for the purpose of another study (animal permission ZH198/17).

Positive and negative samples were selected based on the results from previous ELISAs, and their status confirmed by testing the samples in the same ELISA runs as the mosquito blood meals.

Because mosquitoes do not feed on blood serum, such samples were enriched with whole human blood that was tested negative for *Toxoplasma* antibodies. After centrifugation of the whole human blood for 8 min at 520 × g, the cell pellet was mixed in a 1:1 ratio with the serum samples.

### 2.3. Host-specific ELISA for *Toxoplasma* antibody detection

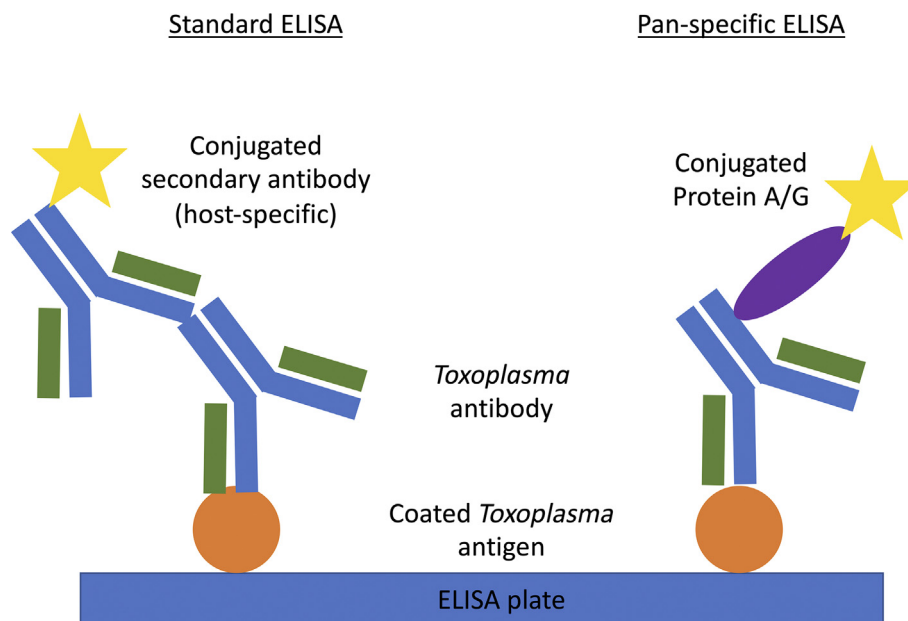
The ELISA for detecting specific anti-*Toxoplasma* antibodies in blood meals of *Ae. japonicus* mosquitoes was performed as described elsewhere (Schreiber et al., 2021) with a different detection antibody, for which host-specific secondary goat-anti-feline IgG antibodies (H+L; 6080-04, Southern Biotech, Allschwil, Switzerland) were used. The abdomens of the mosquitoes were removed with sterile scalpel and forceps, and were homogenized with a pestle in 2 ml Eppendorf tubes with 220 µl dilution buffer (PBS with 0.05% bovine haemoglobin and 0.3% Tween-20). Mosquito homogenates were centrifuged for 3 min at 16,000 × g, and 100 µl of the supernatants were used for the ELISA.

### 2.4. Host-specific ELISA for SARS-CoV-2 antibody detection

The homogenisation of the abdomens of mosquitoes fed on alpaca blood and the ELISA were performed the same way as described above, with the only differences that recombinant SARS-CoV-2 spike protein was coated onto the ELISA plate, and anti-llama IgG-AP (LSBio, LS-C316339, 1:5000) was used for the detection of alpaca IgG.

### 2.5. Pan-specific ELISA for *Toxoplasma* antibody detection

To be able to detect antibodies when the host of the mosquito is unknown, a pan-specific ELISA was tested by using an alkaline phosphatase-conjugated protein A/G (Pierce, ThermoFisher, Switzerland) for mammalian hosts and anti-IgY (Promega, G1151) for bird hosts (Fig. 1). To increase the sensitivity, mosquito abdomens were homogenized in a smaller volume (100 µl dilution buffer), and 50 µl of the supernatant (3 min, 16,000 × g) was used for the ELISA.



