UDC 616.9:579.834:616.15-074:612.017.1 https://doi.org/10.15407/biotech17.01.062

RELATIONSHIP BETWEEN THE SPECTROMETRIC VALUES OF DNA, RNA, AND THE PCR PRESENCE OF A PATHOGEN IN SINGLE TICK SAMPLES

Gudrun Baersch
Sudhir Bhatia

Genekam Biotechnology AG, Duisburg, Germany

E-mail: anfrage@genekam.de

Received 2023/11/15
Revised 2024/01/16
Accepted 2024/02/29

Aim. Ticks are the vectors of many pathogens, which cause diseases with fatal consequences. Polymerase chain reaction (PCR) was used to detect the presence of these different pathogens in ticks, but there is a need of isolated nucleic acid to conduct the molecular assays. In our previous research, we found that some ticks give huge yield of isolated nucleic acid during spectrometric measurements, therefore aim of this study is to find whether there is any relation between spectrometric values of DNA, RNA and presence of *Borrelia burgdorferi* as example pathogen in single tick samples.

Method. DNA and RNA were isolated with mini column method from single tick samples. They were run in real time as well as conventional PCR tests for the presence of *Borrelia burgdorferi*. The nucleic acid yields of isolated nucleic acid samples were measured with a spectrophotometer.

Results. It was found that there were 47 ticks positive for *Borrelia burgdorferi* and 40 were negative. Average isolated DNA and RNA quantity was higher in pathogen positive ticks than those of negative ticks. There was no correlation between the yield of nucleic acid and presence of pathogen in a single tick, but there was tendency that pathogen positive tick gave higher yield of DNA and RNA during the isolation.

Conclusions. This study shows some of *Borrelia burgdorferi* positive ticks give very high yield of DNA and RNA during the isolation. There is no correlation between presence of pathogen and nucleic acid in a single tick, but there is tendency that pathogen positive tick may have higher nucleic acid yield. Therefore, our recommendation is that laboratory should always measure the nucleic acid yield along with conducting the PCR tests.

Key words: *Borrelia burgdorferi*, tick, nucleic acids, polymerase chain reaction.

Ticks are the vectors of a wide range of pathogens, which cause a number of fatal diseases in human beings. Therefore, it is essential to monitor such pathogens. Polymerase chain reaction (PCR) is one of the important methods being used widely for detection of various microbes today [1–4]. It makes possible to detect them in ticks. There are many publications about the detection of pathogens in ticks, but many research groups are using different methods to isolate nucleic acid from ticks, most commonly the pooled tick samples [5–14].

Our group has developed a mechanical crushing method to isolate the DNA/RNA from a single tick. During the publication of this method, we found that there may be some correlation between the presence of high yield of DNA/RNA in a single tick with the presence of a pathogen in it [1]. In this work, we used *Borrelia burgdorferi* as an example. In the literature, there are rare reports of spectrometric values of a larger number of nucleic acid samples in the ticks as most of groups are using pooled samples to detect the presence of pathogens in ticks [5, 15]. It is very important to find the presence of pathogens in a single tick to develop the accurate preventive and therapeutic strategy in a particular area. We have published a simple and inexpensive

method to isolate the nucleic acid from a single tick, which may open new opportunities [1].

Therefore, in this research work, we decided to conduct further studies to find whether there is any correlation between the spectrometric values of isolated DNA / RNA and PCR presence of *B. burgdorferi* in single tick samples.

Materials and Methods

The ticks were sent with a letter at room temperature with German postal service. DNA and RNA were isolated with mini column isolation kit. The mechanical crushing method was used and described in other publication fully [1].

The real time and conventional PCR kits (Genekam, Germany) were used to detect the presence of *B. burgdorferi* in the isolated nucleic acid samples. The machine used for real time PCR was ABI 7500 (Thermo Fischer Scientific, USA) and the results were read at Ct values along with presence of the curves. Conventional PCR was conducted in thermocycler (Biometra, Germany) and the results were seen as band in the gel agarose stained with ethidium bromide. Positive and negative samples were used [1].

The yield of isolated DNA and RNA from each single tick was measured in Nanodrop (Thermo Fischer Scientific, USA). The spectrometer was calibrated with elution buffer of the nucleic acid isolation kit. After that 3 values per sample (single tick) were measured and the average of these values was calculated. These average value of all *B. burgdorferi* positive and negative samples are shown in Table 1 and 2.

