Public Health risk of zoonotic ticks in Abeokuta, Southwest Nigeria

F.A. Akande[1] and B.O. Fagbemi[2]

[1]Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun state. Nigeria; [2]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Ogun state. Nigeria
Email: dayoakande2006@gmail.com

SUMMARY
Ticks are obligate blood-feeders that require a host to survive and reproduce and has been said to be second to mosquito in pathogen transmission. Tick-borne diseases are increasingly becoming an important public health issue because of the possibility of acquiring them through tick bite and the presence of ticks in environment poses risk to humans and livestock alike. This study was carried out to determine the presence of ticks in Abeokuta environment and to screen harvested ticks for some zoonotic pathogens. Ticks were gathered from ten different spots in Abeokuta Ogun state using the cloth dragging method. The gathered ticks were morphologically identified to genus level using stereo microscope and grouped based on their developmental stages. Harvested ticks were screened for 5 pathogens of Public Health importance namely *Anaplasma, Babesia, Borrelia*, *Ehrlichia* and *Rickettsia* by Polymerase Chain Reaction (PCR) using extracted DNA from the ticks. A total of 357 ticks were gathered from the environment, the environments were areas where cattle are being grazed. Using their morphology; 19 (5.3%) of ticks were of the genus *Amblyomma*, 274 (76.6%) were of the Genus *Rhipicephalus* (sub genus *Boophilus*), and 64 (17.9%) were of the Genus *Rhipicephalu*s. Using the developmental stages; 44 (12.3%) of the ticks were adults, 304 (85.2%) were larvae and 9(2.5%) were nymphs. Pathogen prevalence in the harvested ticks was 20.73%. PCR analysis revealed that 2 (0.56%) of ticks were positive for *Coxiella* and *Rickettsia*, 28 (7.84%) for *Anaplasma*, 29 (8.12%) for *Coxiella*, 7 (1.96%) ticks were positive for *Coxiella* and *Anaplasma* and 8 (2.25%) ticks were positive for Rickettsia. None of the ticks had *Babesia* and *Borrelia*. With the presence of *Anaplasma, Coxiella* and *Rickettsia* in the harvested ticks, the risk of acquiring infection from bite of ticks by humans and livestock is very high in the study areas and the possibility of transfer into other areas through movement of goods and services involved in global trade portend danger. Importance of co-infection in the harvested ticks and the dangers they pose in disease transmission to animal and humans are of great public health concern which require Public awareness and education.

Key words: zoonoses, Borrelia, pathogens, Rickettsia

INTRODUCTION

Ticks are obligate hematophagous ectoparasites of wild and domestic animals and humans that are classified in the subclass Acari, order Parasitiformes, suborder Ixodida, and distributed from Arctic to tropical regions of the world. Despite efforts to control tick infestations, ticks and the pathogens they transmit continue to be a serious constraint to human and animal health worldwide (de la Fuente and Kocan, 2006). Questing is the mechanism by which ticks find a host, climb on it, and feed. Ticks quest at variable heights in the vegetation, driven by factors such as temperature and relative humidity (Vail and Smith, 2002; Busby *et al.*, 2012).

Ticks are of great importance because of their ability to transmit a wide spectrum of pathogenic microorganisms, such as bacteria, protozoa, rickettsiae, spirochaetes and viruses. Grouped into *Ixodidæ* and *Argasidae* families, all ticks are obligate blood-suckers and they undergo three stages of development: larval, nymphal and adult. The life cycle of ticks starts when larvae seek hosts, attach, feed, detach, and develop in sheltered microenvironments where they moult to nymphs; nymphs follow the same pattern and

moult to adults (except argasids, which first moult into further nymphal stages); adults seek hosts, mate, feed, and in the case of engorged females, drop off to deposit eggs" (Dennis and Piesman, 2005). Tick hosts are terrestrial vertebrates, and host-seeking activity is called questing. Ticks may have vastly different host preferences, among species and at different developmental stages, as well as seasonal questing that depends on climate and environmental conditions (Dennis and Piesman, 2005).