**Fig. 1** Schematic overview of conventional and pan-specific ELISA. The conjugated protein A/G allows to detect mammalian antibodies. To be able to detect bird antibodies, anti-IgY (not depicted) was added to the pan-specific ELISA, which works like the conventional ELISA (left side of the figure).

Total tachyzoite homogenate of cultivated *T. gondii* strain II (5.04 mg/ml) was prepared as described (Johnson et al., 2007). The antigen was diluted to 10 µg/ml in 0.1 M NaCO<sub>3</sub> buffer at pH 9.6, and 100 µl was coated in each well of 96-well Maxisorp plates (Nunc Roskilde, Denmark) overnight at 4 °C. The ELISA was performed according to the protocol from Schreiber et al. (2021) using 50 µl of the mosquito supernatant, followed by incubation with 100 µl mixture of the enzyme (alkaline phosphatase, AP) conjugated protein A/G (ThermoFisher Sci., Cat. Nr. 32391, 1:15,000) and anti-IgY (Promega, Cat. Nr. G1151, 1:1000). Absorbance of the substrate (pNPP disodium hexahydrate, SIGMA 71768) was read at 405 nm after 30 min using a microplate reader (Multiskan RC, Thermo LabSystems, Waltham, USA).

### 3. Results and discussion

Abdomens of *Ae. japonicus* were investigated with the host-specific ELISA at 0, 6, 12, 24, 36, 48 and 72 h after feeding on cat blood positive for *T. gondii* antibodies. Samples of mosquitoes fed on cat blood without *Toxoplasma* antibodies were only analysed immediately after feeding, showing an optical density (OD) value of  $0.001 \pm 0.003$  (mean  $\pm$  standard error), while the samples from *Toxoplasma*-positive blood had an OD of  $2.111 \pm 0.065$  (Fig. 2). There was an exponential decline in the optical density (OD) over time. Until 36 h after feeding, all samples had elevated OD values, but only 1 of 4 tested at 48 h. Seventy-two h after feeding, the OD values of all samples were similar to the controls. In similar studies using ELISAs with tropical mosquitoes (*Ae. aegypti*, *Culex* spp.) fed on human blood, detection of antibodies, either an added monoclonal antibody or from natural dengue or Japanese virus infections, was possible for 24–48 h (Leighton et al., 2014) or up to 30 h (Barbazan et al., 2009) after feeding. Antibodies against West Nile virus in spiked blood meals were detectable in mosquito blood meals for up to 30 h by using a biotin microsphere immunoassay (b-MIA) or a VecTest inhibition assay (Komar et al., 2015). The time until which detection is possible will depend on the sensitivity of the assay but also on the blood meal size and the digestion rate of the blood meal by the mosquito. The digestion rate of tropical mosquito species probably is higher (O'gower,

1956) and, therefore, detection of antibodies shorter compared to the temperate mosquito *Ae. japonicus* used in this study.

Similar to the *Toxoplasma* antibodies, OD values in ELISA for SARS-CoV-2 antibodies in blood meals were distinctly elevated up to 24 h after the blood meal (later time points not tested, Fig. 3).

