Zoonotic potential of guinea pigs: Outbreak of cryptosporidiosis combined with chlamydiosis in a breeding guinea pig herd

N. Borel[1], S. Ciuria[1], T. Flury[2], W. Basso[3], M. Ruetten[4]

[1]Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zürich;
[2]Small Animal Hospital Flury, Zihlschlacht-Sitterdorf;
[3]Institute for Veterinary Parasitology, Vetsuisse-Faculty, University of Bern; [4]PathoVet AG, Tagelswangen

Meerschweinchen als Zoonose Überträger: Ausbruch von Kryptosporidiose kombiniert mit Chlamydiose in einer Meerschweinchen-Zuchtherde

In einer Meerschweinchenherde mit 26 Zuchttieren starben mehrere Tiere unterschiedlicher Altersklassen (16/26) nachdem drei Tiere aus einer anderen Herde neu integriert wurden. Die Gruppe zeigte Apathie, Anorexie, starken Gewichtsverlust und Konjunktivitis sowie Aborte und Totgeburten. *Chlamydia caviae* konnte bei einem juvenilen Tier zusammen mit einer intestinalen *Cryptosporidium wrairi*-Infektion aus der Bindehaut und Vagina/Uterus nachgewiesen werden. Oozysten wurden histologisch im Dünndarm gefunden, was durch PCR bestätigt wurde. *C. wairi* ist ein an Meerschweinchen angepasster Parasit mit zoonotischem Potenzial, der in grösseren Meerschweinchenherden Durchfall mit häufigen Todesfällen verursacht. *C. caviae* ist ebenfalls ein Zoonoseerreger und häufig die Ursache von Konjunktivitis, Lungenentzündung und Aborten bei Meerschweinchen und kann beim Menschen zu Erkrankungen der oberen Atemwege, Konjunktivitis aber auch schweren Lungenentzündungen führen. Die vermehrten Todesfälle und die klinischen Symptome konnten auf eine Infektion mit *Cryptosporidium wrairi* zurückgeführt werden, erschwert durch eine Co-Infektion mit *C. caviae*. Wir vermuten, dass die Aborte durch *C. caviae* verursacht wurden, aber da die Meerschweinchengruppe mit verschiedenen Antibiotika behandelt wurde, die gegen Chlamydieninfektionen wirksam waren, war es nicht mehr möglich, dies durch PCR-Tests zu überprüfen. Leider verstarben weitere Tiere und schliesslich überlebten 2 von 26 Tieren. Mit diesem Fallbericht möchten wir Tierärzte darauf hinweisen, dass Meerschweinchen eine wichtige Quelle zoonotischer Infektionen für verschiedene Krankheitserreger sein können, zumal sie beliebte Haustiere sind und oft in engen Kontakt mit Kindern kommen, bei denen die Hygiene nicht immer strikt eingehalten werden kann.

Schlüsselwörter: Abort, *Chlamydia caviae*, *Cryptosporidium wrairi*, Massensterben, Zoonose, Meerschweinchen (Cavia porcellus), Molekulardiagnostik

Summary

In a guinea pig herd with 26 breeding animals, several individuals of all age categories died (16/26) after three animals had been newly introduced from another herd. Furthermore, the population suffered of apathy, anorexia, severe weight loss and conjunctivitis, as well as abortions and stillbirths. At the same time, the owner experienced a SARS-CoV-2 infection with pneumonia, which was confirmed by taking a PCR test. *Chlamydia caviae* was detected from the conjunctiva and vagina/uterus in one juvenile animal together with an intestinal *Cryptosporidium wrairi* infection. Oocysts were found histologically in the small intestine, which was confirmed by PCR. *C. wairi* is a parasite adapted to guinea pigs with zoonotic potential, which causes diarrhoea with frequent deaths in larger guinea pig herds. *C. caviae* is also a zoonotic pathogen and often the cause of conjunctivitis, pneumonia and abortions in guinea pigs and can lead to upper respiratory tract disease, conjunctivitis but also severe pneumonia in humans. The increased death cases and the clinical signs could be traced back to an infection with *Cryptosporidium wrairi*, complicated by a co-infection of *C. caviae*. We suspect that the abortions were caused by *C. caviae*, but since the population was treated with various antibiotics effective against chlamydial infections, it was no longer possible to verify this by PCR testing. Unfortunately, more animals succumbed and finally only two animals of the originally 26 were left. With this case report, we would like to point out to veterinarians that guinea pigs can be an important source of zoonotic infections for various pathogens, especially since they are popular pets and often come into close contact with children where hygiene might not always be strictly followed.