Ticks are ranked as the most economically important ecto-parasites of livestock in the tropics, including sub Saharan Africa (SSA) (Uilenberg 1995). Their veterinary importance is related to their blood-feeding, from which both their direct and indirect pathogenicity originates (Jongejan and Uilenberg 2004). In cattle, tick infestation alone can cause anaemia, stress, reduction in weight gain and milk yields, depreciation of hide value, hypersensitivity and toxicosis, leading also to secondary infections (Jongejan and Uilenberg 2004). In addition, some tick species can act as vectors of pathogens causing several tick-borne diseases (TBDs), a serious impairment to cattle health and productivity in SSA (Young *et al;* 1988).

In Nigeria, 90% of the cattle population are kept under the traditional pastoral husbandry of Fulani herders; who graze cattle extensively in pastures and forest, thereby exposing the cattle to various form of ticks in the country (Lawal-Adebowale 2012) these ticks are cat-off the cattle during grazing and are usually found in the grazing areas or along the grazing routes. Genera *Amblyomma, Hyalomma*, and *Rhipicephalus*, (sub-genus *Boophilus* inclusive) are known vectors of the causative agents of the most important bovine Tick-Borne Diseases (TBDs) (Akande et al 2017).

In tropical Africa, tick-borne protozoan diseases (e.g. theileriosis and babesiosis) and rickettsial diseases (e.g. anaplasmosis and heartwater [cowdriosis]) are the part of the main challenges of health and management of domestic ruminants (Uilenberg, 1992). Other known pathogenic bacteria transmitted by ticks are intracellular alpha proteobacteria, which includes the families Anaplasmataceae, Bartonellaceae, and Rickettsiaceae (Dumler *et al*; 2001). Members of the genera Anaplasma and Ehrlichia, from the family Anaplasmataceae, infect mainly monocytes and granulocytes and cause human and animal anaplasmoses and ehrlichioses (Chen *et al;* 1994). The tickborne protozoa of the genus Babesia reproduce in erythrocytes, thus causing babesiosis among humans as well as wild and domestic animals (Homer *et al;* 2000).

The diverse and complex ecology of tick-borne diseases (TBDs) is exceptional among vector-borne diseases. It is estimated that tick species exceed 850 and inhabit every continent (Dennis and Piesman 2005, Benoit *et al;* 2007). Their resilience and persistence in the environment can be traced back in the fossil record, which suggests that they originated 65-146 million years ago (Fuente 2003). Ticks have very little mobility, but they may be transported over long distances by their vertebrate hosts during feeding, in particular, bird hosts may efficiently transport ticks across geographical barriers (Poupon *et al;* 2006; Hasle *et al;* 2009,) and spread tickborne pathogens. Birds, especially ground-feeding species, are at risk of tick infestation and are considered important in the global dispersal of ticks and tick-borne pathogens through their migration within and between continents (Olsen *et al;* 1995, Comstedt *et al;* 2006).

Ticks generate a neurotoxin and they can host bacterial, viral, and protozoan pathogens as well, a greater variety of disease agents than any other arthropod vector (Sonnenshine 1991). Among tickborne bacteria, extracellular spirochetes of the genus Borrelia are widely spread and most studied. Some of these, those that belong to the *Borrelia burgdorferi* sensulato complex, are causative agents of Lyme borreliosis (Parola and Raoult 2001; Korenberg *et al;* 2002).

Because of the wide range of transmitted pathogens, ticks are of considerable medical and veterinary interest worldwide. Emerging tick transmitted diseases like babesiosis, anaplasmosis, ehrlichiosis and rickettsiosis among others have drawn both public and scientific attention to these arthropods (Beugnet, 2002; Kenny *et al;* 2004).

