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Differential IFN-Gamma (IFN-γ), Interleukin 10 (IL-10) and Cardiac Troponin I (cTnI) Responses in Natural Bovine Trypanosomosis in Nigeria

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Abstract

Trypanosomosis is major drawback to profitable livestock production in sub-Sahara African, including Nigeria. Knowledge of the cytokines production in the phase of natural infection may help to better diagnose, treat and prevent bovine trypanosomosis. The purpose of the this study was to determine the levels of interferon-gamma (IFN-γ), interleukin-10 (IL-10) and cardiac troponin–I (cTnI) in the sera of cattle naturally infected with *T. brucei*, *T. congolense* and *T. vivax* and correlate these levels with parasitaemia and PCV of the infected animals. Five milliliter of blood samples were collected via the jugular vein from 411 randomly selected cattle into EDTA and non-citrated bottle. PCV was determined manually using HCT. Trypansomes were detected and characterized by microscopy and PCR, respectively. Serum levels of IFN-γ, IL-10 and cTnI were determined using commercial ELISA kit. Data were summarized using descriptive statistic and significance of differences determined by ANOVA. Of the 62 samples positive for trypanosomes by microscopy, 50 samples were confirmed to species level by PCR. The sera levels of IFN-γ, IL-10 and cTnI of infected

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cattle were higher than non-infected cattle. The differences were not significant ($p < 0.05$) from the non-infected cattle except IL-10. There was no correlation between assayed parameters, the PCV and parasitemia. This is the first report that determines the sera levels of IFN-γ, IL-10 and cTnI in cattle with natural trypanosomosis. Further investigation is required to understand the specific effect of trypanosomes on myocardiac integrity and interaction between the two cytokines in natural trypanosomosis in cattle.

Keywords

Cattle, Cardiac Troponin, Interferon-Gamma, Interleukin-10, Trypanosomosis

1. Introduction

Trypanosomosis is a major disease complex that has caused great set back to profitable farming in sub-Saharan African. The disease complex is caused majorly by *T. brucei brucei*, *T. congolense* and *T. vivax* in cattle [1] [2]. Cattle are differentially susceptible to *T. brucei*, *T. congolense* and *T. vivax* infections [3]-[5] but the mechanism of susceptibility or resistance is not fully understood.

It is generally believed that response to African animal trypanosomoses involves Type-I and/or Type-II immune response [6], or a combination of both against infection(s) of trypanosomes. Interferon gamma (IFN-γ) and interleukin-2 (IL-2) are the main cytokines produced in Type-I immune response while IL-4, IL-5, IL-6, IL-9 and IL-10 are mainly produced in Type-II immune response. Though, different reports are available on the roles of Type-I and Type-II immune responses in different animal trypanosomosis model, the reports are contradicting [7]-[9]. While proliferation of trypanosomes is said to decreased in mice treated with anti-IFN-γ [10] [11] and IFN-γ gene deficiency [10], other reports indicate that IFN-γ stimulates trypanosome proliferation [12]. Furthermore, most reports on the immune responses in animals to trypanosomoses are experimental murine models [13]-[15], few are available on experimental bovine *T. congolense* infections [16]-[20] and no report is available on immune responses of animals to *T. vivax* infection.

Cardiac troponin I (cTnI) is a very sensitive biomarker for assessing the cardiac status [21]. It is considered "gold standard" for the non-invasive diagnosis of myocardial injury in human and animal diseses. But no work has been done to assess the effect of trypanosomosis on the serum level of cTnI in infected cattle.

In Nigeria, single and mixed infections of Trypanosomoses have been reported [2], but reports on the immune responses of cattle to trypanosomoses caused by *T. brucei brucei*, *T. congolense and T. vivax* are minimal, where it exists, it is experimental report [22]. To the best of our knowledge no data is available on cytokines and cTnI responses to natural bovine trypanosomosis. This study therefore, quantified the serum levels of interferon gamma (IFN-γ), interleukin-10 (IL-10) and cardiac troponin (cTnI) in cattle that were naturally infected with *T. brucei*, *T. congolense* and *T. vivax*, and also associated the effect of parasitaemia and PCV on the serum levels of the assayed cytokine and cTnI.

