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Molecular Detection of Torque Teno Virus (TTV) in Plasma of People in Contact with Domestic Village Chickens using Polymerase Chain Reaction (PCR)

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ABSTRACT

Background : Torque teno virus (TTV) was first detected in 1997 in Japan in a patient with post-transfusion hepatitis. The virus was also detected in the liver and blood of people with hepatic pathologies of unknown etiology. In addition to man,TTV was also found to infect domestic and wild animals including cattle, chickens, pigs, cats, and dogs, etc.
Objective : This study was designed to detect TTV DNA among people who have contact with domestic chickens in Khartoum State during the period December 2016toJune 2017.
Method : A total 100 blood samples were collected,out of these 60 blood samples were collected from people who had contact with domestic village chickens and 40 blood samples were collected from people who had no contact and did not eat chickens. The samples were tested for TTV DNA using nested Polymerase Chain Reaction (nested-PCR) .
Result : TTV DNA was detected in 3(5 %) out of 60 blood samples from people who have contact with domestic chickens and all the samples were negative in the group of people who had no contact and did not eat chickens.
Conclusion : We observed low TTV prevalence and no association between contact with domestic chickens and TTV infection prevalence was detected.
Keywords : Torque Teno Virus (TTV), Domestic village chickens, polymerase chain reaction (PCR), Sudan.

I. INTRODUCTION

The Torque teno virus (TTV) was first detected in 1997 in the blood of Japanese patients with post-transfusion hepatitis. [1, 2]The virus was also detected in the liver and blood of people with hepatic pathologies of unknown etiology. [2]

TTV is a small, non-enveloped virus with a single-stranded,circular DNA genome of negative polarity, 3.4-3.9 Kb in length, containing two bigger (ORF1 andORF2) and several smaller open reading frames [8]. TTV is currently classified to Circoviridae family [8]. Despite being a DNA virus, TTV demonstrates an extremely wide sequence divergence. At least 16 genotypes with evolutionary distance >0.30 has been described so far [9].

The association between TTV and liver diseases is still controversial and several studies have been undertaken to identify infection sources. [3–5] Epidemiological studies have evidenced the prevalence of TTV in other pathological conditions such as in autoimmune diseases,[4] respiratory conditions [6] and cancer.[7]

However, information is still lacking on TTV infection and the development of pathologies, as well as the change in the course of a particular disease.[3,5]

TTV is ubiquitous virus revealed in more than 50% of the general human population throughout the world [10-12] and nearly 90% of populations [13]. Co-infection of single individuals with TTV isolates belonging to or several phylogenetic groups occurs frequently [14].
TTV was first characterized as a blood-borne virus and thus referred to as transfusion-transmitted (TT) group of viruses [1]but recent studies suggested the existence of other ways of transmission including parenteral [9], sexual [15,16],mother-to-child[17, 18] and others [19-21].

TTV has been suggested to be a causative agent of several diseases such as acute respiratory diseases [6], liver diseases [11,12], AIDS [22] and cancer [23], albeit, without any convincing support. One current hypothesis suggests a key role of TTV in development of autoimmune reactions [24].But until now no confirmed disease associations were discerned, and to date, there are no reliable commercial serological assays that can be used for large-scale screening.

After the discovery of TTV, five other novel Circoviruses were reported. These include SANBAN virus, TTV-like mini virus (TLMV), SEN virus (SENV), Sentinel virus (SNTV) and YONBAN[25].Clear disease associations for these agents are still awaited. In addition to man,TTV was also found to infect domestic and wild animals including cattle, chickens, pigs, cats, doge….etc. Studies on TTV in chickens are rare and there are no much information about the infection of domestic village chickens with TTV The prevalence of TTV infection in domestic village chickens in different villages in Isfahan (Iran) was estimated to be 40% by using primers from UTR region [26].

II. METHODS AND MATERIAL

Study site:
This study was conducted in Nablus area, North Khartoum, Sudan among people who have contact with domestic chickens. People who have no contactand do not eat chickens were used as controls.The study was carried out between December 2016 and June2017.

Data collection:
Through a structured questionnaire, information on Age,gender, contact with domestic village chickens and any current clinical manifestations, were recorded.

Sample collection:
A total of 60 blood samples were collected from people who had contact with domestic village chickens (27 male and 33 female) and 40 blood samples were collected from people who had no contact with domestic chickens as a control group.Blood samples were centrifuged at 3000 RPM for 5 mints. Obtained plasma samples were stored at -20 ºC until used for DNA extraction.

DNA extraction:
Commercial DNA extraction kits (Analytika Jena, Germany) were used according to manufacturer's instructions. Extracted DNA was stored at -20º C until used.