Increased mobility of animals and the ability of ticks to find niches in new climatic conditions have resulted in rapid extension of the

zoogeographical ranges for many tick species (Glaser and Gothe, 1998; Shaw *et al;* 2001). The increasing number of ticks has also been associated with growing accessibility of natural environments and an increase in the population of wild host species (deer, small mammals and foxes) that now have a closer association with human activity. For example, *Rhipicephalus microplus* has extended its range in South America to South Africa and in about 10 years ago has spread to other West African countries. It has been reported that importation of *Rhipicephalusmicroplus* into new regions poses higher risk for bovine babesiosis (Cumming *and* Guegan 2006, Akande and Fagbemi, 2018).

Ticks are the primary vector for several transmissible pathogens, transmitting more pathogens than any other arthropod vector group with an estimated 10% of all tick species being a vector. Ticks are second only to mosquitoes for vectoring human diseases. Many of the diseases transmitted by ticks are of huge medical and veterinary importance (Jongejan and Uilenberg 2004, Bernette 1995, Pietzsch 2005)**.**

There has been a notable increase in the prevalence of many tick-borne diseases; as a result, ticks are of paramount importance globally, due to the implications on both human and livestock health (Parola and Didier 2005, Randolph 2001)**.** Because of these important roles of ticks and their effects on livestock and human, their presence in the environment and the attendant pathogens in them cannot be overemphasised considering the danger their presence portends and the possibility of continuous transmission of diseases once they are established in the environment, thus the study was done to harvest ticks in the study area usingcattle grazing areas and human routes, to screen the harvested ticks for zoonotic pathogens so as to create awareness on the danger of tick and tick-borne diseases in Nigeria in particular and Africa at large.

MATERIALS AND METHODS

Study areas

The study was conducted in Abeokuta, Ogun state. South-West Nigeria. Ten areas where cattle are being grazed and humans usually pass were used for this study.

Sampling techniques

Questing ticks from the grazing areas were collected by cloth dragging method after each dragging of about 10 metres, the1m^2flannel for collection was put on a flat surface where the ticks were picked with the aid of a forceps (Gilbert et al; 2001).The ticks that attached to the flannel were put into a container with perforated lids this is then transported to the Parasitology laboratory of the Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture. Abeokuta. Ogun state, Nigeria. The ticks were sorted and cleaned after which they were identified and stored in 70% ethanol and were kept at +4^{0}C until DNA extraction.

DNA extraction, storage and analysis

Collected ticks were identified under the stereo-microscope using the keys of Walker *et al;* (2003) and Soulsby (1982); the ticks from grazing areas (questing ticks) were grouped based on the stage of their life-cycle into larvae, nymph and adults. Each identified tick was kept in a separate safe lock tubes for molecular analysis after identification to genus level. DNA was extracted using the InviMag DNA Tissue kit (Qiagen, Germany) according to manufacturer's instructions.

About 100 µl DNA was gotten from the extraction for each tick. These were dispensed into five clear PCR plates for analysis and three clear PCR plates as stock to be stored each contain 5 µl and 25 µl of DNA respectively. Plates were stored at -80^{0}C until further analysis.

PRIMER SETS: The following primer sets were used for pathogens assay (TABLE 1)

Table 1: Primer lists with annealing temperature

Pathogen	Forward or Reverse	Primer	Targeted gene	Annealing Temp (°C)	Type of PCR	Amplicon size	Reference
Anaplasma	F	EHR1	*16SrRNA*	63	Heminested	711bp	Rar*et al;* 2005
	R	new EHR2	*16SrRNA*				
Babesia	F	BJ1	*18SrRNA*	56	Simple	476-520bp	Casati 2006
	R	BN2	*18SrRNA*				
Borrelia	F	newLDf	*16SrRNA*	61	Simple	357bp	Marconi and Garon1992 (Modified)
	R	newLDr	*16SrRNA*				
Coxiella	F	Q5 &Q6	*htpB*	58	Nested	325bp	To *et al;* 1996
	R	Q3&Q4	*htpB*				
Rickettsia	F	Rr17k.1p	*17-KDa*	72	Heminested	539bp	Ishikura*et al;* 2002
	R	Rr17k.539 n	*17-KDa*				