Keywords: Abortion, *Chlamydia caviae*, *Cryptosporidium wrairi*, mass death, zoonosis, guinea pig (Cavia porcellus), molecular diagnostics

https://doi.org/10.17236/sat00383

Eingereicht: 20.06.2022
Angenommen: 05.12.2022

Zoonotic potential of guinea pigs: Outbreak of cryptosporidiosis combined with chlamydiosis in a breeding guinea pig herd

N. Borel, S. Ciuria, T. Flury, W. Basso, M. Ruetten

Case report

An increased numbers of death cases occurred in a herd of guinea pigs (*Cavia porcellus form. domestica)* with 26 individuals of all age categories (new born to 5 years old) at the end of September 2020. The animals belong to one private person who kept the animal as a hobby. The hygiene was good. Deaths occurred after three animals had been introduced from outside, bought from a commercial breeder. One of the three newly introduced animals suffered from conjunctivitis and diarrhoea. The animals were kept in an outdoor enclosure in several groups (barn with access to an open field) and the animals were fed with hay, grass, vegetables, and guinea pig pellets combined with access to various water bowls which were cleand once a day. At the same time, the owner suffered from a confirmed SARS-CoV-2 infection with symptoms such as fever, cough, headache, joint pain and pneumonia. The question arose if COVID-19 was also rampant in the guinea pigs. At that time, it was not known if guinea pigs could be infected or even transmit COVID-19 to humans.

Additionally, to the death cases, the population showed apathy, anorexia, severe weight loss, distended abdomens, inflamed conjunctivas, questionable lung problems and abortions / stillbirths. A juvenile, non-pregnant female, which had been pre-treated with the whole group with chloramphenicol per os (Chloropal forte 50 mg / kg body weight per twice a day; Dr. E. Graeub AG, Bern, Switzerland) to prevent further disease, died in mid-October and was send for pathologic examination.

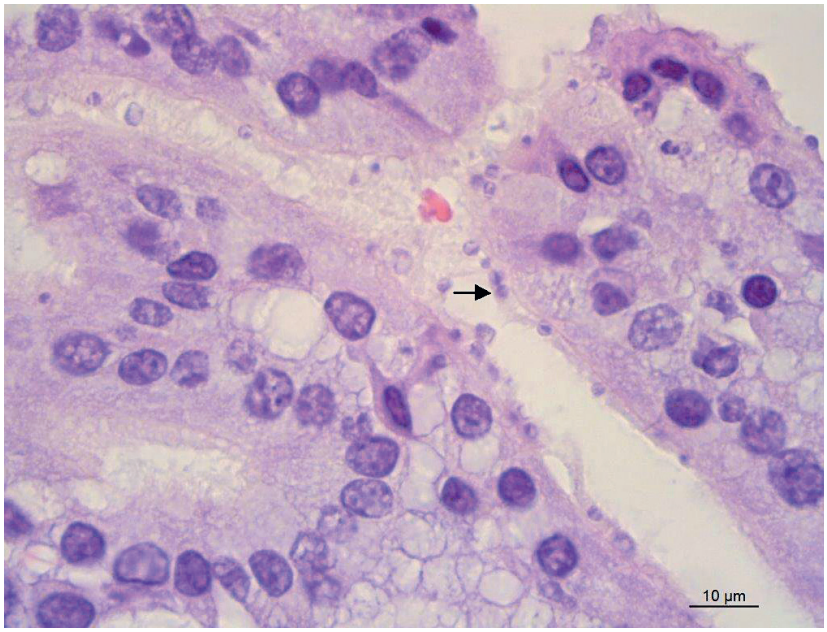

Figure 1: Section of the small intestine of a guinea pigs (Cavia porcellus form. domestica), stained with haematoxylin and eosin (HE). On the villus surface are numerous stages of cryptosporidia (4–6 µm) attached to enterocytes (arrow)

