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Keywords:
Piroplamorida
Ticks
Rickettsia
Babesia
Algeria
Theileria

A B S T R A C T

A molecular survey was undertaken to determine the presence of protozoal and bacterial organisms in 120 ticks and 87 blood samples collected from mammals in north-eastern Algeria. Eight tick species were morphologically identified including 70 *Rhipicephalus* (*Boophilus*) *annulatus*, 23 *Rhipicephalus bursa*, five *Rhipicephalus sanguineus* sensu lato, 11 *Hyalomma impeltatum*, five *Hyalomma scupense*, two *Hyalommma marginatum*, one *Hyalomma anatolicum* and three *Ixodes ricinus*. Quantitative PCR screening of the ticks showed that *Theileria annulata*, "*Candidatus* Ehrlichia urmitei", *Theileria buffeli* and *Anaplasma platys* were detected in *Rh. annulatus*. *Rickettsia massiliae* and *Anaplasma ovis* were detected in *Rh. sanguineus* s.l. and *Rh. bursa*. *Rickettsia aeschlimannii* was detected in *Hy. marginatum*, *Hy. scupense* and *Hy. impeltatum*. Finally, "*Candidatus* Rickettsia barbariae" was detected in *Rh. bursa*. In the screening blood samples, *Theileria equi*, *T.annulata*, *T. buffeli*, *Babesia bovis*, *Anaplasma marginale*, *A. ovis* and *Borrelia* spp. were detected in cattle. *Theileria ovis*, *T. annulata*, and *A. ovis* were detected in sheep. In addition, *A. ovis* and *T. equi* were detected in goats and equidea respectively. In this study, *T. equi* and "*Candidatus* Rickettsia barbariae" were identified for the first time in Algeria as well as potential new species of *Ehrlichia* and *Anaplasma*.

Although molecular detection does not indicate vector/reservoir competence when investigating ticks removed from animals, this study expands the knowledge of the microorganisms detected in ticks in north-east of Algeria.

1. Introduction

Ticks are obligate hematophagous arthropods known to be important vectors of a wide variety of protozoa, fungi, bacteria, viruses and filarial worms of medical and veterinary importance (Aydin et al., 2015; Pereira et al., 2016; Remedio et al., 2015). In Algeria, Medi-terranean spotted fever caused by *Rickettsia conorii conorii* is known to be endemic, and other rickettsial pathogens have been detected in ticks, including *Rickettsia aeschlimannii*, *Rickettsia massiliae*, *Rickettsia helve-tica*, *Rickettsia monacensis*, *Rickettsia slovaca*, *Rickettsia africae* and *Rickettsia sibirica mongolitimonae* (Kernif et al., 2012). In addition, other tick-borne pathogens such as *Borrelia* spp., *Anaplasma* spp., and *Coxiella burnetii*, have also been detected in ticks and/or cattle (Aouadi et al., 2017; Boucheikhchoukh et al., 2018; Dahmani et al., 2015; Leulmi et al., 2016; Rjeibi et al., 2016; Ziam et al., 2015). Very few studies have been conducted on *Theileria* spp. and *Babesia* spp. in cattle and ticks from Algeria (Aouadi et al., 2017; Ziam et al., 2015). These studies reported the presence of *Theileria orientalis, T. annulata*, *T. ovis*, *Babesia ovis*, and *B. bovis* in cattle and ticks (Aouadi et al., 2017; Ziam et al., 2015). These apicomplexan protozoa are causative agents of piroplasmoses which are among the most economically important haemoparasitic tick-borne diseases of ruminants worldwide (Adjou Moumouni et al., 2015; Aydin et al., 2015; Toma et al., 2017; Dib et al., 2008; Pereira et al., 2016).