Data analysis

Frequency distribution and percentages were used to present the data gotten while chi square was used for bivariate analysis to find association between ticks stages and pathogen found in them

RESULTS

Table 2: Tick borne pathogen distribution in the harvested questing ticks

PATHOGENS	FREQUENCY (%)
Anaplasma	28 (7.84)
Coxiella	29 (8.12)
Coxiella + Anaplasma	7 (1.96)
Coxiella + Rickettsia	2 (0.56)
Rickettsia	8 (2.25)
TOTAL	74 (20.73)

DISCUSSIONS AND CONCLUSIONS

It has been suggested that theileriosis, Babesiosis, Anaplasmosis and heartwater which are the disease caused by some of the pathogens isolated in this work are most important tick-borne diseases (TBDs) of livestock in sub-Saharan Africa resulting in extensive economic losses to farmers in endemic area (Eygelaar *et al.,* 2015). Thus, there is need for better control programme for this TBDs because of their importance and their classification as emerging zoonosis especially Anaplasmosis and Babesiosis in Humans.

The zoonotic implication of the pathogens harvested cannot be over emphasized as some authors have suggested that the presence of pathogens like *Coxiella burnetii* poses a great threat to animals and human in the areas where they are found (Mediannikov *et al.,* 2010; Kanga-waladjo *et al.,* 2010).*Coxiella burnetii* has recently been declared a source of bioterrorism (Madariaga *et al*, 2003), It is an occupational hazard for veterinarians, animal handlers and their close associate. It is a known cause of abortion and fetal waste in ruminant production. It has also been reported an emerging vector borne pathogen with high zoonotic potential (Shannon et al 2005, Rodolakis *et al;* 2007, Hazlet *et al;* 2013)

Questing ticks have been said to pose a great risk of infection to human and animals in the areas where they are found as they help maintain pathogens in the environment and at times help in sustaining the pathogens through various developmental stages till, they climb on a susceptible host who they can infect with pathogen in them. The high prevalence of pathogen prevalence observed in the questing

ticks poses a potential danger for continuous transmission and maintenance of pathogens in the study areas. (Childs and Paddock, 2003)

Co infection observed in the ticks might be due to a single blood meal on cattle that are carrying more than one pathogen or by transfer of blood between co- feeding ticks (Piesman and Happ, 2001).

Having pathogens from different genera in the questing ticks harvested from this study is a pointer to concept of Parasite Globalization, which affirms that parasite do not have boundary, daily movement of humans, animals and commodities along national and/or international trade routes and boundaries can help in their establishment in areas where they were not known before (De Deken *et al.,* 2007)**.**

Co-infection has seen in this work calls for serious attention as it portends danger, because of its requirement in terms of treatment which must be done regularly with attendant cost implications and resultant development of resistance to drug and acaricide usage. It has also been reported that coinfection produces severe symptoms in some group of animals which might also call for a lot of spending on herd maintenance and treatment (Suskwat *et al.,* 2001, Gal *et al.,* 2007), thus coinfection has seen in this study is a source of worry because the livestock and human populations are at risk of having more than one pathogen at a time and the cost of treatment which might require more than a group of drug at times.

This investigation has shown presence of emerging and re-emerging tick-borne pathogens in the environment studied. Various species of ticks found in the study can transmit one pathogen or the other to any host they invade, either livestock or human beings. The demonstration of some important tick-borne pathogens from the questing ticks are pointers to the fact that these pathogens can be of zoonotic and of public health importance which put available host at risk of continuous infection if not controlled.

Increasing movement of commodities, animals and humans aids in dissemination of arthropods especially ticks and by extension the pathogens they vectored. This makes transmission of ticks and tick-borne pathogens very easy and a serious problem to combat in Nigeria and other African countries nowadays. High prevalence of pathogens in the harvested questing ticks are indicators of the relatively hazardous environment we are living in which makes active prevention a needed tool to combat continuous spread.

