



Short Communication

Molecular Survey of *Campylobacter jejuni* in Broiler Chicken Farms in East Coast of Peninsular, Malaysia

Abdul Muhaimin Wahab¹, Basit Zeshan^{2,*}, Naveed Ahmed², Muhammad Afzal² and Muhammad Naveed²

¹Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, (UMK), Kota Bharu, Kelantan, Malaysia

²Department of Microbiology, Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan

ABSTRACT

Campylobacter spp. is responsible for food borne illness in humans while *Campylobacter jejuni* is the most common species for majority of human enteritis cases. The present study was conducted to determine the prevalence of *C. jejuni*, risk factors associated with the occurrence, identification control and preventive measure to reduce the prevalence in broiler chicken farms in Kelantan state located at east coast region of peninsula Malaysia. Eighty cloacal swab samples were collected from 4 different broiler chicken farms in district Tumpat, Machang and Bachok. The samples were processed for identification of *C. jejuni* followed by PCR to detect the presence of *C. jejuni*. Overall, 65% in total (80 samples) of cloacal swab samples showed positive reactions, where prevalence in farms A, B, C and D was 70%, 70%, 75% and 45%, respectively. Among, 2 of 5 identified risk factors through questionnaire showed significant difference which were open house system and untreated water source. Other risk factors which includes small scale, not using probiotic and poor biosecurity were not significant enough in association to occurrence of *C. jejuni*. Based on the risk factors that have been identified, control measure to reduce the occurrence was; by changing the open housing system into close housing system, using treated water source, use of probiotics, implementing strict biosecurity and good hygiene practices. In conclusion, the prevalence of *C. jejuni* in broiler chicken farms in Kelantan was high. Therefore, the farmer should always aware with this public health threat by avoiding those potential risk factors that are associated with the colonization of *Campylobacter*.

Article Information

Received 02 July 2018

Revised 23 June 2019

Accepted 06 August 2019

Available online 26 August 2020

Authors' Contributions

AMW collected samples, performed lab work and drafted the initial manuscript. BZ and MN supervised the work and helped in preparation of manuscript. NA and MA prepared the figures and manuscript.

Key words

Campylobacter, Molecular survey, Chicken farm, Kelantan.

Campylobacter is one of the pathogen that causes most of the foodborne illnesses other than *Salmonella* species. It is a major contaminant in poultry meat, poultry products and the environment. A low infective dosage is enough to cause campylobacteriosis in human. According to the World Health Organisation (WHO), *Campylobacter* is 1 of 4 main global causes of diarrhoeal diseases and it is considered to be the most common bacterial cause for human gastroenteritis in the world. The high incidence of *Campylobacter* associated diarrhoea, its duration and possible complications makes it highly important from a socio-economic perspective. In developing countries, *Campylobacter* infections are frequently found in children under the age of 2 years, sometimes leads to death. The illness is characterized by acute watery or bloody diarrhea and abdominal pain. The food safety measures ensure that the food is safe to

be consumed by humans without causing illness to them. Nevertheless, human illnesses associated with the consumption of food contaminated with pathogenic organisms or toxins are frequently reported. In humans, *C. jejuni* infection usually causes acute enteritis and abdominal pain which lasts for a week or more. Even though, it can be self-limiting, other complications may arise including bacteraemia, Guillain-Barre syndrome, reactive arthritis, and abortion (Skirrow, 2000).

Campylobacter species is prevalent in poultry farms and poultry product causing major sporadic gastroenteritis in humans. This happens due to handling and consumption of undercooked poultry meat. At low infective dosage which is 500 colony forming units (CFU), is enough to cause an infection to humans. Human campylobacteriosis is manifested by acute watery or bloody diarrhoea and abdominal pain. The great majority cases have been associated with *C. jejuni* followed by *C. coli* and *C. lari*. Among these, *C. jejuni* and *C. coli* are the most common pathogens responsible for the majority of human enteritis cases.