The aim of this study was to update the repertoire of protozoan and bacterial diseases in domestic animals and their infesting ticks in

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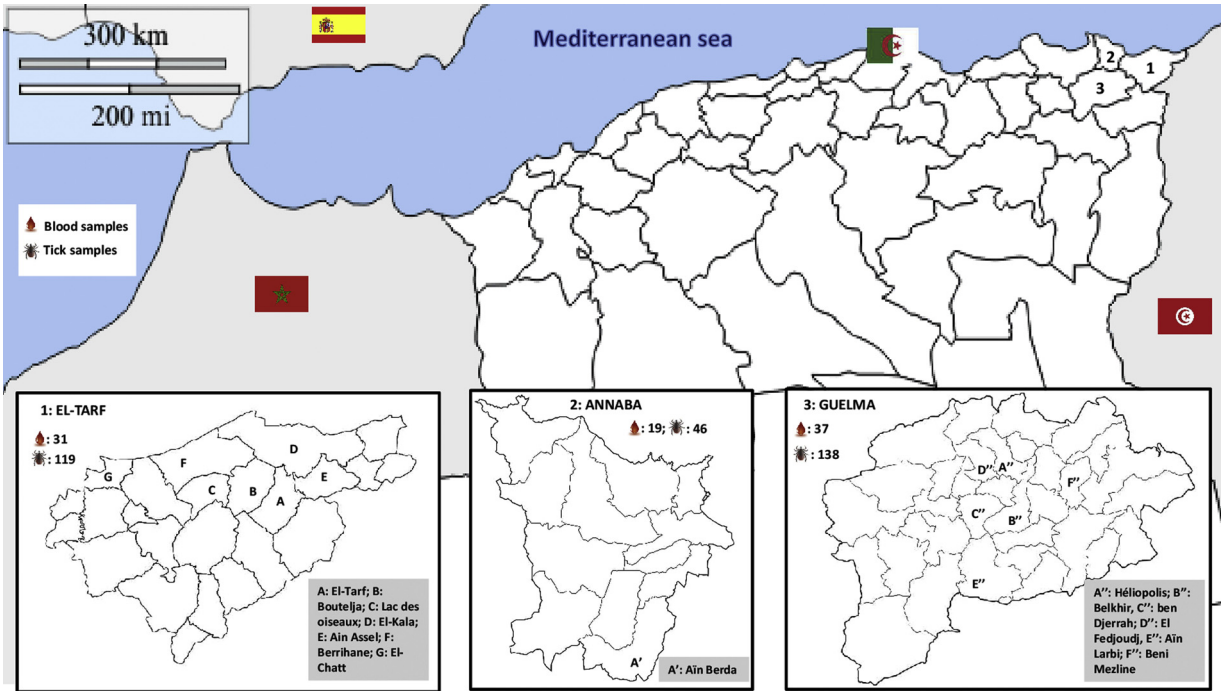

Fig. 1. Map of Algeria with sample collection sites.

northern Algeria, using molecular methods.

2. Materials and methods

2.1. Study area, tick collection and blood sampling

From March 2016 to February 2017, 303 ticks and 87 blood samples were collected from cattle in the provinces of Guelma (36 ° 27 '0 "N 7 ° 25' 59.999" E), Annaba (36 ° 53 '60" N 7 ° 46 '0.001 "E) and El-Tarf (36 ° 46' 1.2" N 8 ° 19 '1.2 "E) in north-eastern Algeria (Fig. 1).

Blood samples were collected and stored in EDTA tubes at −20 °C and ticks were removed from animals and stored at room temperature in 70 % ethanol. The morphological identification of ticks was carried out using a microscope and identification keys (Walker, 2003). The sample collection was authorised by the Animal Ethics Committee of El-Tarf University (Law No. 88-08 of 26 January 1988 on the activities of veterinary medicine and the protection of animal health). Molecular analysis was performed on 120 selected ticks and all blood samples.

2.2. Detection of microorganisms

DNA was extracted individually from each sample (tick and blood) using the EZ1 DNA Tissue Kit (Qiagen, Hilden, Germany). Both sample types were screened by quantitative real-time PCR (qPCR) for *Rickettsia* spp., *Bartonella* spp., *Anaplasmataceae* spp., *C. burnetii*, *Borrelia* spp., *Theileria* spp. and *Babesia* spp., by using specific primers and probes (Table1). All the genus-qPCR positive samples for the different micro-organisms were then tested using specific qPCR and/or subjected to standard PCR prior to sequencing to identify the microorganism species (Dahmani et al., 2015; Diarra et al., 2017; Tahir et al., 2016). Phylogenetic trees were drawn based on the alignment of the different genes using Bioedit and TOPALi 2.5 software (Biomathematics and Statistics Scotland, Edinburgh, United Kingdom) using the Tnr + Γ substitution model. The nucleotide sequences were compared with GenBank entries using BLASTn and filed in GenBank under the following accession numbers: MH319801, MH327771, MH327772, MH327773, MH327774, MH321192, MH321193, MH321194, MH321195 and MH321197.