When evaluating the pan-specific ELISA, pilot experiments revealed that the conjugated protein A/G, which should bind IgG from a wide range of different mammals according to information provided by the manufacturer, could not detect *Toxoplasma* antibodies in cat blood. However, experiments with human blood and pig and sheep sera (enriched with human blood) were successful. *Toxoplasma* antibodies in blood meals of human origin were investigated at 0, 6, 12, 24, 36, 48 and 72 h after feeding to reveal the time limit of detection. OD values were clearly higher than the control up to 48 h after feeding and a few slightly elevated even 72 h after feeding (Fig. 4). To confirm that the assays also work with blood from other hosts, chicken blood as well as enriched pig and sheep sera was investigated at 0, 12 and 24 h after feeding. *Toxoplasma* antibodies could also be detected in mosquitoes fed on blood of these three species (Fig. 5). However, the assay using both Prot A/G-AP and anti-IgY-AP in the same reaction mix resulted in a considerable loss of sensitivity for *Toxoplasma* antibodies in sheep enriched serum. Because the anti-IgY was developed in goat, which is phylogenetically most similar to sheep, the protein A/G most likely binds to the same site and therefore goat IgG is adsorbing the free soluble protein A/G from the mix which then cannot bind the sheep IgG. Possible solutions to this problem could be to add the protein A/G-AP first and adding the anti-IgY-AP after a wash step, or to increase the amount of protein A/G. This requires further evaluation.

Testing more host species and more individuals as well as different suppliers of the conjugated protein A/G could reveal why these differences in detection between hosts occurred. Other methods to detect antibodies independent of host species include the b-MIA and VecTest-inhibition assay. The b-MIA test relies on biotin labelled antibodies followed by detection with phycoerythrin-labelled streptavidin that binds biotin and is a good alternative to the ELISA although more sophisticated laboratory equipment is required (Komar et al., 2015). The VecTest is commercially available (Vector Test Systems, Inc., Thousand Oaks, USA)

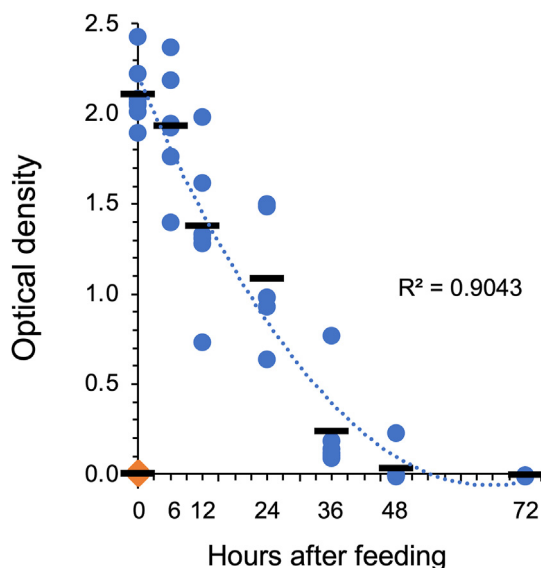


Fig. 2 Host-specific detection of *Toxoplasma* antibodies in blood-fed mosquitoes. *Aedes japonicus* were fed on a cat blood sample that was positive (blue circles) or negative (orange squares) for *Toxoplasma gondii* antibodies. Groups of 4–7 mosquitoes were removed at different time points and analysed by ELISA. Horizontal bars indicate means. Curves are from a best-fit nonlinear regression.

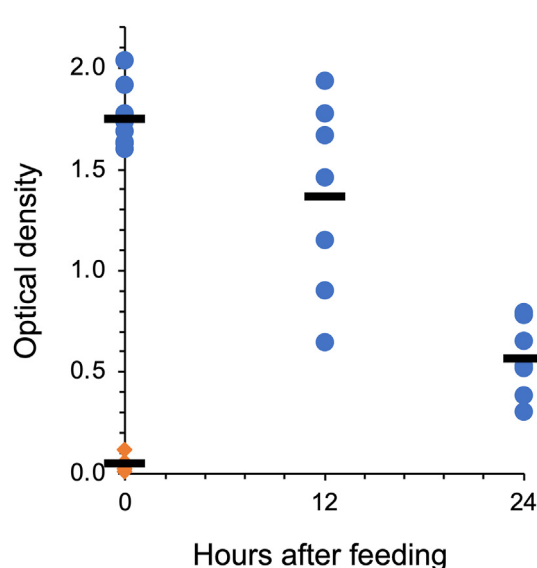
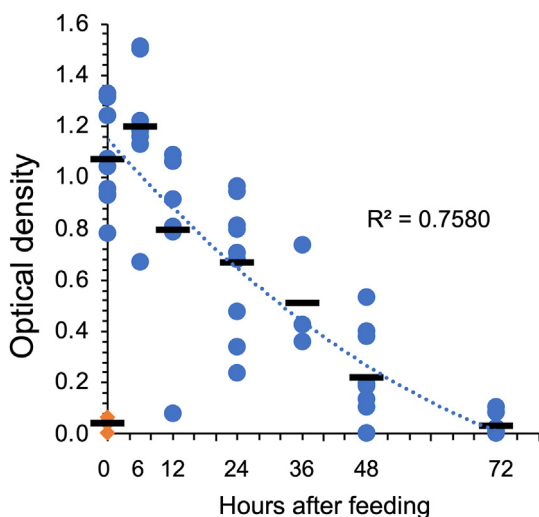