* Corresponding author: dr.basitzeshan@ucp.edu.pk
0030-9923/2021/0001-0001 \$ 9.00/0

Copyright 2021 Zoological Society of Pakistan

The polymerase chain reaction (PCR) assays have been widely employed for identification of the pathogens owing to their sensitivity and cost effectiveness. A number of PCR assays have been described for detection of *Campylobacter* from food and fecal samples (Al Amri *et al.*, 2007; Debruyne *et al.*, 2008; Persson and Olsen, 2005; Sails *et al.*, 2003). However, there are limited studies on occurrence of *Campylobacter* in broiler chicken farms in Malaysia. Moreover, there is limited data on the occurrence and risk factors of *Campylobacter* in broiler chicken farms in Kelantan. However, with development of technology and strategies implementation, transmission of those bacterial infections to humans can be prevented. The present study was conducted to assess prevalence of *C. jejuni* in faecal samples obtained from live chicken at various farms located within Kelantan state. The PCR assay was used to detect the *C. jejuni* in the collected faecal swabs and faeces samples. The results of our study could provide useful information on the future control and prevention strategies for this important food-born pathogen.

Materials and methods

Eighty cloacal swabs were collected from 4 different broiler chicken farms (20 samples from each) at District Tumpat, Bachok and Machang, Kota Bharu. Each cloacal swab was put into Amies transport medium and was labelled with farm identity number (ID) and sample ID. The samples were transported to laboratory within 2 h after collection in ice pack to maintain temperature at 2-8°C and to avoid them from sunlight.

Cloacal swabs were mixed with 50 ml of enrichment broth to make a homogenous suspension. The mixture was incubated under microaerophilic conditions at 37°C for 3h and then further for 18h at 42°C. Subsequently, it was centrifuged passively to remove debris. One ml of supernatant obtained was further centrifuged at 10000xg for 10min. The resulting pellet was re-suspended in 100µl of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0), boiled for 10min followed by immediate cooling on ice. After centrifugation at 10000xg for 10min, a 5µl of supernatant was directly used as template in 25µl PCR reaction (Al Amri *et al.*, 2007).

The reaction mixture comprised of 1x PCR buffer, 1.0mM MgCl₂, 0.2mM dNTP mixture, 10pmol of each of the primers, 1µl of *Taq* DNA polymerase enzyme, 5µl of template DNA in 25µl of total reaction mixture. The primers used were based on *mapA* gene of *C. jejuni* (Denis *et al.*, 1999). The sequences of forward and reverse oligonucleotide primers are as follows: Forward 5'-CTATTTTATTTTGGAGTGCTTG-3' and Reverse 5'-GCTTTATTTGCCATTTGTTTATTA-3'.

The cyclic conditions for PCR were same as described by (Denis *et al.*, 1999). In brief; the thermal cyclers were set with following program: 1 cycle of 10 min at 95°C, 1 min and 30 sec at 59°C, 1 min at 72°C, and final extension steps of 10 min at 72°C will generate 589-bp DNA fragments corresponding to the *C. jejuni*. *C. jejuni* (ATCC 33560) was used as the positive controls and nuclease-free water was used as the negative control. PCR products were stained with 1% solution of GelRed stain and were visualised under UV light after gel electrophoresis using 1.2% agarose gel. Prevalence was calculated accordingly (Stevenson and EpiCentre, 2008).

$$\text{Prevalence of chicken infected with } \textit{Campylobacter} = (\text{No of chicken positive } \textit{C. jejuni} \div \text{total number of samples taken}) \times 100$$

Risk factor that may associate with occurrence of *C. jejuni* were identified through questionnaire. After the risk factor identified, the association between risk factor and disease occurrence was determined by using 2×2 table where both risk factor and disease occurrence were binary variables (yes or no).

Chi-square was used to evaluate the significance difference between the risk factors that have been identified through questionnaire. A *p*-value of <0.05 is considered as statistically significant. Microsoft Excel and SPSS statistical software were used for data analysis.