3. Results

3.1. Tick identification and sample collection

A total of 120 ticks belonging to eight species were morphologically collected and identified, including *Rh. annulatus*, which was the most prevalent with 58.3 % (70/120), followed by *Rh. bursa* (23/120), *Hy. impeltatum* with (11/120), *Hy. scupense* (5/120), *I. ricinus* (3/120), *Rh. sanguineus* s.l. (5/120), *Hy. marginatum* (2/120) and *Hy. anatolicum* (1/120) (Table 1). Of the 87 blood samples, 35, 27, 17 and 8 were collected from cattle, sheep, horses and goats respectively.

Table 1
Numbers of ticks used for pathogens identification.

Tick species	Number collected from cattle	Number collected from sheep	Number collected from goats	Number collected from equidae	Total
Rhipicephalus annulatus	29	14	7	20	70
Rhipicephalus bursa	0	14	9	0	23
Hyalomma impeltatum	8	3	0	0	11
Hyalomma scupense	0	1	4	0	5
Ixodes ricinus	0	2	1	0	3
Rhipicephalus sanguineus s.l.	0	2	3	0	5
Hyalomma marginatum	0	1	1	0	2
Hyalomma anatolicum	0	0	1	0	1
Total	37	37	26	20	120

Table 2
Numbers of ticks or blood samples which tested positive for various pathogens analysed in the present study.

	Total tested	Numbers of samples positive for													
		R. massiliae	*R. aeschlimannii*	Other *Rickettsia*	*A. marginale*	*A. ovis*	*A. platys*	*Candidatus* Ehrlichia urmitei	*T. annulata*	*T. equi*	*T. ovis*	*T. buffeli*	*B. bovis*	*Borrelia* spp.	*Bartonella* spp.	*C. burnetii*
Ticks	120	2 *Rh. bursa*, 2 *Rh. sanguineus* s.l.	1 *Hy. impeltatum*, 2 *Hy. scupense*, 1 *Hy. marginatum*	1 *Rh. bursa*		1 *Rh. sanguineus* s.l., *1 Rh. bursa*	1 *Rh. annulatus**	2 *Rh. annulatus**	4 *Rh. annulatus**, 2*Rh. annulatus*	–	–	1 *Rh. annulatus**	–	–	–	–
Blood	87	–	–		4 Cattle	3 Goat, 1 Sheep, 1 Cattle	–	–	4 Cattle, 1 Sheep	1 Cattle, 2 Equidea	4 Sheep	1 Cattle	1 Cattle	2 Cattle	–	–

* Engorged tick specimen.

3.2. Detection of microorganisms

Piroplasmorida were detected in seven (7/70; 10 %) *Rh. annulatus* ticks and fourteen blood samples. Sequencing showed that six out of seven sequences obtained from ticks were 99.42 % identical to *T. annulata* (KX273857) and one was 99.24 % identical to *T. buffeli* (HM538197). For the blood samples the sequencing showed that five of fourteen sequences were 99.15–99.89% identical to *T. annulata* (KX273857, KT367878), four were 99.89–100% identical to *T. ovis* (FJ603460), three were 99.04 % identical to *T. equi* (KY952232), one was 99.25 % identical to *T. buffeli* (HM538197) and one was 99.35 % identical to *B. bovis* (EF601930) (Table 2). The phylogenetic position of Piroplasmida identified in our study is illustrated in Fig. 2.

Similarly, three *Rh. annulatus*, one *Rh. sanguineus* s.l., one *Rh. bursa* and nine blood samples were positive for *Anaplasma* spp. The sequences obtained from *Rh. sanguineus* s.l. and *Rh. bursa* were 100 % identical to *A. ovis* (KY498325). Two of the three sequences obtained from *Rh. annulatus* were 98.07 % identical to "*Candidatus* Ehrlichia urmitei" (KM021422) and one was 97.69 %, identical to *A. platys* (KM021414). In animals, four sequences were 99.57–100% identical to *A. marginale* (CP006847, KY498335) and five were 99.5–100% identical to *A. ovis* (KM021411) (Table 2). The phylogenetic tree based on the 23S gene (485 bp) shows the position of the Anaplasmatacae genotypes of this study compared to the other genotypes in Supplementary Fig. 1.