Fig. 3 Host-specific detection of SARS-CoV-2 antibodies in blood-fed mosquitoes. *Aedes japonicus* were fed on alpaca blood that was positive (blue circles) or negative (orange squares) for SARS-CoV-2 antibodies. Groups of 4–7 mosquitoes were removed after 0, 12 and 24 h and analysed by ELISA. Horizontal bars indicate means.



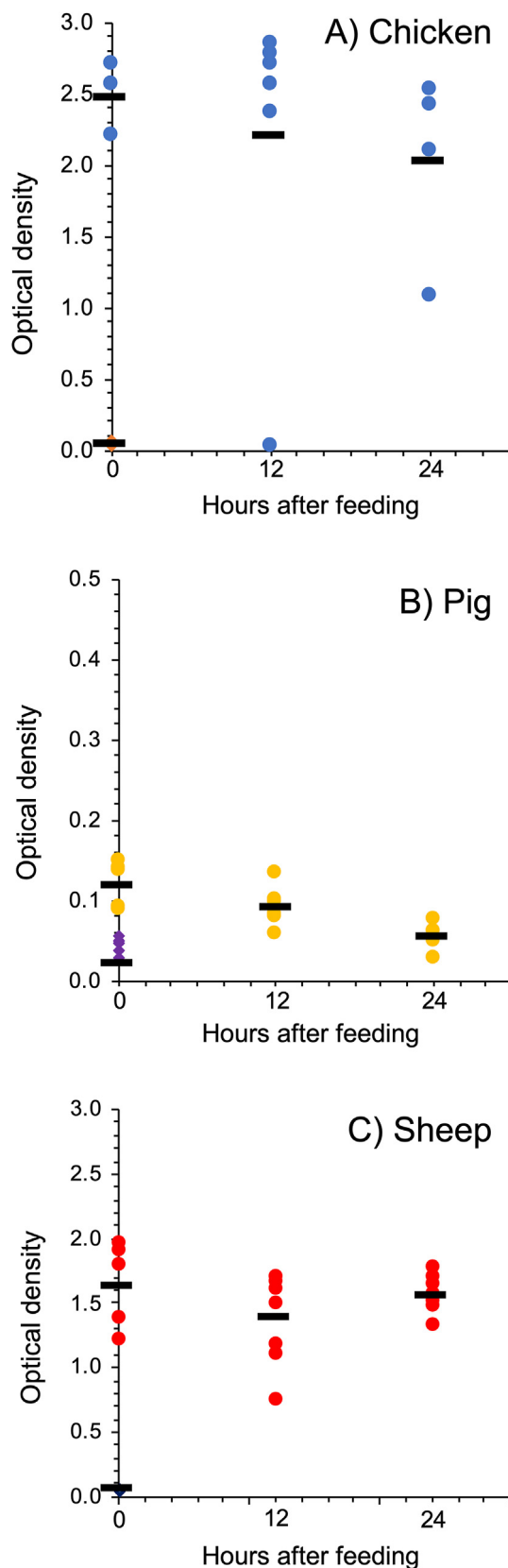
**Fig. 4** Pan-specific detection of *Toxoplasma* antibodies in blood-fed mosquitoes. *Aedes japonicus* were fed on human blood that was positive (blue circles) or negative (orange squares) for *Toxoplasma gondii* antibodies. Groups of 4–7 mosquitoes were removed at different time points and analysed by a pan-specific ELISA using a conjugated protein A/G and anti-IgY-AP. Horizontal bars indicate means. Curves are from a best-fit nonlinear regression.

