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Acute Phase Proteins, Hematobiochemical Profiles, Acid–base Balance and Blood Gas Alterations in Camel Calves Infested with Ticks

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ABSTRACT

This experiment was designed to assess the status of acute phase proteins (APPs) serum amyloid A (SAA) and haptoglobin, cardiac marker troponin I (cTnI), hematobiochemical profiles, acid–base and blood gas alterations in postnatal camel calves with tick infestation. Twenty-one camel calves (*Camelus dromedarius*), aged 1-4 weeks and weighing 30-65 kg were used. They were presented because of off milk, lethargy, recumbency and convulsions. Ten age-matched apparently healthy camel calves were used a control group. From both diseased and control groups, 3 blood samples were collected; one on EDTA for hematological analysis, the second on heparin for the determination of acid-base, blood gases, biochemical metabolites, electrolytes and lactate and the third on plain tubes for serum harvesting. Treatment has consisted of subcutaneous injection by ivermectin twice 15 days apart at a dose of 1mL per 50kg BW. Diseased calves were also sprayed with diazinon twice 72h apart at a concentration of 1mL/1L water. *Hyalomma dromedarii* predominated in all the diseased camel calves. Seventeen calves were presented in lateral recumbency position while the remaining 4 were admitted in sternal recumbency. The means ±SD of cTnI were 2.23±1.0 ng/mL versus 0.012±0.014 ng/mL in healthy animals. Serum concentrations of SAA in camel calves were 6.57±2.39 ng/mL in diseased versus 0.59±0.45 ng/mL in controls. Serum concentration of Hp were markedly elevated in camel with tick infestation (2.33±0.54mg/L in diseases group versus 0.25±0.26 mg/L in controls). There were significant increases in total white blood cells, lymphocytes and neutrophils in diseased camel calves compared to healthy ones. Red blood cells, hemoglobin and hematocrit were significantly lower in diseased group versus healthy calves. Serum concentrations of albumin, calcium, blood urea nitrogen and phosphorus differed significantly between diseased and healthy calves. The serum activity of alkaline phosphatase, aspartate aminotransferase, γ-glutamyl transferase and creatine kinase were significantly higher in tick infected camel calves versus healthy animals. The blood pH and anion gap were different significantly between diseased and healthy group. Highly significant differences were measured for lactate values in tick-infected calves competed to healthy group. In conclusion, this study showed that postnatal camel calves infested with *Hyalomma dromedarii* ticks had different clinical presentations either in lateral or sternal positions. Diseased calves have a prove of cardiac injury as a result of cTnI increases and acute phase reaction documented by significant alterations in SAA and haptoglobin. Unsound camel calves have also leukocytosis, anemia, metabolic acidosis and severe lactic acidosis.

Key words: Biomarkers, Camels, *Hyalomma dromedarii*, Pathophysiology, Ticks.

INTRODUCTION

Ticks are known as one of the principal blood sucking ectoparasites in camels (Perveen et al. 2023). In tropical and subtropical areas, many diseases are transmitted in this creature by ticks resulting in threatening the human as well as the camel health and dozens of published articles pointed out to this fact (Li et al. 2015; Devaux et al. 2020;

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Onyiche et al. 2020; Getange et al. 2021; Bendary et al. 2022; Ashour et al. 2023a,b; Aldujaily et al. 2023; Weidinger et al. 2023; El-Alfy et al. 2024).

In the locality where this study has been carried out, Saudi Arabia, *Hyalomma dromedarii* is the most found ticks, then *Hyalomma impeltatum* and finally *Hyalomma excavatum* (Tharwat and Al-Sobayil 2014b; Alanazi et al. 2020; Tharwat 2020a; Zakham et al. 2021; Ali et al. 2023; Aljasham et al. 2023). In a study conducted by Alanazi et al. (2020), out of 170 camels, 116 (68%) were infested by ticks and 13 of them were found to be positive for at least one tick-borne pathogen. In order, these pathogens include *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Anaplasma sp*., and *Hepatozoon canis*. None of the tested camels was infected with more than one pathogen and none was infected with *Babesia* spp. and *Theileria* spp. (Alanazi et al. 2020).

Acute-phase proteins (APPs) are proteins widely used for assessing traumatic injuries, inflammation or infection (Murata et al. 2004; Tharwat 2020a, 2023; Tharwat et al. 2014a; Tharwat and Al-Sobayil 2015a, 2015b; 2018; 2022). Those biomarkers are therefore frequently measured in camels and others to estimate the severity of disease status and expecting disease outcome (Azma et al. 2015; Brodzki et al. 2019; Hedia et al. 2021; Darwish 2023; Isik et al. 2023). Of the APPs, serum amyloid A (SAA) and haptoglobin have been used in camels to assess the health status and diseased process (Tharwat and Al-Sobayil 2015a, b, 2018; Tharwat 2020a).

Cardiac troponin I (cTnI) is a sensitive marker for cardiac insult in humans and also in animals. It is used for the assessing the disease progress and also as an aid for prognostication of myocardial damage (Tharwat and Al-Sobayil 2018; Rehman et al. 2023; Zhang et al. 2023). This cardiac marker cTnI has been used in camels for the evaluation of either healthy or diseased camels (Tharwat and Al-Sobayil 2014b; Tharwat et al. 2014b, 2024; Tharwat 2023).