Total number of 47 *B. burgdorferi* positive single ticks were used for measuring the nucleic yield. The average of isolated DNA and RNA from these 47 ticks was 80.27 ng/µl and 60.42 ng/µl per single tick respectively. These average values show that yield of DNA per tick is more than that of RNA. The DNA yield per sample varies from minimum 0.2 ng/µl to maximum 856.7 ng/µl, hence there is a wide variation of isolated DNA yield between the single tick samples. Similarly, there was a broader variation range of RNA yield, where the minimum value varies from −0.15 to maximum value 591.3 ng/µl. These values were calculated with the use of MS Excel.

Total number of 40 *B. burgdorferi* negative single ticks were used for measuring the nucleic acid outputs. It is found that average DNA yield each tick was 57.11 ng/µl, where the average yield per tick for isolated RNA was 41.52 ng/µl. These results show that DNA yield in *B. burgdorferi* negative samples was lower than that of RNA. These results have similar pattern as with those of *B.b.* positive samples. The DNA yield varies 0 to 646.5 ng/µl. One sample, which was *B. burgdorferi* negative, but it was positive for tick borne encephalitis virus (data not shown). This sample has DNA yield 2.0 ng/µl and RNA yield was 1.3 ng/µl. RNA yield varies between from minimum 0.3 to maximum 296.1 ng/µl.

Three different groups depending upon the yield of isolated nucleic acid were created to find the percentage range among these groups. These were 0–20 ng/µl, 20–200 ng/µl and more than 200 ng/µl. The number of samples with more than 200 ng/µl DNA among positive tick were 5/47 total = 10.64% against those with more than 20 ng/µl DNA up to 200 ng/µl DNA yield were 20, which is 40.43%. The highest percentage was found in the group lower than 20 ng/µl. (Fig. 1) It was found that 68.18% negative ticks are under 20 ng/µl (Fig. 2). These figures were generated with the use of MS Excel.

Number of ticks with higher DNA and RNA yield are more in *B. burgdorferi* positive ticks against the number of *B. burgdorferi* negative tick. This can be expressed as percentage and the probability, hence there is tendency that higher yield of DNA and RNA are in *B. burgdorferi* positive samples. There is no true co relation between the nucleic acid yields in *B. burgdorferi* positive and negative samples.

Results and Discussion

In this research work, we analyzed total 87 single tick samples to find a relation between spectrometric values of isolated DNA, RNA and *B. burgdorferi* presence in single tick samples. In our previous publication [1], where we found that there was hardly to find publication about studies for the total yield of DNA and RNA from one single tick. In this publication, we thought that the highly DNA yield from one single tick should be corelated with presence of the *B. burgdorferi*. However, our results of this research work do not support fully this idea, whereas the results are supporting that there is a tendency that higher DNA and RNA yields from single tick may be an indication of presence of the pathogen in a tick. Therefore, it is essential that it should to measure nucleic

Table 1

The spectrometric values of DNA and RNA yield of different pathogen positive single ticks

Tick No.	DNA, ng/ml	RNA, ng/ml
1	856.7	591.3
2	556.8	303.3
3	292.8	312.0
4	245.3	68.5
5	212.5	170.9
6	194.3	189.4
7	188.2	155.5
8	184.3	152.1
9	110.6	89.1
10	97.4	76.9
11	97.2	94.8
12	83.3	62.0
13	72.6	59.9
14	66.5	52.1
15	62.4	50.6
16	51.6	30.2
17	41.7	37.7
18	41.1	25.2
19	29.5	16.0
20	26.7	23.1
21	26.1	20.8
22	24.7	20.2
23	20.3	16.6
24	20.2	12.1
25	19.1	15.6
26	19.0	44.0
27	17.5	9.7
28	17.2	12.7
29	15.5	13.5
30	13.8	12.4
31	13.3	6.0
32	12.6	9.4
33	10.7	8.6
34	9.5	7.2
35	5.1	4.7
36	5.1	5.6
37	4.9	4.3
38	4.4	6.3
39	4.1	3.0
40	3.7	1.2
41	3.6	4.0
42	2.1	2.0
43	2.0	2.2
44	1.7	2.1
45	1.2	1.0
46	0.2	−0.15
47	−16.2	34.3

Table 2

The spectrometric values of DNA and RNA yields of different *Borrelia burgdorferi* negative single ticks