The animal had severe conjunctivitis , had no subcutaneous fat, but no changes were visible in the in the lungs and the vulva was macroscopically normal. The small intestine contained watery content, which continued into the rectum. Despite the lack of lung changes, the animal was tested for SARS-Cov-2 to rule out an asymptomatic carrier state of COVID-19. The result turned out negative. Histologically, intracellular but extracytoplasmic protozoa of approx. 4–6 µm attached to the cell surface directly under the cell membrane of enterocytes could be seen (fig. 1). Additionally, a coproscopic examination using Ziehl-Neelsen (ZN) staining showed few ZN-positive oocysts in the faeces. In order to confirm this finding, DNA was extracted from 150 mg of faeces with a commercial kit (Quick-DNA Fecal / Soil Microbe Miniprep kit, Zymo Research, Irvine, USA) and a PCR was carried out which amplified a partial sequence of the 18S rRNA gene from apicomplexa coccidia. An approximately 280 bp PCR product was purified (DNA Clean & Concentrator Kit, Zymo Research, Irvine, USA) and then sequenced with the primers COC1 and COC2 (Microsynth, Balgach Switzerland). The corresponding sequence (without primer regions) showed a 99,6 % (228/229 bp) agreement with GenBank ™ sequences from *Cryptosporidium wrairi* from the USA (U11440.1) and was deposited at the GenBank™ access nr OL354438.

At necropsy, swabs from the conjunctiva and the vagina were taken, as well as fresh tissue from the eyelid and uterus/ vagina were removed for PCR testing for *Chlamydia* sp. The DNA extraction from the swab samples was carried out with the Maxwell 16 Buccal Swab LEV DNA Purification Kit (Promega, Madison, WI, USA) and the extraction from the fresh material (eyelid, vagina/uterus) with the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Hilden). The extracted DNA was then examined by a 23S rRNA *Chlamydiaceae*-specific PCR[10]. This method enables a broad screening for the currently known chlamydial species of the *Chlamydiaceae* family. In a second step, the chlamydial species was determined using a conventional 16S rRNA PCR and subsequent sequencing. The sequencing of the two swab samples as well as the fresh tissues (eyelid, uterus) resulted in *C. caviae* (99,6 % sequence homology). Subtyping using a conventional PCR, which amplifies the outer membrane protein A (*ompA*) gene of *Chlamydia*, resulted in 100 % homology to the strain *C. caviae* KY777669.1, which was also identified in the zoonotic cases from The Netherlands.[15]

Thus, the death of the guinea pig was attributed to an infection with *Cryptosporidium wrairi*. The additional chlamydial finding was considered as a secondary infection complicating the ongoing herd problem and was most likely the cause of the concurrently reported abortions in pregnant and older animals.

The housing was disinfected at the end of October 2020, but unfortunately, already 19 of the 26 animals had died in

the meantime. From mid-October 2020 on, the remaining animals were treated with Chloropal forte (50 mg/kg twice a day) Dr. E. Graeub AG), Baycox ® (0,3 ml/kg once a day, Bayer, Basel, Swizterland), Oxytetracycline eye ointment (Jenapharm®, Jena, Germany) and Engemycin 10 % (25 mg/kg once a day, MSD Animal Health GmbH, Zurich, Switzerland) by the private veterinarian. Of the seven remaining animals, two more pregnant / discarding animals died in November 2020. The autopsy revealed the presence of acute purulent endometritis with dissemination of the infection into the liver and lungs as well as a disseminated intravascular coagulopathy (DIC). Both animals were negative for *Chlamydia* by PCR but had been pre-treated with antibiotics. Due to advanced autolysis, the intestines could not be further assessed by histopathology limiting conclusions about the outcome of the treatment on *C. wrairi*. The five remaining animals (age: a few months to 1 year) were again tested for *Chlamydia* by PCR on conjunctival and rectal swabs with negative results. The therapy as described before was continued, unfortunately without success. The animals died.

Discussion

Pets can be carriers of zoonotic diseases caused by viruses, bacteria, fungi or parasites. Since the first SARS-CoV-2 cases occurred, pets have been discussed again and again as potential carriers. However, it can be assumed that the risk of SARSV-CoV-2 infection in pets (especially dogs and cats) is low. Rather, it seems to be the case that pets are given an infection by their owners than the other way around and that they just participate with the disease and quarantine of their owner.[18] The guniea pigs resulted COVID- 19 negative, therefore we assume they are not highly sensitive to the infection.