2. Materials and Methods

2.1. Study Population

A total of 411 cattle consisting of 308 cattle kept under traditional management system of free grazing (nomadic) and 103 cattle from various abattoirs and slaughter slabs were randomly selected for sampling between October and December, 2010. Those animals that tested positive for trypanosomes by microscopy and confirmed by polymerase chain reaction (PCR) were selected for this study. The study was approved by the Ethical Committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria.

2.2. Sample Collection

Blood samples were collected from the jugular vein of each cattle into 5ml tubes containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant and 5ml tubes without the EDTA for serum analysis. The samples were transported in mobile refrigerator to the laboratory within 3 hours of collection. The blood samples without the

anticoagulant were set on tray slanted and allowed to stay for 24 hours in the laboratory for serum harvest. Sera were collected in clean and sterile eppendorf tubes and stored in −20˚C freezer until use while the blood, in the EDTA bottles were stored at 4˚C prior to parasitological examination and DNA extraction.

2.3. Parasitological Diagnosis and PCV Determination

Parasitemia was determined using rapid matching method [23]. From each tubes containing anticoagulant, blood was transfer into three capillary tubes which were sealed at one end with plasticin. The capillary tubes were spun in microhaematocrit centrifuge, Haematospin 1400 (Hawksley and Son Ltd. UK) at 220 g for 5 minutes at room temperature. After centrifugation, the packed cell volume (PCV) was determined using hematocrit reader. The buffy coat and upper most layer of red blood cells of one capillary tube was extruded onto a microscope slide and examined with a phase-contrast microscope (Olympus CX21, Pennsylvania, USA) at ×400 magnification for the presence motile trypanosomes. Not less than 50 fields were examined before positive or negative was declared for each sample. While the haematocrit centrifugation technique (HCT) positive samples were further processed as thin smear stained with Giemsa (EMD Chemicals, USA) for trypanosome species identification, thick blood smear was also prepared, stained with Giemsa and all examined under ×100 oil immersion objective lens (×1000 magnification).

2.4. DNA Extraction and PCR Diagnosis

DNA was extracted from the blood in EDTA bottle using Quick-gDNA™ MiniPrep (Zymo Research Corporation, Irvine, CA 92614, U.S.A) as described by the manufacturer. The primers sets (Bioneer Inc, USA); TBR1 & 2, TCS1 & 2, TCF1 & 2, TCK1 & 2 and ILO1264 & 1265, as shown in **Table 1**, were selected for amplification of *T. brucei*, *T. congolense-savannah*, *T. congolense-forest*, *T. congolense-kilifi and T. vivax* DNA, respectively. Polymerase chain reaction amplification was performed in 20 µl final reaction volume containing equivalent of 20 ng of genomic DNA, 10mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 µM KCl, 200 µM each of dNTPs, 40 ng of each of the primers and 1unit of *Thermus aquaticus* DNA polymerase (Bioneer USA) as described in the previous work [2]. Ten microliter of the PCR products were electrophoresed through 1% agarose gel in 1 × TAE (40 mM TRIS-acetate and 1 mM EDTA) at 90 V for 80 min. along with 10 µl of biological marker, GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, UT, USA). Gels were stained with GelRedR Nucleic Acid Stain (PHENIX Research Product, Candler, NC, USA) at 5 µl/100 ml of the agarose gel suspension. After electrophoresis, the PCR products were visualized using ultra violet transilluminator (Spectroline^R TC 312 E). The PCR products of *T. brucei*, *T. vivax* and *T. congolense* (savannah and forest) strains were sequenced using Big dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the sequences were aligned with available published sequences of *Trypanosoma species* from GenBank.

Table 1. Names and sequences of primers set optimized for *Trypanosoma* species detection.

Parasite target	Primer designation	Primer sequences 5'-3'	References
T. brucei	TBR 1	CGAATGAATAAACAATGCGCAGT	
	TBR 2	AGAACCATTTATTAGCTTTGTGC	Masiga *et al.* (1992)
T. congolense (s)	TCS 1	AGA ACC ATT TAT TAG CTT TGT GC	
	TCS 2	CGAGCGAGAACGGGCAC	Majiwa and Otieno (1990)
T. congolense (f)	TCF 1	GGACACGCCAGAAGGTACTT	
	TCF 2	GTTCTCGCACCAAATCCAAC	Masiga *et al.* (1992)
T. congolense (k)	TCK 1	GTGCCCAAATTTGAAGTGAT	
	TCK 2	ACTCAAAATCGTGCACCTCG	Masiga *et al.* (1992)
T. vivax	ILO 1264	CAGCTCGCCGAAGGCCACTTGGCTGGG	
	ILO 1265	TCGCTACCACAGTCGCAATCGTCGTCTCAAGG	Masake *et al.* (1997)

Note: *T. congolense* (s); *T. congolense* savannah-type, *T. congolense* (f); *T. congolense* forest-type and *T. congolense* (k); *T. congolense* kilifi-type.