Rickettsia massiliae was identified in two *Rh. sanguineus* s.l. and two *Rh. bursa* and *R. aeschlimannii* in two *Hy. scupense*, one *Hy. impeltatum* and one *Hy. marginatum* by specific qPCR. The sequencing of three ticks which were positive using pan-rickettsial qPCR and negative using specific qPCR allowed us to obtain a sequence only from *Rh. bursa*, which was 99.28 % identical to "*Candidatus* Rickettsia barbariae" (KU645284). All blood samples were negative for *Rickettsia* spp. (Table 2).

All tested ticks were negative for *Borrelia* spp. using qPCR. Three blood samples were positive for *Borrelia* spp. and sequencing showed that the sequences were 99.5 % identical to "uncultured *Borrelia* sp." detected in *Haemaphysalis megaspinosa* and *Haemaphysalis japonica* from Japan (LC170035 and AB897891). The phylogenetic tree based on the 16S rRNA gene (1355 bp) shows the position of the *Borrelia* sp. of this study compared to the other *Borrelia* spp. in Supplementary Fig. 2.

All tested samples were found to be negative for *C. burnetii* and *Bartonella* spp. (Table 2).

4. Discussion

In this study we detected the presence of several pathogens in domestic animals and their infesting ticks using robust molecular approaches including appropriate controls. *T. equi* is an obligate haemoparasite transmitted by ticks that can cause equine piroplasmosis characterised by fever, anaemia, jaundice, hepatosplenomegaly, intravascular haemolysis and haemoglobinuria, or even death (Malekifard et al., 2014). In this study, *T. equi* was detected in blood of cattle and equids for the first time in Algeria. In Africa, *T. equi* had been detected in Tunisia (Ros-García et al., 2013), Kenya (Hawkins et al., 2015), Egypt (Mahmoud et al., 2016), Nigeria (Adamu et al., 2014) and South Africa (Rosa et al., 2014). *T. annulata* is an obligate intracellular protozoan parasite infecting monocytes/macrophages and B cells (Glass et al., 1989). Here, *T. annulata* was identified in *Rh. annulatus* ticks and in the blood of cattle and sheep. The presence of *T. annulata* in cattle was previously detected in eastern and northern central Algeria, in Egypt, Ethiopia, Tunisia and Sudan (Elsify et al., 2015; Gebrekidan et al., 2014; M'ghirbi et al., 2008; Taha et al., 2013; Ziam and Benaouf, 2004; Ziam et al., 2015). *Theileria buffeli* is known as a widely distributed benign haemoparasite of cattle usually transmitted by *Hae. punctata* ticks throughout the Mediterranean basin (Grech-Angelini et al., 2016). In our study, *T. buffeli* was detected in cattle and *Rh. annulatus* ticks. *T. buffeli* was reported for the first time in Algeria by