but the subjectivity inherent to this assay and the potential for erroneous results that were reported previously (Komar et al., 2015) make this a less preferred test. Leighton et al. (2014) suggested, but not tested, a pan-specific detection method by using an antibody-capture ELISA where an Fc binding protein is coated onto the plate and binds to antibodies in the samples. As this captures the total IgG, the test might therefore be less sensitive. Although xenosurveillance of pathogens with host-specific methods can be useful especially when the reservoir host is known, a pan-specific method would be required when cross-species transmission is expected, e.g. in the case of zoonotic agents.

The mosquito blood meal can also be used to identify the blood host species, which can be done with various techniques including ELISA. However, a genetic identification by PCR/sequencing of the cytochrome B locus is most appropriate (Kent & Norris, 2005). Previous studies have shown that the host species in mosquito blood meals can be identified by PCR up to 48–72 h after feeding (Ngo & Kramer, 2003; Kent & Norris, 2005). In a preliminary test, we showed that the pellet that remains after removal of the supernatant for the ELISA can be used for this approach (Supplementary Fig. S1).

Capturing blood-fed mosquitoes is difficult which could limit the use of xenosurveillance. After taking a blood meal, mosquitoes rest and are rarely attracted to the lures used in mosquito traps. Blood-fed mosquitoes can be captured with resting traps (Qiu et al., 2007; Brugman et al., 2017), barrier screens (Burkot et al., 2013) or aspirators (Vazquez-Prokopec et al., 2009; Schönenberger et al., 2016) but catch rates with these methods are low. In a study by Mweresa et al. (2014), the use of fermenting molasses in mosquito traps substantially increased the capture of blood-fed *Anopheles gambiae* and *An. funestus* mosquitoes. Molasses could therefore be an interesting lure for xenosurveillance studies but its effect on other mosquito species needs to be evaluated. Xenosurveillance with mosquitoes is also biased because of mosquito host selection, and it may therefore not be representative for the whole animal population (Takken & Verhulst, 2013).

Future studies should focus on the optimization of collection techniques for blood-fed mosquitoes and the improvement of a pan-specific ELISA to establish a detection method that is suitable for most if not all hosts. The SARS-CoV-2 pandemic highlights the importance of pathogen surveillance and calls for better and novel surveillance methods. The detection of antibodies directed against pathogens, as presented here, could be valuable as they are detectable for a much longer time in the blood than the pathogen itself (Zielinski et al., 2011).



**Fig. 5** Pan-specific detection of *Toxoplasma* antibodies in mosquitoes fed on different hosts. *Aedes japonicus* were fed on chicken (A), pig (B) or sheep (C) blood that were positive (blue circles) or negative (orange squares) for *Toxoplasma gondii* antibodies. Groups of 4–7 mosquitoes were removed after 0, 12 and 24 h and analysed by a pan-specific ELISA by using a conjugated protein A/G and anti-IgY-AP. Horizontal bars indicate means.



#### 4. Conclusions

Antibodies against different pathogens can be detected in the blood meal of mosquitoes up to 72 h after the blood meal. The use of a pan-specific ELISA allows for the detection of antibodies in blood from different hosts, which is useful when the host is unknown. In wildlife for example, xenosurveillance by antibody detection in mosquito blood meals can be an additional surveillance tool because it is difficult to sample the potential reservoirs.

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#### Ethical approval

The use of anonymized human blood samples does not require ethical approval according to the Kantonale Ethikkommission (KEK, Zürich, Switzerland). Sheep and pig blood serum samples were from previous studies at the institute for which ethical approval was obtained (ZH 106/2010, 106/2010, 130/2012). Whole EDTA blood from alpacas was obtained under animal permission ZH198/17.