Tick No.	DNA, ng/ml	RNA, ng/ml
1	1.2	1.0
2	17.2	12.7
3	8.15	5.3
4	3.2	2.3
5	54.2	57.9
6	83.2	37.4
7	50.3	77.4
8	58.3	108.5
9	283.2	149.9
10	79.8	59.6
11	6.3	16.3
12	7.1	9.2
13	646.5	367.1
14	1.1	0.7
15	7.5	5.0
16	13.0	12.1
17	5.7	4.4
18	2.0	1.3
19	157.5	68.6
20	65.4	50.8
21	8.8	7.9
22	0.7	0.6
23	2.5	0.8
24	5.4	3.7
25	4.4	3.5
26	2.3	2.5
27	3.0	2.9
28	3.6	3.9
29	33.8	20.7
30	5.1	5.1
31	7.2	4.5
32	355.8	296.1
33	340.4	271.1
34	20.0	14.8
35	16.2	11.9
36	4.8	3.3
37	0	-0.3
38	57.9	48.0
39	12.5	9.3
40	3.6	3.7
41	1.0	0.6
42	14.4	11.5
43	53.3	47.4
44	5.5	5.7

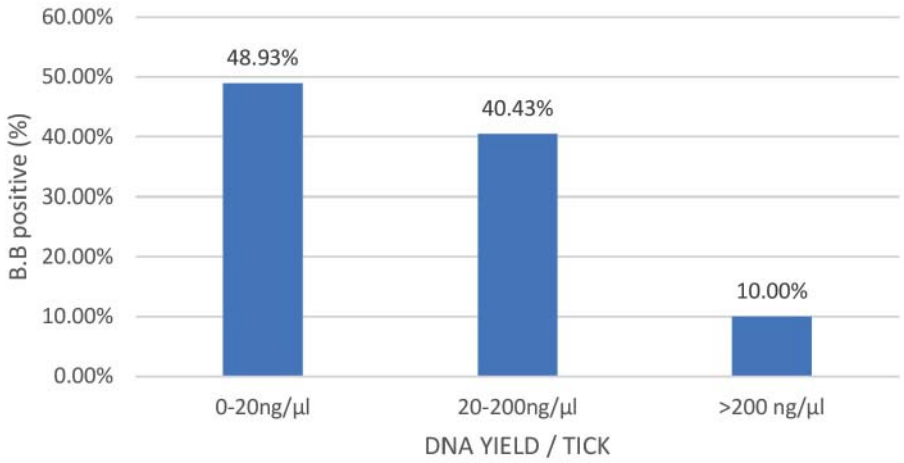

Fig. 1. **Percentage of different DNA yields of *Borrelia burgdorferi* positive ticks**

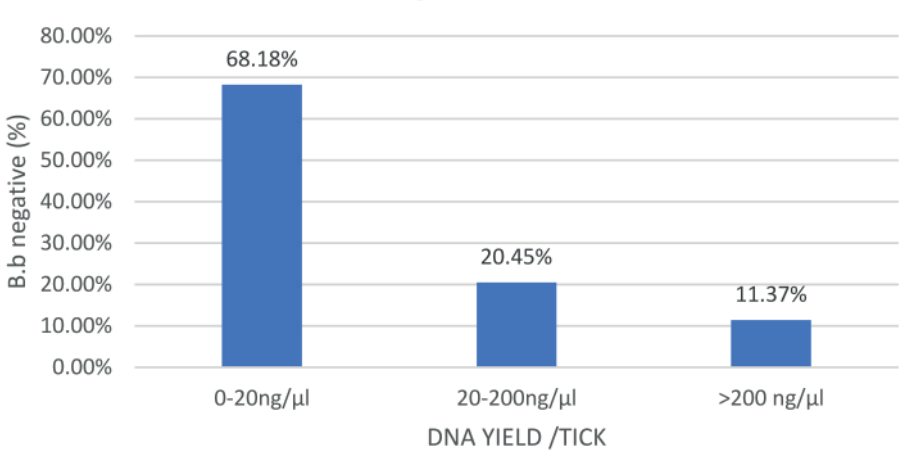

Fig. 2. **Percentage of different DNA yields of *Borrelia burgdorferi* negative ticks**

acid yield from each tick. At present, most of research groups are using pooled sample, but these groups can use our single tick isolation method as standard, so the results can be compared easily.

In the literature, there are a few reports, where the spectrophotometric values are measured from ticks. In one report, there are yield of isolated DNA from pooled tick, which was 2000 ng/µl as this is very high yield from pooled ticks. Such high yield may lead to false results in PCR as very high concentration of nucleic acid can lead to failure of PCR.

The results of this publication are showing also that it is essential to measure the yield of isolated nucleic acid so that user can use optimal concentration of DNA/RNA during the PCR analysis because too high concentration may lead to questionable results.