Cryptorsporidium spp. are widespread in humans and animals all over the world, and often associated with mild diarrhea, but can lead to deaths under poor hygienic conditions or in immunosuppressed individuals. Infection sources are pet food and undercooked vegetables.[16] C. wrairi is a parasite adapted to guinea pigs[19] and occurs more frequently in larger herds conventionally kept with insufficient hygiene than in individually hobby keepings.[1,9,8,3] Both juvenile and adult animals are susceptible.[1,9,8] *C. wrairi* has only rarely been described in humans, but it was reported infecting immunocompetent individuals[2,12] and other animal species, including cattle,[2,11] dwarf hamsters *(Phodopus sp.)*,[8] and red-bellied squirrels *(Callosciurus erythraeus)*.[6]

Other zoonotic pathogens are bacteria, such as *Chlamydia*, which occur in humans and animals worldwide. The best known is *Chlamydia psittaci*, the causative agent of avian chlamydiosis, a notifiable animal disease (https://www.blv.admin.ch/blv/de/home/tiere/tierseuchen/uebersicht-seuchen/alle-tierseuchen /chlamydiosis-der-voegel-beim-tier-und-beim-menschen.html). Ornamental birds (especially psittacids), feral pigeons, but also poultry such as chickens, turkeys and ducks are important sources for infection of humans.[13,17] *C. caviae* infects guinea pigs leading to conjunctivitis, but can also cause rhinitis, pneumonia or abortions and can also infect humans[1,9,3,15,4]. A case report from Switzerland from 2006 describes a zoonotic infection (mild conjunctivitis) in a guinea pig collection and its owner.[14] Chlamydia infections are diagnosed in all animal species using swab samples from the conjunctiva, nose, throat, vagina or rectum, with so-called "flocked swabs" enabling chlamydia-infected epithelial cells to be obtained. These swabs can be used without a transport medium (dry swabs) and are very well suited for molecular diagnostics. PCR diagnostics is the method of choice because it is a very sensitive and specific method. It is important to determine the chlamydia species involved in the infection, which can vary depending on the animal species. This is done either directly by species-specific PCR methods or a screening procedure for chlamydia (based on the search for *Chlamydiaceae*) is carried out first, whereupon further typing is carried out in the positive case. (For questions please contact Prof. Nicole Borel, institute of veterinary pathology of vetsuisse faculty Zurich.).

Conclusion

With this case report, we would like to draw attention to the fact that guinea pigs have zoonotic potential and are often cared for by children for whom hygiene may not be a priority. Newly bought animals should first be tested for potential diseases, clinically examined or kept in quarantine for several days before introduced into the existing herd.

Acknowledgement

Thanks go to Theresa Pesch and Barbara Prähauser for processing of the samples and to Dr. Hanna Marti and Dr. Jasmin Kuratli for help with data interpretation and evaluation for the Chlamydia diagnostics (Institute for Veterinary Pathology, Department of Infectious Pathology with a focus on Chlamydia)

Zoonotic potential of guinea pigs: Outbreak of cryptosporidiosis combined with chlamydiosis in a breeding guinea pig herd

N. Borel, S. Ciuria, T. Flury, W. Basso, M. Ruetten

Potentiel zoonotique des cobayes : foyer de cryptosporidiose associée à une chlamydiose dans un groupe de cobayes reproducteurs.

Dans un groupe de cobayes de 26 animaux reproducteurs, plusieurs individus de toutes les catégories d'âge sont morts (16/26) après l'introduction de trois animaux provenant d'un autre groupe. En outre, la population a souffert d'apathie, d'anorexie, de perte de poids sévère et de conjonctivite ainsi que d'avortements et de mortinatalité. La présence de *Chlamydia caviae* a pu être détectée dans la conjonctive et le vagin/utérus d'un animal juvénile, ainsi qu'une infection intestinale à *Cryptosporidium wrairi*. Des oocystes ont été trouvés histologiquement dans l'intestin grêle, ce qui a été confirmé par PCR. *C. wairi* est un parasite adapté aux cobayes avec un potentiel zoonotique, qui provoque des diarrhées avec des morts fréquentes dans les grands groupes de cobayes. *C. caviae* est également un agent pathogène zoonotique et est souvent à l'origine de conjonctivites, de pneumonies et d'avortements chez les cobayes ; il peut entraîner des maladies des voies respiratoires supérieures, des conjonctivites mais aussi des pneumonies graves chez l'homme. L'augmentation des cas de décès et les signes cliniques pourraient être attribués à une infection par *Cryptosporidium wrairi*, compliquée par une co-infection par *C. caviae*. Nous soupçonnons que les avortements ont été causés par *C. caviae*, mais comme la population a été traitée avec divers antibiotiques efficaces contre les infections à chlamydia, il n'était plus possible de le vérifier par des tests PCR. Malheureusement, d'autres animaux ont succombé et il ne restait finalement que deux animaux sur les 26 d'origine. Avec ce rapport de cas, nous aimerions attirer l'attention des vétérinaires sur le fait que les cochons d'Inde peuvent être une source importante d'infections zoonotiques pour divers pathogènes, d'autant plus qu'il s'agit d'animaux de compagnie populaires qui sont souvent en contact étroit avec des enfants avec lesquels l'hygiène n'est pas toujours strictement respectée.