#### CRedit author statement

SS, FG, AM and NV contributed to the conceptualization and methodology. SS, NV, RW and NV performed the investigation and formal analysis. SS and NV analysed data. SS and NV wrote the original draft. All authors reviewed and edited the manuscript, and approved the final manuscript.

#### Data availability

Data supporting the conclusions of this article are included within the article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

#### Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crpvbd.2022.100076>.

#### References

- Barbazan, P., Palabodeewat, S., Nitatpattana, N., Gonzalez, J.-P., 2009. Detection of host virus-reactive antibodies in blood meals of naturally engorged mosquitoes. *Vector Borne Zoonotic Dis.* 9, 103–108. <https://doi.org/10.1089/vbz.2007.0242>.
- Basso, W., Hartnack, S., Pardini, L., Maksimov, P., Koudela, B., Venturini, M.C., et al., 2013. Assessment of diagnostic accuracy of a commercial ELISA for the detection of *Toxoplasma gondii* infection in pigs compared with IFAT, TgSAG1-ELISA and Western blot, using a Bayesian latent class approach. *Int. J. Parasitol.* 43, 565–570. <https://doi.org/10.1016/j.ijpara.2013.02.003>.
- Bitome-Essono, P.-Y., Ollomo, B., Arnathau, C., Durand, P., Mokoudoum, N.D., Yacka-Mouele, L., et al., 2017. Tracking zoonotic pathogens using blood-sucking flies as “flying syringes”. *Elife* 6, e22069. <https://doi.org/10.7554/eLife.22069>.
- Boothroyd, J.C., 2009. Expansion of host range as a driving force in the evolution of *Toxoplasma*. *Mem. Inst. Oswaldo Cruz* 104, 179–184. <https://doi.org/10.1590/S0074-02762009000200009>.
- Brinkmann, A., Nitsche, A., Kohl, C., 2016. Viral metagenomics on blood-feeding arthropods as a tool for human disease surveillance. *Int. J. Mol. Sci.* 17, 1743. <https://doi.org/10.3390/ijms17101743>.
- Brugman, V., Hernández-Triana, L., England, M., Medlock, J., Mertens, P., Logan, J., et al., 2017. Blood-feeding patterns of native mosquitoes and insights into their potential role as pathogen vectors in the Thames estuary region of the United Kingdom. *Parasit. Vectors* 10, 163. <https://doi.org/10.1186/s13071-017-2098-4>.
- Burkot, T.R., Russell, T.L., Reimer, L.J., Bugoro, H., Beebe, N.W., Cooper, R.D., et al., 2013. Barrier screens: a method to sample blood-fed and host-seeking exophilic mosquitoes. *Malar. J.* 12, 49. <https://doi.org/10.1186/1475-2875-12-49>.
- Glor, S.B., Edelhofer, R., Grimm, F., Deplazes, P., Basso, W., 2013. Evaluation of a commercial ELISA kit for detection of antibodies against *Toxoplasma gondii* in serum, plasma and meat juice from experimentally and naturally infected sheep. *Parasit. Vectors* 6, 85. <https://doi.org/10.1186/1756-3305-6-85>.
- Johnson, T.M., Rajfur, Z., Jacobson, K., Beckers, C.J., 2007. Immobilization of the type XIV myosin complex in *Toxoplasma gondii*. *Mol. Biol. Cell* 18, 3039–3046. <https://doi.org/10.1091/mbc.e07-01-0040>.
- Kent, R.J., Norris, D.E., 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am. J. Trop. Med. Hyg.* 73, 336–342. <https://doi.org/10.4269/ajtmh.2005.73.336>.