2.5. Quantification of Cytokines (IFN-γ and IL-10) and Cardiac Troponin-I (cTnI)

Cytokines and the cardiac troponin I were quantified in serum. Bovine Interferon-gamma (IFN-γ) ELISA was carried out using AbD serotec Bovine Interferon-γ ELISA kit (Catalog number MCA5638KZZ), interleukin-10 (IL-10) ELISA was carried out using Cusabio Biotech Co., Ltd. IL-10 ELISA kit (Catalog number CSB-E12917B) and Cardiac troponin I ELISA was carried out using Life Diagnostic, Incorporation diagnostic ELISA kit (Catalog number 2010-8-HS) following manufacturer's suggested protocols as contained in the kit manuals. The absorbance at 450 nm was measured with micro-titer plate ELISA reader (BioTek ELx 800, Highland Park, USA) and the concentration of the cytokines and the cardiac troponin in each well was calculated by comparison with a standard curve plotted using Microsoft excel program.

2.6. Statistical Analysis

Data were presented as the mean ± SEM. Significance of differences was determined by ANOVA and association within the measured parameters was done using Pearson correlation analysis in SPSS version 19 software. Differences were considered significant at $p < 0.05$.

3. Result

3.1. Parasitological and PCR Results

Parasite detection using microscopy observation showed 62 samples infected by one or more species of trypanosomes. Out of this, 14, 19 and 25 samples were single infection of *T. brucei*, *T. congolense-savannah* type and *T. vivax*, respectively. Using PCR, 7, 22 and 21 of those samples detected as single infections by parasitology were confirmed as *T. brucei*, *T. congolense*(s) and *T. vivax*, respectively.

3.2. Parasitaemia and PCV

The mean ± SEM parasitaemia of infected and non-infected cattle were not significantly different. The mean ± SEM of PCV of *T. vivax*-infected cattle was relatively lower than *T. brucei* and *T. congolense*-infected cattle, but non-infected cattle was had highest PCV that significantly different from others (**Table 2**).

3.3. Interferon Gamma (IFN-γ)

The serum levels of interferon gamma (IFN-γ) of *T. brucei*, *T. congolense*, *T. vivax* and non-infected cattle were 0.022 ± 0.002 pg/ml, 0.219 ± 0.183 pg/ml, 0.065 ± 0.023 pg/ml and 0.018 ± 0.005 pg/ml, respectively. Though the serum IFN-γ values of infected cattle were generally higher than non-infected cattle, the changes were not significantly different ($p = 0.412$). There was no correlation between the levels of interferon gamma expressed in the serum and, the PCV and the parasitaemia ($r = -0.134$ and -0.15)

3.4. Interleukin-10 (IL-10)

The mean sera levels of Interleukin-10 (IL-10) of *T. brucei*, *T. congolense*, *T. vivax* and non-infected cattle were 13.75 ± 11.95 ng/ml, 11.44 ± 18.05 ng/ml, 16.28 ± 20.27 ng/ml and 41.56 ± 34.64 ng/ml, respectively. While there was no significant ($p > 0.05$) different between the mean values of interleukin-10 of the trypanosome infected cattle, the mean values of infected cattle were significantly lower ($p = 0.049$) than non-infected cattle.

Table 2. Parasitaemia and the PCV of trypanosomal infected cattle.

Infection	Parameter	
	Parasitaemia (mean ± SEM)	PCV (mean ± SEM)
Trypanosoma brucei	$2.31 \times 10^6 \pm 1.02 \times 10^6$	32.8% ± 3.25%
Trypanosoma congolense(s)	$5.13 \times 10^5 \pm 1.45 \times 10^6$	33.1% ± 2.59%
Trypanosoma vivax	$1.37 \times 10^6 \pm 6.30 \times 10^5$	30.8% ± 2.08%

Note: *Trypanosoma congolense*(s); *Trypanosoma congolense* savannah-type.