Table I.- Prevalence of *C. Jejuni* and in the cloacal swab samples from different broiler chicken farms.

	No. of cloacal swab samples taken	No. of cloacal swab samples showed positive <i>C. jejuni</i>
Farm A	20	14 (70%)
Farm B	20	14 (70%)
Farm C	20	15 (75%)
Farm D	20	9 (45%)
Total	80	52 (65%)

Results and discussion

Table I shows prevalence of *C. jejuni* in different broiler chicken farms. From a total of 80 cloacal swab samples at 4 different broiler chicken farms, 52 (65%) were positive of *C. jejuni* through PCR in which out of 20 samples in each farm. But the total positive *C. jejuni* percentage showed high incidence of disease due to broiler consumption in north eastern Kelantan. And also these results showed the visible difference between the percentages of open house and close house farming of broiler and all other conditions which were responsible for colonization of *C. jejuni* in these farms. Out of all these four farms the highest prevalence was found in farm

C (75%) which is an open house farm and the lowest prevalence was in a close house farm D (45%). A number of recent studies in Malaysia (2010 – 2015) found 51% to 96% chickens were infected with *Campylobacter* (Saleha, 2004). Besides that, the neighboring country which is Thailand also recorded high prevalence of *Campylobacter* in chicken with 85% in farm (Padungtod *et al.*, 2002). [Supplementary Figure S1](#) shows the 500-600kb PCR product of *mapA* gene in different samples from different poultry farms.

The odd occurrence *C. jejuni* was 3.09 times higher in open house system compared to close house system. There is significant difference between prevalence of *Campylobacter* in open house and close house system farms (p -value < 0.05) ([Table II](#)). While the prevalence of *C. jejuni* is 1.95 times higher in small scale farm compared to medium scale farm. There is no significant difference between prevalence of *Campylobacter* in small scale farm and medium scale farm (p -value > 0.05) ([Table II](#)). The odd occurrence *C. jejuni* was 3.09 times higher in untreated water source compared to treated water source. There is significant difference between prevalence of *Campylobacter* in farm that practices untreated water and treated water (p -value < 0.05) ([Table III](#)). While the *C. jejuni* was 1.95 times prevalent in higher poor biosecurity farm compared to fair biosecurity farm. There is no significant difference between prevalence of *Campylobacter* in poor biosecurity farm and fair biosecurity farm (p -value > 0.05) ([Table III](#)).

Other risk factor such as small scale, not using probiotic and poor biosecurity were not significant to

associate with occurrence of *C. jejuni* in farm even though that prevalence were recorded high in this study. Nevertheless, those risk factors should not be simply neglected such as using probiotic and practices good biosecurity. They are also a part of good management of farm not only to reduce *C. jejuni* but also other diseases and problems.

There are five risk factors that have been identified through the questionnaire which are open house system management, use of untreated water source, small scale farm, not using probiotic and poor biosecurity. Among five identified risk factors through questionnaire, two of them show significant value in term of risk factor that associate with occurrence of *C. jejuni* in broiler chicken farm. The two risks are significant (p -value < 0.05); open house system farm (OR = 3.09) and untreated water source (OR = 3.09). This is supported by from study conducted by (Kapperud *et al.*, 1993) in which the feeding broilers with untreated water was found to be independently associated with increased risk of *Campylobacter* colonization with OR = 3.42, p -value = 0.045 (Saleha, 2004) through her study found that 1.5% of untreated water supplies were positive for *Campylobacter*. Apart from that, chickens raised under closed-house system were not colonized by *Campylobacter* compared to those raised under open-sided house system. (Keener *et al.*, 2004) in his comprehensive review article on *Campylobacter* suggesting chlorination of poultry drinking water as one of various on-farm strategies to reduce colonization of *Campylobacter* in poultry. Therefore, giving treated water instead of untreated water to poultry can reduce the incidence of *Campylobacter*.