Isolates that were gotten from this work are not indigenous or peculiar to Nigeria thus parasite globalization is a reality. Scientists and relevant stakeholders must be ready to wage war against this because parasites no longer have barrier or boundary. The one-health approach must be encouraged among veterinarian and other health and livestock workers bearing in mind the importance of zoonoses. Veterinarians, farmers, farm workers, visitors to the farm and their relatives are at the risk of being bitten and infected with any of this ticks and pathogens thus exposing people to occupational hazards which may be zoonotic and of public health importance. With the difficulty associated with control of these ticks there is need for development of strict prevention and control methods to prevent their further spread in the country and the neighbouring countries.

Ticks are a source of worry to be combatted early in all African nations especially the tropics.

REFERENCES

Bennett CE. Ticks and Lyme disease, *Adv Parasitology* , 1995, vol. 36 (pg. 343-405) https://doi.org/10.1016/S0065-308X(08)60494-7

Benoit, J.B., J. A. Yoder, G. Lopez-Martinez, M. A. Elnitsky, R. E. Lee and D. L. Denlinger. 2007. Habitat requirements of the seabird tick, Ixodes uriae (Acari: Ixodidae), from the Antarctic Peninsula in relation to water balance characteristics of eggs, nonfed and engorged stages. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 177(2): 205-215, DOI: 10. 1007 /s00360-006-0122-7.

Beugnet, F. (ed.) 2002. Guide to major vector-borne diseases. Merial S.A.S., 203 pp.

Busby, A. T., Ayllón, N., Kocan, K. M., Blouin, E. F., de la Fuente, G., Galindo, R. C., Villar, M., and de la Fuente, J. (2012). Expression of heat-shock proteins and subolesin affects stress

responses, *Anaplasmaphagocytophilum* infection and questing behavior in the tick, *Ixodes scapularis*. *Med. Vet. Entomology.* 26, 92–102.

Casati S, Sager H, Gern L, Piffaretti JC. 2006. Presence of potentially pathogenic Babesia sp. for human in Ixodes ricinus in Switzerland. Ann. Agric. Environ. Med. 13:65–70.

Chen, S.M., Dumler, J.S., Bakken, J.S., Walker, D.H., 1994. Identification of a granulocytotropicEhrlichia species as the etiologic agent of human disease. J. Clin. Microbiol. 32, 589–595.

Childs, J.E. and Paddock, C.D. (2003): The ascendancy of *Amblyommaamericanum* as a vector of pathogens affecting humans in the United States. Ann Rev Entomol, 48: 307-337.

Comstedt P, bergstrom S, olsen b, garpmo U, Marjavaaral, Mejlon H, barbourag, bunikisj. Migratory Passerine birds as Reservoirs of lyme borreliosis in Europe. Emerg infect Dis 2006; 12(7): 1087–95.

Cumming G.S. and Guegan J.F. 2006. Food webs and disease: is pathogen diversity limited by vector diversity? Eco Health3:163–170

de la Fuente, J., and Kocan, K. M. (2006). Strategies for development of vaccines for control of ixodid tick species. *Parasite Immunol.* 28, 275–283.

Dennis, D.T. and J.E. Piesman. 2005. Overview of tick-borneinfections of humans. In tick-borne diseases of humans, Jesse L. Goodman, David T appen Dennis, and DanielE. Sonenshine, 3-11. ASM Press.

Eygelaar D, Jori F, Mokopasetso M, Sibeko KP, Collins NE,et al. (2015) Tick-borne haemoparasites in African buffalo (*Synceruscaffer*) from two wildlife areas in Northern Botswana. Parasite and Vectors 8:1-11.

Fuente, J.D.L.2003. The fossil record and the origin of ticks (Acari: Parasitiformes: Ixodida). Vector-borne and Zoonotic Diseases 29: 331-344.