There was no correlation between the levels of interleukin-10 expressed in the serum and the PCV and the parasitaemia (r = 0.048 and 0.122).

3.5. Cardiac Troponin (cTrI)

The mean values of serum cTrI of *T. brucei*, *T. congolense*, *T. vivax* and non–infected cattle were 0.11 ± 0.02 ng/ml, 0.14 ± 0.07 ng/ml, 0.16 ± 0.42 ng/ml and 0.10 ± 0.03 ng/ml, respectively. Though the serum cTnI values of infected cattle were generally higher than non-infected cattle, the changes were not significantly different (p = 0.44). There was no correlation between the levels of cardiac troponin expressed in the serum and, the PCV and the parasitaemia (r = −0.187 and 0.008).

4. Discussion

The immune response to African animal trypanosomoses has been studied extensively in experimental mice and bovine model, but no attention has been paid to natural infections in cattle. In this study, we assessed the level of interferon-gamma (IFN-γ), interleukin-10 (IL-10) and cardiac troponin-I (cTnI) in the sera of naturally infected cattle in Nigeria.

The circulating levels of IFN-γ and IL-10 assayed in this study, exhibited relatively higher levels in the infected cattle than non-infected cattle. Though, report on the plasma/serum level of IFN-γ and IL-10 in naturally infected cattle is not available. [6] [14] Reported elevated and decreased levels of IFN-γ in highly susceptible BALB/c and resistant C57BL/6 mice, respectively, infected with *T. congolense*, [5] reported significantly higher plasma IL-10 and IFN-γ levels in BALB/c mice infected with STIB247 strain of *T. brucei.* Though these reports are all from murine trypanosomosis model, they are in agreement with our findings except the report of [14]. However, in experimental bovine trypanosomoses, emphasis had been more on *T. congolense* infections [1] [18] [20] than *T. vivax* infection [24]. IFN-γ increased significantly in experimental *T. congolense*-infected cattle [25] [26], but the increase in *T. vivax*-infected cattle was small and transitory [24] whereas IL-10 is elevated in susceptible Boran and trypanotolerant N'Dama cattle infected with *T. congolense* [27]. These reports are in partial agreement with our findings as we recorded significant changes in the levels of IL-10 when compared with the non-infected cattle.

The serum levels of cTnI of infected cattle were slightly elevated than the levels in non-infected cattle. This finding could not be compared due to paucity of data. Though, cTnI level in the serum of cattle naturally or experimentally infected with trypanosomes, has not been documented up to date in Nigeria and elsewhere, it's increase has been reported in bovine theileriosis [28], canine babesiosis, ehrlichiosis and canine heart worm diseases [29]-[31]. We reported higher levels of cTnI in infected cattle than non-infected cattle. Contrary to our expectation, the increase was highest in *T. vivax* infection, a strict intravascular parasite, than infection of *T. congolense* and *T. brucei*, both which are capable individually, of attaching to red cells and extravascular foci, respectively. Neither PCV nor parasitaemia had positive correlation with the levels of cytokines and cTnI measured in this study, a finding that is in variant with the report of [32] who indicated that increased levels of cytokines are associated with anemia.

We report for the first time in Nigeria the sera levels of cardiac troponin-I (cTnI) and cytokines (IFN-γ and IL-10) in natural bovine trypanosomoses. Elevation of cardiac troponin may be an indication of myocardiac injury due to trypanosomes. Both cytokines measured were higher than non-infected animals. This is suggestive of both cytokine being critical in immune-modulation in bovine trypanosomoses. The significantly higher serum levels of IL-10 than IFN-γ in the infected animals support the recent report of [13], who indicated that murine trypanosomosis elicit both Type I and Type II immune responses. The process involves sequential switch from Type I cytokine (IFN-γ and TNF-α) production during the early stage of infection to Type II cytokine (IL-10 and/or IL-4) production during the late stage of infection.

5. Conclusion

In this study, we provide information on serum levels of IL-10, IFN-γ and cTnI in natural bovine trypansomosis. However, the low number of animals considered and absence of follow up makes further investigation imperative to ascertain the specific effect of trypanosomes on myocardiac integrity and interaction between the two cytokine in natural bovine trypanosomosis.

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