Nested polymerase chain reaction (Nested PCR):
Nested PCR was performed by processing the extracted DNA with primers that are specific for N22 coding region of TTV. The primers used consisted of external primer: forward 5'- ACA GAC AGA GGA GAA GGC AAC ATG – 3' and reverse 5' – CTG GCA TTT TAC CAT TTC CAA AGTT – 3' and the internal primers: forward 5' – GGC AAC ATG TTA TGA TAG ACT GG – 3'and reverse 5'- CTG GCA TTT TAC CAT TTC CAA AGT T-3' . The reaction was performed in 25 µl volume using Maxim PCR PreMix tubes (i-Taq). The volume included: 5 µl master mix, 1 µl of forward external primer, 1 µl of reverse external primer, 15 µl of distilled water and 3 µl of DNA. The tubes were transferred to the PCR machine (ESCO 6.5) The PCR was performed in 35 cycles consisting of denaturation for 1 minute at 94°C, annealing for 1 minute at 60°C and extension for 1.5 minutes and at 72°C.For second round PCR the reaction was performed in 25 µl volume using Maxim PCR PreMix tubes (i-Taq). The volume included: 5 µl master mix, 1 µl forward internal primer, 1 µl reverse internal primer, 16 µl of distilled water and 2 µl of DNA product from first PCR round was used as template DNA. The tubes were transferred to the thermo cycler using PCR machine (ESCO 6.5) using same program.(letters sizes,bont, are not uniform).

Gel electrophoresis

Five µl of the amplified products from both protocols were subjected to direct analysis by Gel Electrophoresis in 2% agarose, the gel was prepared by adding 1.5 g of agarose to 75 ml 1X Tris Borate EDTA buffer. The product was visualized by staining with 0.15% ethidium bromide and documented using UV gel documentation system (INGeNius). The expected sizes for the amplified DNA of TTV for conventional and nested PCR were 199 bp and 271 bp respectively.

Statistical analysis:

Collected data were analyzed using statistical package for social science (SPSS version 12.0). A p value of ≤ 0.05 was considered significant.

III. RESULTS AND DISCUSSION

Results

During the study period, 60 people in contact with domestic chickens (27 male and 33 female) and 40 people who had no any contact with domestic chickens were enrolled. Out of these TTV virus was detected in 3 (5%) of people in contact with domestic chicken'. (Table1) . While we found 3 TTV DNA positive in the case group, no positive result were detected in the control group. Data show no significant difference between the case and the control groups.
(Table1) .
Based on age group, the distribution of patients positive for TTV were (66.7%) and (33.3%) in the age groups 20-40 year and 40-60 year old respectively. (Table1).
According to gender, TTV DNA positive in 1/27 (3.7%)of the male patients and (6.1%) 2/33 of female patients but with no significant difference between male and female. (Table1).

Table1:characteristics of TT-virus DNA positive in People contact with domestic chickens according to their age and sex (case group).

Variable	**People in contact with domestic chickens (TTV+ve%)**	**TTV +ve number** (% out of total positives)*	**P value**
Age (yrs)			Not significant at level ≥0.05
10-20	15 (25%)	0(0%)	
20-40	14(23.3%)	2(66.7%)	
40-60	26(43.3%)	1(33.3%)	
60-80	5 (8.3%)	0(0%)	
Sex			Not significant at level ≥0.05
Male	27(3.7%)	1(1.6%)	
Female	33(6.1%)	2(3.3%)	

*calculated from total **People contact with domestic chickens** tested (number=60)

Discussion

TTV is a novel single-stranded DNA virus that is transmitted both parenteraly and non-parenteraly. Epidemiological studies have shown the virus to be widely distributed in different populations with parenteral risk exposure. TTV prevalence in apparently healthy population ranging from 7% to 83% was reported in different geographical areas of the world. [28, 29]

The presnt research is the first study to explore the prevalence of TTV among people in contact with domestic village chickens in Sudan.

We found that contact with domestic chickens wasn't a significant risk factor for being infected with TTV virus. Studies on TTV in chickens are rare and there are no much information about the infection of domestic village chickens with TTV in Sudan, Rana Mohammed (2016) detected the virus in 15 (17.6%) out of 85 chicken sera tested using conventional PCR and 38 out of 85 (44.7 %) were positive using Real-time PCR (unpublished data).

However, many investigators reported on the presence of TTV in domestic village chickens that may play a role in transmission of the virus to other domestic animals and human and vice versa, e.g.in Iran [26].

In this study, there was no association between age & sex and TTV infection. These results are in agreement with Abe et al., (1999)[30] and Pistello et. al., (2001)[31], who found no difference in the prevalence of TTV infection regarding age or sex.

Furthermore, in this study TTV virus shows a low prevalence compared to other studies done in healthy people in Sudan. Fifty one (61%) out of 83 healthy blood donors and 68 (83%) out of 81 healthy blood donors were shown to be positive for TTV using nested PCR to detect N22 region and single step PCR to detect UTR region respectively (33). Furthermore, a recent study TTV DNA was detected in 24 (28.9 %) of chronic HBV patients, but no detrimental effects was noted in this patients (32).

IV. CONCLUSION

The present study represents the first report on the molecular detection of TTV DNA in people in contact with domestic chickens in Sudan. Results of the study suggest that contact with domestic chickens does not appear to be a significant risk factor for acquiring TTV infection.

The mode of transmission and prevalence of this virus in domestic chickens in Sudan need further investigations.

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