Gal A, *et al;*.2007. Coinfection with multiple tick-borne and intestinal parasites in a 6-week-old dog. Can. Vet. J.48:619– 622**.**

Gilbert, L., Norman, R., Laurenson, K. M., Reid, H. W. and Hudson, P. J. (2001). Disease persistence and apparent competition in a three-host community: an empirical and analytical study of large-scale wild populations. Journal of Animal Ecology 70, 1053–1061.

Glaser, B., Gothe, R. 1998. Hundeturismus und -import: Eine Umfrage in Deutschland zuAusmaßsowie Spektrum und Präferenz der Aufenthalts-, bzw. Herkunftsländer. Tierärztl. Prax. 26, 197- 202.

Hasle G, Horak IG, Grieve G, Leinaas HP, Clarke F. Ticks collected from birds in northern South africa, 2004–2006. Onderstepoort Journal of Vet 2009; 76: 167–75.

Hazlett, M.J., Mcdowall, R., Delay, J., Mcewen, B., Dreumel, T., Van Spinato, M., Slavic, D., Carman, S., Cai, H.Y., 2013. A prospective study of sheep and goat abortion using real-time polymerase chain reaction and cut point estimation shows Coxiella burnetii and Chlamydophila abortus infection concurrently with other major pathogens. J. Vet. Diagnostic Investig. 25, 359–368**.**

Homer MJ, Aguilar-Delfin I, Telford SR, Krause PJ, Persing DH. Babesiosis. Clin Microbiol Rev. 2000;13: 451–69**.**

Ishikura MS, Ando S, Shinagawa Y, Matsuura K, Hasegawa S, Nakayama T, Fujita H, Watanabe M. Phylogenetic analysis of spotted fever group rickettsiae based on GltA, 17-kDA, and rOmpA genes amplified by nested PCR from ticks in Japan. Microbiol Immunol 2003; 47:823-832.

Jongejan F, Uilenberg G. The global importance of ticks, *Parasitology*, 2004, vol. 129 (pg. S3 - S14)https://doi.org/10.1017/S0031182004005967**.**

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology. 129, Suppl, 3-14**.**

Kamga-Waladjo AR, *et al;*.2010. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop. Anim. Health Prod.42:953–959.

Kenny, M.J., Shaw, S.E., Hillyard, P.D., Forbes, A.B. 2004b. Ectoparasite and haemoparasite risks associated with imported exotic reptiles. Vet. Rec. 154, 434-435.

Korenberg EI, Kovalevsky YV, Gorelova NB. Ecology of Borrelia burgdorferi sensulato in Russia. In: Gray G, Kahl O, Lane RS, editors. Lyme borreliosis epidemiology and control. Oxford (UK): CAB International; 2002. p. 175–200.

Lawal-Adebowale O.A (2012) Dynamic of Ruminant Livestock Management in the Context of Nigerian Agricultural system. Livestock Production, Dr. Khalid Javed (Ed.), ISBN: 978-953-51-0814-6, InTech, DOI: 10.5772/52923. http://www.intechopen.com/book/livetock-production/dynamics-of-ruminant-livestock-management-in-the-context-of-the-nigerian-agricultural-system.

Madariaga, M. G., Rezai, K., Trenholme, G. M., and Weinstein, R. A. (2003). Q fever: a biological weapon in your backyard. Lancet Infect. Dis. 3, 709–721. doi: 10.1016/S1473-3099(03)00804-1

Marconi, R. T., L. Lubke, W. Hauglum, and C. F. Garon. 1992. Species-specific identification of and distinction between Borrelia burgdorfen genomic groups by using 16S rRNA-directed oligonucleotide probes. J. Clin. Microbiol. 30:628-632.

Mediannikov O, *et al;*. 2010. *Coxiella burnetii*in humans and ticks in rural Senegal. PLoSNegl. Trop. Dis. 4: e654.