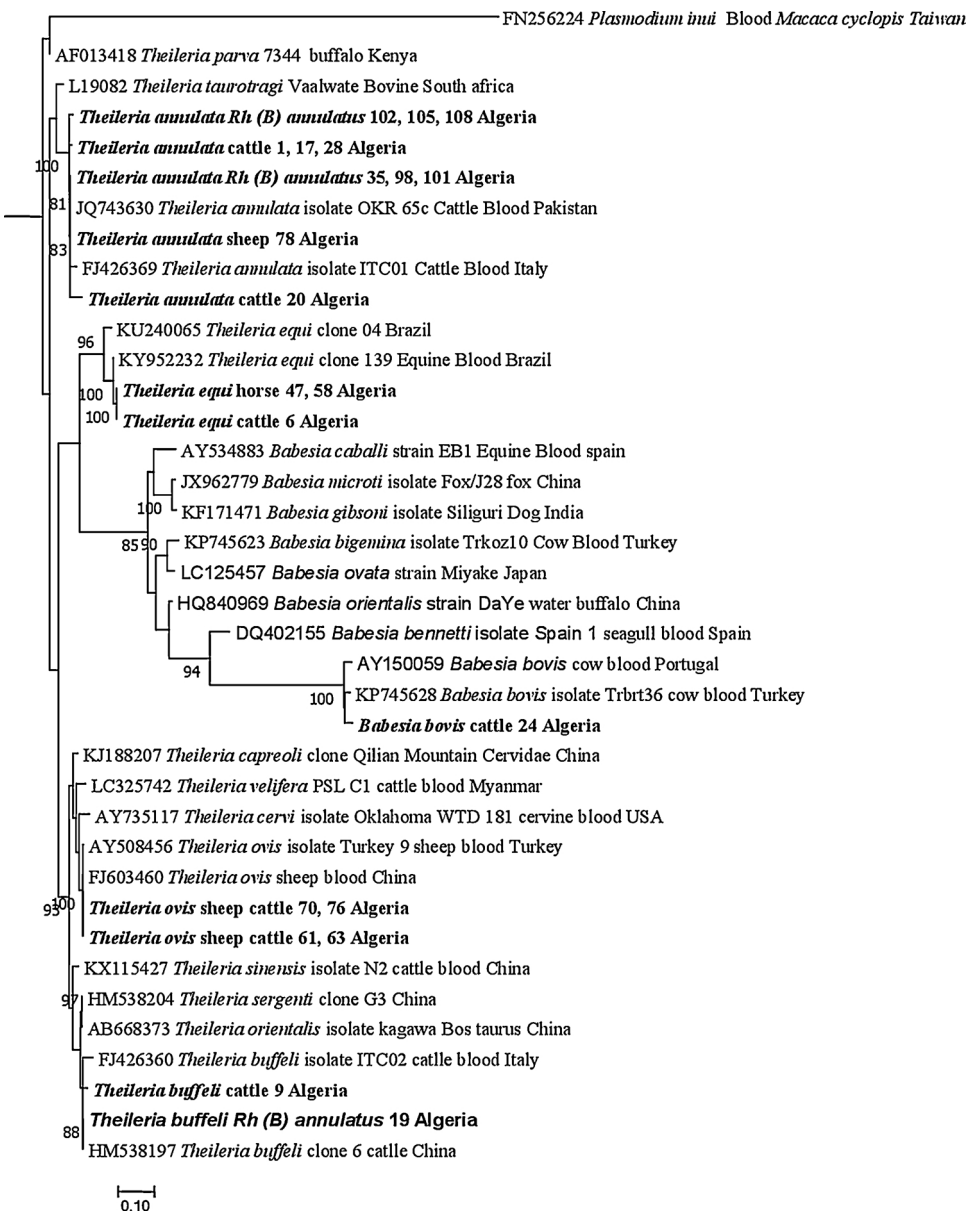

Fig. 2. Phylogenetic tree showing the relationships between Piroplasmida identified in the present study relative to other species based on a comparison of a 965 bp fragment of the 18S gene.

Ziam et al. (2015). It has also been found in cattle from Tunisia (M'ghirbi et al., 2008) and in buffalo from South Africa and Mozambique (Chaisi et al., 2013). *T. ovis* was detected only in sheep, in line with previous studies reporting the detection of this pathogen in sheep, goats and *Rhipicephalus* ticks in Algeria (Aouadi et al., 2017). It is a causative agent of subclinical infection in small ruminants and is widespread throughout in the world (Jalali et al., 2014).

B. bovis, the agent responsible for bovine babesiosis, is a highly prevalent protozoan intra-erythrocyte parasite of economic importance in cattle and is transmitted by ticks (Mtshali and Mtshali, 2013). In this study, *B. bovis* was found in cattle from eastern Algeria, which was consistent with previous results reported by Ziam and Benaouf (2004). It has also been detected in cattle from Kenya (Adjou Moumouni et al., 2015).