One comparison between the mRNA vaccine against the coronavirus and concentration of *B. burgdorferi* positive tick DNA, a vaccine dose contains 50 µg in 500 µl of solution (100 ng/µl), but a tick has up to 856 ng/µl and many ticks have more than 200 ng/µl DNA in this study. It shows that some ticks contain many times more nucleic acid than the amount being used as vaccine today. There is urgent need of more research about the role of such high dose inoculated in the persons from the tick bite and how such doses effect the clinical outcome. There are many laboratories, which are publishing the research about the presence of pathogens in ticks as well as patients suffering from these infections. These should measure the spectrometric yields of isolated nucleic acid of such ticks to contribute for the better scientific and clinical understanding. These values may help to generate better vaccines for tick borne pathogens, but more research work is needed here.

In these studies, we used only one pathogen (*B. burgdorferi*), but this work shows that one has to conduct the further studies about the presence of other pathogens in order to have better overview.

This publication shows the importance of spectrometric values of nucleic acid yields from the ticks and some correlation or tendency with the presence of pathogens in a tick. It is highly recommended for other research workers to measure such values and conduct further studies of molecular analysis to find such correlations. The result report of tick analysis should also contain the yield of isolated nucleic acid along with the molecular detection of the presence of pathogens.

Conclusion

In this research work, we established for the first timea correlation between the spectrometric value of nucleic acid (DNA and RNA) of a single tick and presence of the pathogens. This indicates that there is a tendency of presence of pathogen in high nucleic acid yielding ticks, but the studies were unable to establish a real correlation between the higher nucleic acid yield and presence of *Borrelia burgdorferi* in single tick. It is highly recommended to measure the spectrometric values of isolated nucleic acid along with molecular detection of pathogens, hence both parameters should be the parts of tick analysis report. Such parameters may lead to develop better understanding the clinical outcome of tick-borne infections as well as control of these tick infections.

Funding
There was no external funding.

Conflict
There was no conflict of interests.

Acknowledgement
The authors would like to thank Kornelia Niklis for assistance for laboratory work and Mr Helge Schreiner for collection of samples, calculations and graphics.

REFERENCES

1. *Bhatia S., Baersch G.* A simple, effective and inexpensive method to isolate the nucleic acid (DNA/RNA) from a single tick for molecular detection of various pathogens. *Eur Res J.* 2023, 10(1):1–7. https://doi.org/10.18621/eurj.1315058
2. *Muller M. G., George A. R., Walochnik J.* Acinetobacter baumannii in Localised Cutaneous Mycobacteriosis in Falcons. *Vet Med Int* 2010, Published online 2010 Sep 5. https://doi.org/10.4061/2010/321797
3. *Bhatia S.* Pitfalls found in SARS CoV-2 specific test performance during the comparison between WHO recommended method and a commercial test. *Atlantic J Med Sci Res.* 2023, 3(1):22–26. https//doi.org/10.5455/atjmed.2022.12.024
4. *Liu Y.C., Ohyashiki J.H, Ito Y., Iwaya K., Serizawa H., Mukai K, Goto H., Usui M, Ohyashiki K.* Chlamydia psittaci in ocular adnexal lymphoma: Japanese experience. *Leukemia Research*. 2006, 30(12):1587–1589. https://doi.org/10.1016/j.leukres.2006.01.015
5. *Reifenberger G.C., Thomas B.A., Rhodes D.V.L.* Comparison of DNA Extraction and Amplification Techniques for Use with Engorged Hard-Bodied Ticks. *Microorganisms*. 2022, 10(6):1254. https://doi.org/10.3390/microorganisms10061254
6. *Laroche M., Weeks, E.N.I.* Vector-borne bacterial diseases: a neglected field of infectious diseases research. *Med Vet Entomol.* 2023, 37:177–178. https://doi.org/10.1111/mve.12646
7. *Harvey E., Rose K., Eden J. S., Lo N., Abeyasuriya T., Shi M., Doggett S.L., Holmes E.C.* Extensive diversity of RNA viruses in Australian ticks. *J Virol.* 2019, 93(3):e01358-18. https://doi.org/10.1128/jvi.01358-18
8. *Hubbard M.J., Cann, K.J., Wright D.J.M.* Validation and rapid extraction of nucleic acids from alcohol-preserved ticks. *Exp Appl Acarol.* 1995, 19:473–478. https://doi.org/10.1007/bf00048266
9. *Stanley H., Rhodes D.V.L.* Presence of Rickettsia Species in Ticks Collected from Companion Animals in Northeastern Georgia, United States. *Vet Sci* 2021, v.8:37. https://doi.org/10.3390/vetsci8030037
10. *Cafiso A., Chiappa G., Luzzago C., Bonato D., Koleci X., Bazzocch C.* Protocol optimization for simultaneous DNA and RNA co-extraction from single hard tick specimens. *MethodsX*. 2021, v.8:101315. https://doi.org/10.1016/j.mex.2021.101315
11. *Crowder C.D., Rounds M.A., Phillipson C.A., Picuri J.M., Matthews H.E., Halverson J.,*