Table II.- Analysis of risk factors for open house v/s close house systems and small scale v/s medium scale in association to incidence of *Campylobacter jejuni*.

	Total	Positive <i>C. jejuni</i>	Incidence of <i>C. jejuni</i>	OR	χ^2	p-value
Open house	60	43	71.67%	3.09	4.6886	0.030363*
Close house	20	9	45%			
Small scale	40	29	72.5%	1.95	1.978	0.159599**
Medium scale	40	23	57.5%			

*The result is significant. **The result is not significant.

Table III.- Analysis of risk factor for poor v/s fair biosecurity and untreated water v/s treated water in association to incidence of *Campylobacter jejuni*.

	Total	Positive <i>C. jejuni</i>	Incidence of <i>C. jejuni</i>	OR	χ^2	p-value
Untreated water	60	43	71.67%	3.09	4.6886	0.030363*
Treated water	20	9	45%			
Poor biosecurity	40	29	72.5%	1.95	1.978	0.159599**
Fair biosecurity	40	23	57.5%			

*The result is significant. **The result is not significant.

Findings of present study showed that broiler chickens in farm were highly colonized by *C. jejuni*. Due to high prevalence in the farm level, it is possible to have contamination during slaughtering and processing plant. In order to prevent and reduce the contamination, it is advisable for farmer to adhere good animal husbandry practices (GAHP) and good biosecurity to ensure the level of colonization in the farm can be reduced therefore reduce the possible contamination that can occur during the post harvesting period.

Conclusion

As conclusion, broiler chickens are highly colonized with *C. jejuni* in the farm with prevalence ranging from 45% to 75%. Broiler chicken reared under open house system and given untreated water source tends to associate with high occurrence of *C. jejuni* in the farm with prevalence of 71.67% in each of both factors. Farm D was the lowest prevalence with 45% is because of the farm practices close house system and use treated water source.

Acknowledgments

We are grateful the supports of the Faculty of Veterinary Medicine, University of Malaysia Kelantan, Malaysia.

Funding

This research was funded by Faculty of Veterinary Medicine, University of Malaysia Kelantan, Malaysia.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20180702090700>

Statement of conflict of interest

Authors have declared no conflict of interest.

References

- Al Amri, A., Senok, A.C., Ismaeel, A.Y., Al-Mahmeed, A.E. and Botta, G.A., 2007. *J. med. Microbiol.*, **56**: 1350-1355. <https://doi.org/10.1099/jmm.0.47220-0>
- Debruyne, L., Samyn, E., De Brandt, E., Vandenberg, O., Heyndrickx, M. and Vandamme, P., 2008. *Res. Microbiol.*, **159**: 88-93. <https://doi.org/10.1016/j.resmic.2007.11.020>
- Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G. and Colin, P., 1999. *Lett. appl. Microbiol.*, **29**: 406-410. <https://doi.org/10.1046/j.1472-765X.1999.00658.x>
- Kapperud, G., Skjerve, E., Vik, L., Hauge, K., Lysaker, A., Aalmen, I., Ostroff, S. and Potter, M., 1993. *Epidemiol. Infect.*, **111**: 245-256. <https://doi.org/10.1017/S0950268800056958>
- Keener, K., Bashor, M., Curtis, P., Sheldon, B. and Kathariou, S., 2004. *Comp. Rev. Fd. Sci. Fd. Safe.*, **3**: 105-116. <https://doi.org/10.1111/j.1541-4337.2004.tb00060.x>
- Padungtod, P., Hanson, R., Wilson, D.L., Bell, J., Linz, J.E. and Kaneene, J.B., 2002. *J. Fd. Protect.*, **65**: 1712-1716. <https://doi.org/10.4315/0362-028X-65.11.1712>
- Persson, S. and Olsen, K.E., 2005. *J. med. Microbiol.*, **54**: 1043-1047. <https://doi.org/10.1099/jmm.0.46203-0>
- Sails, A.D., Fox, A.J., Bolton, F.J., Wareing, D.R. and Greenway, D.L., 2003. *Appl. environm. Microbiol.*, **69**: 1383-1390. <https://doi.org/10.1128/AEM.69.3.1383-1390.2003>
- Saleha, A., 2004. *Int. J. Poult. Sci.*, **3**: 129-134.
- Skirrow, M.B., 2000. Clinical aspects of campylobacter infection. In: *Campylobacter* (eds. I. Nachamkin and M. Blaser). American Society for Microbiology Press, pp. 69-88.
- Stevenson, M. and EpiCentre, I., 2008. *An introduction to veterinary epidemiology*. Massey University, New Zealand.