Olsen B, JaensonTgT, bergstrom S. Prevalence of borrelia burgdorferi sensulato-infected ticks on migrating birds. Applied Environmental Microbiology 1995; 61(8): 3082–7.

Parola P, Didier R. Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat, *Clin Infect Dis*, 2001, vol. 32 pg. 897-928https://doi.org/10.1086/319347.

Piesman, J., Happ, C.M., 2001. The efficacy of co-feeding as a means of maintaining Borrelia burgdorferi: a North American model system. Journal of Vector Ecology 26, 216–220.

Pietzsch ME, Medlock JM, Jones L, et al. Distribution of *Ixodes ricinus* in the British Isles: investigation of historical records, *Med Vet Entomol* , 2005, vol. 19 (pg. 306-314)https://doi.org/10.1111/j.1365-2915.2005.00575.x.

Poupon Ma, lommano E, Humair PF, Douet V, Raiso, Schaad M, jennil, gernl. Prevalence of borrelia burgdorferi sensulato in ticks collected from migratory birds in Switzerland. Appl. Environ Microbiol 2006; 72: 976–9.

Randolph SE. The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe, *Philos Trans R Soc Lon B*, 2001, vol. 356 (pg. 1045-1056)https://doi.org/10.1098/rstb.2001.0893

Rar VA, Fomenko NV, Dobrotvorsky AK, Livanova NN, Rudakova SA, Fedorov EG, et al. Tickborne Pathogen Detection, Western Siberia, Russia. Emerg Infect Dis. 2005; 11(11):1708-1715. https://dx.doi.org/10.3201/eid1111.041195

Rodolakis, A; Berri, M; Hechard, C; Caudron, C; Souriau, A; Bodier, CC; Blanchard, B; Camuset, P; Devillechaise, P; Natorp, JC; Vadet, JP and ArricauBouvery, N (2007). Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci., 90: 5352-5360.

Shannon, J. G., D. Howe, and R. A. Heinzen. 2005. Virulent *Coxiella burnetii* does not activate human 031182000058388.

dendritic cells: role of lipopolysaccharide as a shielding molecule. Proc. Natl. Acad. Sci. U. S. A.102:8722-8727.

Shaw, S.E., Day, M.J., Birtles, R.J., Breitschwerdt, E.B. 2001. Tick-borne infectious diseases of dogs. Trends in Parasitology. 17, 74-80.

Sonenshine, D. E., 1991. Biology of ticks Vol 1. Oxford University Press, New York, 13-64.

Suksawat J, *et al;* 2001. Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand. J. Vet. Intern. Med.15:453–462.

To, H., Kato, N., Zhang, G. Q., Otuka, H., Ogawa, M., Ochiai, O, et al (1996). Q fever pneumonia in children in Japan. J Clin Microbiol 34, 647–651.

Uilenberg G. (1992). -Veterinary significance of ticks and tick-borne diseases. In Tick vector biology. Medical and Veterinary aspects (B .Fivaz, T.Petney& I. Horak , eds) . Springer-Verlag, Berlin, 23-33.

Uilenberg G: International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Vet Parasitol. 1995, 57: 19-41. 10.1016/0304-4017(94)03107-8.

Vail, S. C., and Smith, G. J. (2002). Vertical movement and posture of blacklegged tick (Acari: Ixodidae) nymphs as a function of temperature and relative humidity in laboratory experiments. *J. Med. Entomol.* 39, 842–846.

Walker A.R., Bouattour A., Camicas J.L., Estrada-Peña A., Horak I. G., Latif A.A., Pegram R. G. and Preston P.M. 2003. Ticks of Domestic Animals in Africa: a Guide to Identification of Species. Edinburgh, Scotland, U.K.: Bioscience Reports.

Young AS, Groocock CM, Kariuki DP: Integrated control of ticks and tick-borne diseases of cattle in Africa. Parasitology. 1988, 96: 403-432. 10.1017/S0