Schutzer S.E., Ecker D.J., Eshoo M.W. Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. *J Med Entomol*. 2010, 47(1):89–94. https://doi.org/10.1603/033.047.0112
12. *Shan J., Jia Y., Hickenbotham P., Teulières L., Clokie M.R.J.* Combining citizen science and molecular diagnostic methods to investigate the prevalence of Borrelia burgdorferi s.l. and Borrelia miyamotoi in tick pools across Great Britain. *Front Microbiol*. 2023, 14:1126498. https://doi.org/10.3389/fmicb.2023.1126498
13. *Jones A.M., Van de Wyngaerde M.T., Machtinger E.T., Rajotte E.G., Thomas C., Baker T.C.* Choice of Laboratory Tissue Homogenizers Matters When Recovering Nucleic Acid From Medically Important Ticks- J Med Entomol 2020; 57(4):1221–1227. https://doi.org/10.1093/jme/tjaa006
14. *Day C.A., Butler R.A., Durick H.E., Chandler J.G., Paulsen D.J., Mordoh S.L., Foggin C., Parry R., Moyo I., Ruso L., Kennedy M.A., Trout Freyxell R.T.* An ecological and epidemiological single-season survey of Anaplasma and Ehrlichia positive ticks in Victoria Falls National Park, Zimbabwe. *Med Vet Entomol*. 2023, 37(2):195— 208. https://doi.org/10.1111/mve.12640
15. *Ammazzalorso A.D., Zolnik C.P., Daniels T.J., Kolokotronis S.* To beat or not to beat a tick: comparison of DNA extraction methods for ticks (Ixodes scapularis). *PeerJ*. 2015, 3:e1147. https://doi.org/10.7717%2Fpeerj.1147

ЗВ'ЯЗОК МІЖ СПЕКТРОМЕТРИЧНИМИ ЗНАЧЕННЯМИ ДНК, РНК ТА НАЯВНІСТЮ ПЛР-ПРОДУКТУ ПАТОГЕНУ В ЗРАЗКАХ КЛІЩІВ

Gudrun Baersch, Sudhir Bhatia

Genekam Biotechnology AG, Duisburg, Germany

E-mail: anfrage@genekam.de

Мета. Кліщі є векторами багатьох патогенів, які викликають хвороби з фатальними наслідками. Для виявлення наявності цих патогенів у кліщів використовується полімеразна ланцюгова реакція (ПЛР), але для проведення молекулярних аналізів потрібна ізольована нуклеїнова кислота. У наших попередніх дослідженнях ми виявили, що деякі кліщі дають великий вихід ізольованої нуклеїнової кислоти під час спектрометричних вимірювань, тому метою цього дослідження є виявлення наявності зв'язку між спектрометричними значеннями ДНК, РНК та наявністю *Borrelia burgdorferi* як прикладу патогена у зразках одиничного кліща.

Метод. ДНК та РНК були ізольовані з використанням міні-колонок зі зразків одиничного кліща. Вони були тестовані в режимі реального часу, а також звичайними ПЛР-тестами на наявність *Borrelia burgdorferi*. Вихід ізольованої нуклеїнової кислоти вимірювався спектрофотометрично.

Результати. Було виявлено, що 47 кліщів були позитивними на *Borrelia burgdorferi*, і 40 — негативними. Середня кількість ізольованої ДНК та РНК була вище у кліщів, позитивних на патоген, ніж у негативних. Не було виявлено кореляції між виходом нуклеїнової кислоти та наявністю патогена в одному кліщі, але була тенденція, що позитивні на патоген кліщі характеризуютьсяя більшим виходом ДНК та РНК.

Висновки. Дослідження демонструє, що деякі кліщі, позитивні на *Borrelia burgdorferi*, дають підвищений вихід ДНК та РНК під час ізолювання. Встановлено відсутність кореляційної залежності між наявністю патогена та нуклеїновою кислотою в одиничному кліщі, але є тенденція до того, що позитивні на патоген кліщі можуть мати вищий вихід нуклеїнової кислоти. На підставі отриманих результатів можна рекомендувати профільним лабораторіям здійснювати визначення виходу нуклеїнових кислот додатково до проведення ПЛР-тестів.

Ключові слова: *Borrelia burgdorferi*, кліщ, нуклеїнові кислоти, полімеразна ланцюгова реакція.