Supplementary Material

Short Communication: Molecular Survey of *Campylobacter jejuni* in Broiler Chicken Farms in East Coast of Peninsular, Malaysia

Abdul Muhaimin Wahab¹, Basit Zeshan^{2,*}, Naveed Ahmed², Muhammad Afzal² and Muhammad Naveed²

¹Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, (UMK), Kota Bharu, Kelantan, Malaysia

²Department of Microbiology, Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan

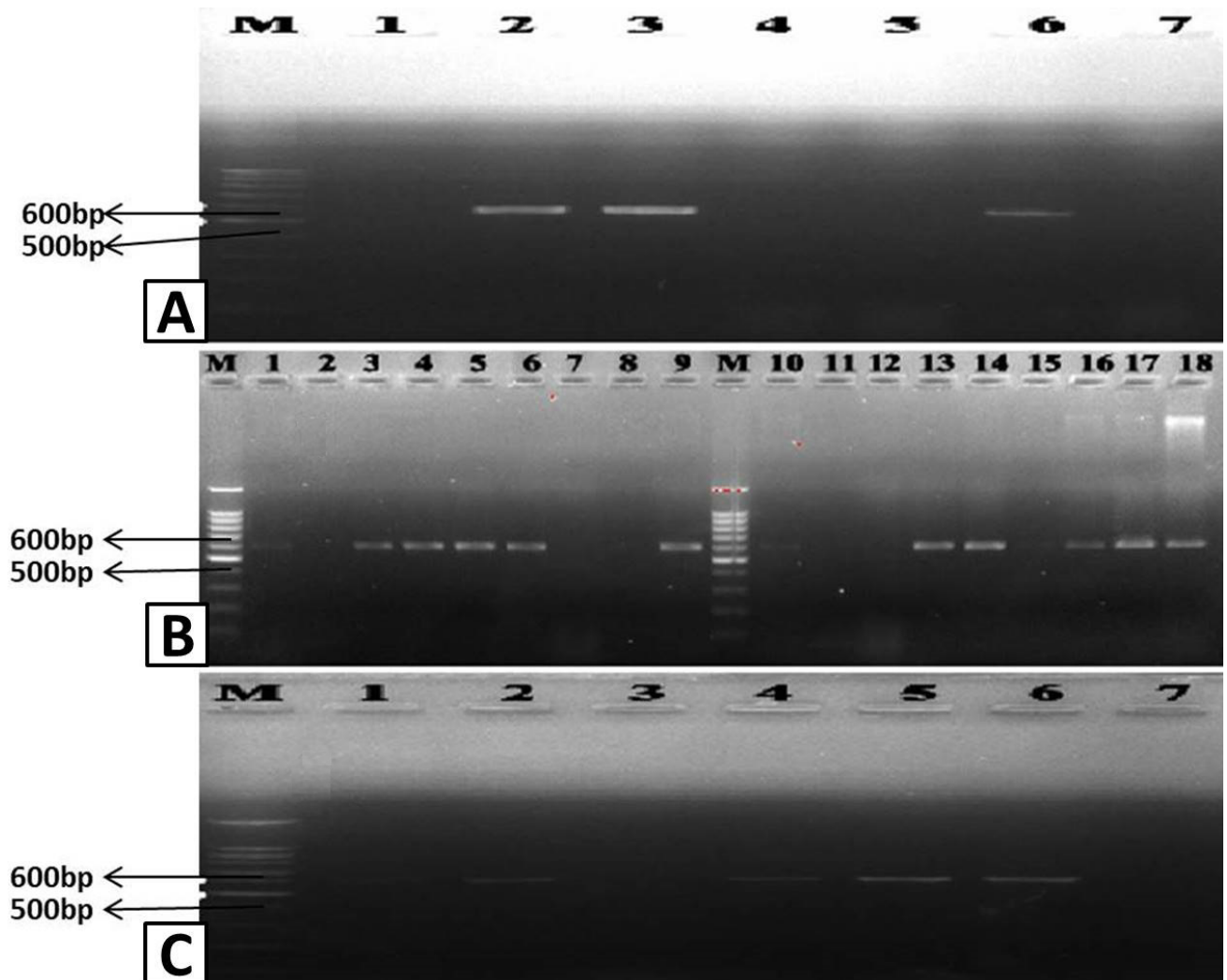


Supplementary Fig. S1. GelRed-stained agarose PCR gel (farm A). A: M, 1kb DNA ladder; lanes 1 and 10, positive control; lanes 2 and 11, negative control, distilled water substituted for DNA template; lane 3, A1; lane 4, A2; lane 5, A3; lane 6, A4; lane 7, A5; lane 8, A6; lane 9, A7; lane 12, A8; lane 13, A9; lane 14, A10; lane 15, A11; lane 16, A12; lane 17, A13; lane 18, A14. B: M, 1-kb DNA ladder; lane 1, positive control; lanes 2 and 11, negative control, distilled water substituted for DNA template; lane 3, B1; lane 4, B2; lane 5, B3; lane 6, B4; lane 7, B5; lane 8, B6; lane 9, B7; lane 12, B8; lane 13, B9; lane 14, B10; lane 15, B11; lane 16, B12; lane 17, B13; lane 18, B14.