In this study, we detected several *Anaplasma* species, including *A. marginale*, an obligate intracellular bacterium responsible for bovine anaplasmosis worldwide, manifested by anaemia and jaundice (Parola and Raoult, 2001). As previously reported in Algeria by Ziam and Benaouf (2004), this bacterium was detected in cattle. In addition, we detected *A. ovis* in the blood of small ruminants, *Rh. sanguineus* s.l. and *Rh. bursa*. Previous studies had already reported the presence of *A. ovis* in these ticks (Aouadi et al., 2017; Aubry and Geale, 2011; Dahmani et al., 2017). *Anaplasma ovis* is the causative agent of anaplasmosis in small ruminants and is known to be transmitted by several tick species (Dumler et al., 2001). *Anaplasma ovis* has already been reported in small ruminants and ticks from Algeria and Tunisia (Aouadi et al., 2017; Belkahia et al., 2014, 2017; Ben Said et al., 2015) and in ticks from Ethiopia (Teshale et al., 2016). Interestingly, we identified a new *Anaplasma* genotype close to *A. platys*, the agent of canine anaplasmosis which exclusively infects platelets and periodically causes deep thrombocytopenia in dogs (Nair et al., 2016), transmitted by *Rh. sanguineus* s.l. ticks (Sanogo et al., 2003). Strains which are genetically close to *A. platys* have already been reported in cattle and small rumi-nants (Ben Said et al., 2017, 2018) and in camels (Belkahia et al., 2015; Selmi et al., 2019) from Tunisia. Further analyses are needed, to know whether it is specifically *A. platys* or genetically-related strains. We also found a potential new species of *Ehrlichia* which is phylogenetically close to "*Candidatus* Ehrlichia urmitei" in *Rh. annulatus*. Little is known

about this species since it was recently detected for the first time in *Amblyomma variegatum*, *Rhipicephalus microplus* and *Hyalomma truncatum* collected from cattle in Côte d'Ivoire (Ehounoud et al., 2016). More recently, it has also been detected in *Rh. microplus* and *Rh. bursa* collected in France and Mali respectively (Dahmani et al., 2017; Diarra et al., 2017).

In this study, *R. massiliae* was detected in *Rh. bursa* and *Rh. sanguineus* s.l., thus confirming its presence in the country. Similarly, in our study, *R. aeschlimannii* DNA was found in *Hy. impeltatum*, *Hy. scupense* and *Hy. marginatum*. In Algeria, previous studies have reported the presence of *R. aeschlimannii* in several tick species of the *Hyalomma* genus (Bitam et al., 2006, 2009; Djerbouh et al., 2012; Leulmi et al., 2016). Both *R. massiliae* and *R. aeschlimannii* are spotted fever group rickettsial, known to be agents of emerging rickettsioses in humans. "*Candidatus* Rickettsia barbariae" was also found in one *Rh. bursa*. This bacterium was first detected in *Rh. sanguineus* sensu lato group ticks collected from Italian sheep (Mura et al., 2008). Since then, several studies have reported the presence of "*Candidatus* Rickettsia barbariae" in ticks and fleas from Europe and Asia (Chochlakis et al., 2012; Socolovschi et al., 2012; Zhao et al., 2016). Our study is the first to report the presence of "*Candidatus* Rickettsia barbariae" in Africa. The pathogenicity for animals or humans of this bacterium remains unknown.

The spirochaete *B. theileri*, the causative agent of bovine borreliosis in cattle, associated with fever and anaemia (Smith et al., 1985), was detected in two cattle blood samples. In Algeria, this species has been reported in blood from sheep and goats (Aouadi et al., 2017), but no studies have thus far indicated its presence in Algerian cattle. In Africa, *B. theileri* has also been reported in *Rhipicephalus geigyi* collected from cattle in Mali (McCoy et al., 2014).

5. Conclusion

In this study, we reported the presence of numerous pathogens in mammals and their ticks in north-eastern Algeria. Although we contribute towards broadening the knowledge of the repertoire of microorganisms, our results do not show infection status of the region due to the small amount of material collected. Also, it is not possible to draw conclusion on the competence of these vectors/reservoirs, given that these data do not suggest that the tick species mentioned in the document can serve as a competent vector for all detected pathogens. Nevertheless, other studies are needed to show the exact distribution of ticks and to appreciate the extent of the distribution of microorganisms in Algeria.

Acknowledgments

We thank all the farmers who agreed to participate to our study and the contributors from the various institutions for the collection of field samples and laboratory analysis. This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program « Investissements d'avenir », reference ANR-10-IAHU-03, the Région Provence Alpes Côte d'Azur and European funding FEDER PRIMI.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2019.101330.

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