Mots clés: Avortement, *Chlamydia caviae*, *Cryptosporidium wrairi*, mort massive, zoonose, cobaye (Cavia porcellus), diagnostic moléculaire.

Potenziale zoonotico dei porcellini d'India: focolaio di criptosporidiosi combinata a clamidiosi in un allevamento di porcellini d'India.

In un allevamento di porcellini d'India composto da 26 animali da riproduzione, diversi animali di diverse fasce d'età (16/26) sono morti dopo l'introduzione di tre nuovi animali provenienti da un altro allevamento. Gli animali presentavano apatia, anoressia, grave perdita di peso e congiuntivite, nonché aborti e nati morti. In un giovane animale è stata rilevata la presenza di *Chlamydia caviae* e di un'infezione intestinale provocata da *Cryptosporidium wrairi* nella congiuntiva e nella vagina/utero. Le oocisti sono state rilevate istologicamente nell'intestino tenue e la loro presenza è stata confermata via PCR. *C. wairi* è un parassita che si adatta ai porcellini d'India con un potenziale zoonotico che causa diarrea e frequenti decessi in grandi allevamenti di porcellini d'India. *C. caviae* è anche un patogeno zoonotico ed è spesso la causa di congiuntiviti, polmoniti e aborti nei porcellini d'India e può causare malattie del tratto respiratorio superiore, congiuntiviti ma anche gravi polmoniti nell'uomo. L'aumento dei decessi e dei segni clinici potrebbe essere attribuito ad un'infezione da *Cryptosporidium wrairi*, aggravata da una coinfezione con *C. caviae*. Si sospetta che gli aborti siano stati causati da *C. caviae*, ma poiché alla popolazione sono stati somministrati diversi antibiotici efficaci contro le infezioni da clamidia, non è stato più possibile verificare mediante test PCR. Purtroppo, altri animali sono morti e alla fine sono sopravvissuti solo 2 dei 26 animali. Con questo caso, vorremmo richiamare l'attenzione dei veterinari che i porcellini d'India possono essere un'importante fonte di infezioni zoonotiche per vari agenti patogeni, soprattutto perché sono animali domestici molto diffusi e spesso sono a stretto contatto con i bambini, la cui igiene non può essere sempre rigorosamente mantenuta.

Parole chiave: aborto, *Chlamydia caviae*, *Cryptosporidium wrairi*, mortalità di massa, zoonosi, porcellino d'India (Cavia porcellus), diagnostica molecolare

Literaturnachweis

[1] Angus KW and Hutchison G Infectivity of a strain of *Cryptosporidium* found in the guinea-pig (*Cavia porcellus*) for guinea-pigs, mice and lambs. Journal of Comparative Pathology, 1985: 95 (2):151–165.

[2] Azami M, Moghaddam DD, Salehi R, and Salehi M. The Identification of *Cryptosporidium* species in Isfahan, Iran by PCR-RFLP Analysis of the 18S rRNA Gene ISSN 0026–8933, Molecular Biology, 2007: 41 (5): 851–856. © Pleiades Publishing, Inc., 2007. Published in Russian in Molekulyarnaya Biologiya, 2007:41(5):934–939.

[3] Beck W and Pantchev N. Praktische Parasitologie bei Heimtieren, 2. überarb. Auflage, Schlütersche Verlag, Hannover, 2013: 369. ISBN: 9783899930887.

[4] Borel N, Polkinghorne A, and Pospischil A. A Review on Chlamydial Diseases in Animals: Still a Challenge for Pathologists? Veterinary Pathology 2018:*55*: 374–390.

[5] Buxton D, Barlow RM, Finlayson J, Anderson IE, and Mackellar A. Observations on the pathogenesis of *Chlamydia psittaci* infection of pregnant sheep. J Comp Pathol 1990: 10: 221–237.