- Komar, N., Panella, N.A., Young, G.R., Basile, A.J., 2015. Methods for detection of West Nile virus antibodies in mosquito blood meals. *J. Am. Mosq. Contr. Assoc.* 31, 1–6. <https://doi.org/10.2987/14-6468R.1>.
- Lackie, A.M., Gavin, S., 1989. Uptake and persistence of ingested antibody in the mosquito *Anopheles stephensi*. *Med. Vet. Entomol.* 3, 225–230. <https://doi.org/10.1111/j.1365-2915.1989.tb00220.x>.
- Leighton, B.J., Roitberg, B.D., Lowenberger, C.A., Belton, P., 2014. Host antibodies in mosquito bloodmeals: a potential tool to detect and monitor infectious diseases in wildlife. *J. Med. Entomol.* 45, 470–475. <https://doi.org/10.1093/jmedent/45.3.470>.
- Mwera, C.K., Omusula, P., Otieno, B., Van Loon, J.J., Takken, W., Mukabana, W.R., 2014. Molasses as a source of carbon dioxide for attracting the malaria mosquitoes *Anopheles gambiae* and *Anopheles funestus*. *Malar. J.* 13, 160. <https://doi.org/10.1186/1475-2875-13-160>.
- Ngo, K.A., Kramer, L.D., 2003. Identification of mosquito bloodmeals using polymerase chain reaction (PCR) with order-specific primers. *J. Med. Entomol.* 40, 215–222. <https://doi.org/10.1603/0022-2585-40.2.215>.
- O'gower, A., 1956. The rate of digestion of, human blood by certain species of mosquitoes. *Aust. J. Biol. Sci.* 9, 125–129. <https://doi.org/10.1071/B19560125>.
- Pauvolid-Corrêa, A., Komar, N., 2017. VectorTest™ West Nile Virus Antigen Assay in an inhibition platform as field screening tool for *Flavivirus* group-specific antibodies in Brazilian equines. *J. Am. Mosq. Control Assoc.* 33, 237–240. <https://doi.org/10.2987/17-6645R.1>.
- Qiu, Y.T., Spitzen, J., Smallegange, R.C., Knols, B., 2007. Monitoring systems for adult insect pests and disease vectors. In: Takken, W., Knols, B. (Eds.), *Emerging pests and vector-borne diseases in Europe*. Wageningen Academic Publishers, Wageningen, pp. 329–353.
- Schönenberger, A., Wagner, S., Tuten, H., Schaffner, F., Torgerson, P., Furrer, S., et al., 2016. Host preferences in host-seeking and blood-fed mosquitoes in Switzerland. *Med. Vet. Entomol.* 30, 39–52. <https://doi.org/10.1111/mve.12155>.
- Schreiber, N., Basso, W., Rioud, B., Willi, B., Torgerson, P.R., Deplazes, P., 2021. Antibody kinetics and exposure to *Toxoplasma gondii* in cats: a seroepidemiological study. *Int. J. Parasitol.* 51, 291–299. <https://doi.org/10.1016/j.ijpara.2020.09.011>.
- Takken, W., Verhulst, N.O., 2013. Host preferences of blood-feeding mosquitoes. *Annu. Rev. Entomol.* 58, 433–453. <https://doi.org/10.1146/annurev-ento-120811-153618>.
- Vazquez-Prokopec, G.M., Galvin, W.A., Kelly, R., Kitron, U., 2009. A new, cost-effective, battery-powered aspirator for adult mosquito collections. *J. Med. Entomol.* 46, 1256–1259. <https://doi.org/10.1603/033.046.0602>.
- Verhulst, N.O., Brendle, A., Blanckenhorn, W.U., Mathis, A., 2020. Thermal preferences of subtropical *Aedes aegypti* and temperate *Ae. japonicus* mosquitoes. *J. Therm. Biol.* 91, 102637. <https://doi.org/10.1016/j.jtherbio.2020.102637>.
- Zielinski, C.E., Corti, D., Mele, F., Pinto, D., Lanzavecchia, A., Sallusto, F., 2011. Dissecting the human immunologic memory for pathogens. *Immunol. Rev.* 240, 40–51. <https://doi.org/10.1111/j.1600-065X.2010.01000.x>.