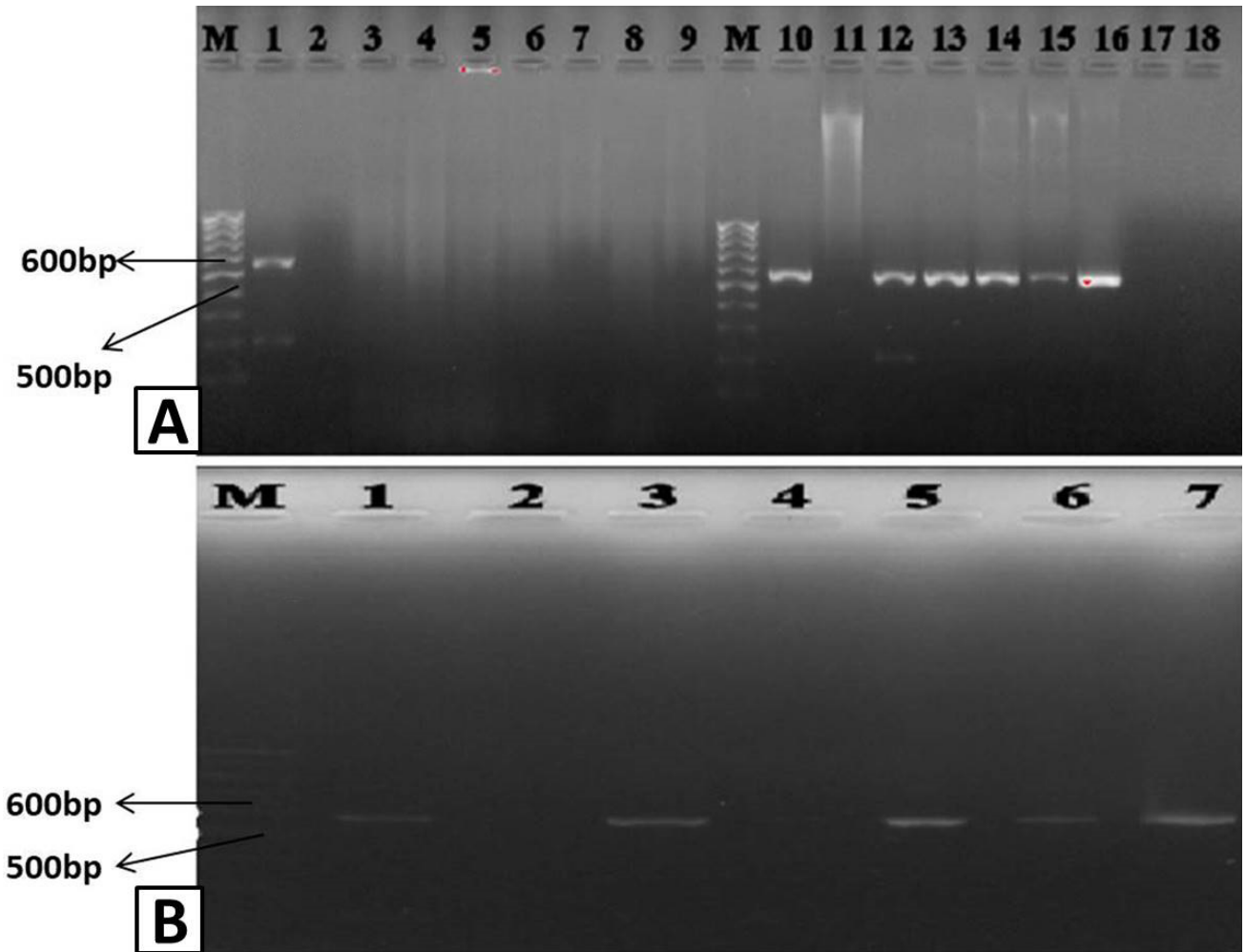
* Corresponding author: dr.basitzeshan@ucp.edu.pk

0030-9923/2021/0001-0001 \$ 9.00/0

Copyright 2021 Zoological Society of Pakistan



Supplementary Fig. S2. GelRed-stained agarose PCR gel (farm B). A: M, 1-kb DNA ladder; lane 1, positive control; lanes 2, B15; lane 3, B16; lane 4, B17; lane 5, B18; lane 6, B19; lane 7, B20. B: GelRed-stained agarose PCR gel (farm C). M, 1-kb DNA ladder; lanes 1 and 10, positive control; lanes 2 and 11, negative control, distilled water substituted for DNA template; lane 3, C1; lane 4, C2; lane 5, C3; lane 6, C4; lane 7, C5; lane 8, C6; lane 9, C7; lane 12, C8; lane 13, C9; lane 14, C10; lane 15, C11; lane 16, C12; lane 17, C13; lane 18, C14. C: GelRed-stained agarose PCR gel (farm C). M, 1-kb DNA ladder; lane 1, positive control; lanes 2, C15; lane 3, C16; lane 4, C17; lane 5, C18; lane 6, C19; lane 7, C20.



Supplementary Fig. S3. GelRed-stained agarose PCR gel (farm D). **A:** M, 1-kb DNA ladder; lanes 1 and 10, positive control; lanes 2 and 11, negative control, distilled water substituted for DNA template; lane 3, D1; lane 4, D2; lane 5, D3; lane 6, D4; lane 7, D5; lane 8, D6; lane 9, D7; lane 12, D8; lane 13, D9; lane 14, D10; lane 15, D11; lane 16, D12; lane 17, D13; lane 18, D14. **B:** GelRed-stained agarose PCR gel (farm D). M, 1-kb DNA ladder; lane 1, positive control; lanes 2, D15; lane 3, D16; lane 4, D17; lane 5, D18; lane 6, D19; lane 7, D20.