[6] Chai Y, Deng L, Liu H, Yao J, Zhong Z, Xiang L, Fu H, Shen L, Zhou Z, Deng J, Hu Y, Peng G. First detection of Cryptosporidium spp. in red-bellied tree squirrels (*Callosciurus erythraeus*) in China. Parasite. 2019;26:28. doi: 10.1051/parasite/2019029. Epub 2019 May 13. PMID: 31081495.

[7] Hen S, Chai Y, Deng L, Liu H, Zhong Z, Fu H, Hu Y, Shen L, Zhou Z, Geng Y, Peng G. *Cryptosporidium* spp. in pet dwarf winter white Russian hamsters (*Phodopus sungoris sungoris*) in China. J Parasitol. 2021;107(5):770–777. doi: 10.1645/20-102. PMID: 34547101.

[8] Chen J, Wang W, Lin Y, Sun L, Li N, Guo Y, Kvac M, Ryan U, Feng Y, Xiao L. Genetic characterizations of *Cryptosporidium* spp. from pet rodents indicate high zoonotic potential of pathogens from chinchillas. One Health. 2021 May 24;13:100269. doi: 10.1016/j.onehlt.2021.100269. eCollection 2021 Dec. PMID: 34113708.

[9] Chrisp CE, Reid WC, Rush HG, Suckow MA, Bush A, and Thomann MJ. Cryptosporidiosis in Guinea Pigs: an Animal Model. Infection and Immunity, 1990:58: 674–679.

[10] Ehricht R; Slickers P, Goellner S, Hotzel H, Sachse K. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol. Cell. Probes 2006: 20: 60–63.

[11] Firoozi Z, Sazmand A, Zahedi A, Astani A, Fattahi-Bafghi A, Kiani-Salmi N, Ebrahimi B, Dehghani-Tafti A, Ryan U, Akrami-Mohajeri F. Prevalence and genotyping identification of *Cryptosporidium* in adult ruminants in central Iran. Parasit Vectors. 2019;12(1):510. doi: 10.1186/s13071-019-3759-2. PMID: 31666095

[12] Hernández-Castro C, Dashti A, Köster PC, Bailo B, López A, Llorente MT, Sánchez S, Carmena D. *Cryptosporidium wrairi* infection of zoonotic origin in an immunocompetent toddler, Spain. 2021. DOI: 10.13140/RG.2.2.34978.35520 (Preprint).

[13] Longbottom D, and Coulter LJ. Animal chlamydioses and zoonotic implications. J Comp Pathol 2003:128: 217–244.

[14] Lutz-Wohlgroth L, Becker A, Brugnera E., Huat ZL, Zimmermann D, Grimm F, Haessig M, Greub G, Kaps S, Spiess B, *et al.* Chlamydiales in guinea-pigs and their zoonotic potential. J Vet Med A Physiol Pathol Clin Med 2006: 53: 185–193.

[15] Ramakers BP, Heijne M, Lie N, Le TN, van Vliet M, Claessen VPJ, Tolsma PJ.P, De Rosa M, Roest HIJ, Vanrompay ., *et al.* Zoonotic *Chlamydia caviae* presenting as community-acquired pneumonia. N Engl J Med 2017:377: 992–994 DOI: 10.1056/NEJMc1702983.

[16] Ryan U, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology. 2014;141(13):1667–85. doi: 10.1017/S0031182014001085. Epub 2014 Aug 11. PMID: 25111501.

[17] Sachse K, Laroucau K, and Vanrompay D. Avian Chlamydiosis. Curr Clin Microbiol Reports 2015:2: 10–21 DOI: 10.1007/s40588-014-0010-y.

[18] Schubert F, People with COVID Often Infect Their Pets New unpublished studies show that dogs and cats with COVID-positive owners frequently have SARS-CoV-2 antibodies, scientific American, 2021.

[19] Xiao L, Fayer R, Ryan U, Upton SJ. *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin Microbiol Rev. 2004:17(1):72–97. doi: 10.1128/CMR.17.1.72-97.2004. PMID: 1472645.

Korrespondenzadresse

Maja Ruetten
Dr med vet., Dipl. ECVP, FVH für Pathology (GST)
Pathovet AG
Buckstrasse 2
CH-8317 Tagelswangen
E-Mail: maja.ruetten@pathovet.ch

Zoonotic potential of guinea pigs: Outbreak of cryptosporidiosis combined with chlamydiosis in a breeding guinea pig herd

N. Borel, S. Ciuria, T. Flury, W. Basso, M. Ruetten