In adult camels, we have reported the effects of tick infestation on the serum levels of APPs and bone biomarkers together with variable of hematology, biochemistry and changes in acid-base and blood gases (Tharwat and Al-Sobayil 2014b). In continuation to our previous work, this study was planned to evaluate the status of APPs SAA and haptoglobin, cardiac biomarker cTnI, hematobiochemical profiles, acid–base balance and blood gas alterations in postnatal camel calves with tick infestation.

MATERIALS AND METHODS

Ethical approval

Because only venipuncture was applied to camel calves, the *Institutional Animal Ethics Committee's* approval was not required as there were no invasive methods that would harm animals.

Animals and blood sampling

Twenty-one camel calves (*Camelus dromedarius*), 13 females and 8 males, aged 1-4 weeks and weighing 30-65 kg were used. They were admitted to the University Veterinary Hospital of Qassim University because of off milk, lethargy, recumbency and convulsions. Study period was from 2018-2023. Duration of illness ranged from 2 to 5 days. Ticks were palpated deeply and, in some cases, visible and in the skin over the entire body, particularly the neck, thorax and abdomen. Ten age-matched healthy camel calves were used as a control group. From both diseased and control groups, 3 blood samples were collected; one on EDTA for hematological analysis, the second on heparin for the determination of acid-base, blood gases, biochemical metabolites, electrolytes and lactate and the third on plain tubes for serum harvesting. Treatment has consisted of subcutaneous injection by ivermectin twice 15 days apart (IVOMEC Super®, MERIAL, Paulinia, Brazil) with a dosage of 1mL/50kg of weight (200µg ivermectin and 2 mgclorsulon per one kg of BW). Diseased calves were also immersed in diazinon twice 3 days apart (Diazin 600, Veterinary Pharmaceuticals Co., Ltd., Dammam, Saudi Arabia) with a concentration of 1mL per 1L of water.

Determination of hematobiochemical variables

The complete blood count panels including total white blood cells (WBCs), neutrophils, lymphocytes, monocytes, basophils, eosinophils, red blood cell counts (RBCs), hemoglobin (Hg), hematocrit (HCT) and RBCs indices comprising of mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration were measured in EDTA samples by a veterinary analyzer (VetScan HM5, Abaxis, California, USA). Different biochemical parameters and electrolytes including albumin, calcium, blood urea nitrogen (BUN), phosphorus, magnesium, sodium, potassium and chloride were also evaluated in heparin sample using a veterinary analyzer (VetScan VS2, Abaxis, California, USA). The activity of γ-glutamyl transferase (GGT), alkaline phosphates (ALP), creatine kinase (CK) and aspartate aminotransferase (AST) were also determined in the second blood sample.

Determination of blood gas parameters

Complete acid-base and blood gas panel was measured immediately after the collection of heparinized blood sample by a portable veterinary analyzer (I-STAT®, Abaxis, California, USA). This panel included (1) pH (2) oxygen partial pressure (PO_2) (3) carbon dioxide partial pressure (PCO_2) (4) total carbon dioxide (TCO_2) (5) excess of base (BE) (6) oxygen saturation (So_2) (7) bicarbonate (HCO_3) (8) anion gap (AG) and (9) lactate (Tharwat 2015; 2021a,b; 2023; Tharwat et al. 2014b, 2024; Tharwat and Al-Sobayil 2014a,b,c).

Measurements of serum amyloid A and haptoglobin

The inflammation biomarkers serum amyloid A (SAA) and haptoglobin (Hp) were tested in serum samples by commercial kits (Multispecies SAA ELISA kit and haptoglobin colorimetric kit, Tridelta Ltd., Ireland) as reported (Tharwat et al. 2014a; Tharwat and Al-Sobayil 2015a,b; 2022; Tharwat and Al-Sobayil 2018; Tharwat 2020a; 2023).

Statistical analysis

Results were presented as mean±SD, and it was evaluated by a statistical package (SPSS 2017). Student's *t* test was used for comparison between diseased and controls and the significance was fixed at $P \leq 0.05$.

RESULTS

Hyalomma dromedarii predominated in all the 21 camel calves. The clinical presentation of camel calves affected with tick infestation is shown in Fig. 1. Seventeen (81%) animals were presented in lateral recumbency position while the remaining 4 (19%) were admitted in sternal recumbency position. In the 17 calves admitted with lateral presentation, 10(58.8%) have opisthotonos combined with muscular spasm of the fore and hind limbs, 4(23.5%) have opisthotonos with flaccidity of the extremities and the remaining 3(17.6%) have complete flaccidity of the head, neck and extremities. All the 4(100%) calves presented at sternal recumbency have opisthotonos with stargazing position. After treatment, 15 out of the 21(71.4%) diseased calves has recovered safely while the remaining 6(28.6%) did not recover and diet. Unfortunately, none of the diseased camels examined postmortem.

Difference in serum cTnI between diseased camel calves and healthy controls is presented in Fig. 2A. The means ±SD of cTnI were 2.23±1.0 ng/mL versus 0.012±0.014 ng/mL in healthy animals with a statistically significant difference (P<0.0001). Fig. 2B shows serum concentration in camel calves with tick infection compared to control animals (6.57±2.39 ng/mL in diseased versus 0.59±0.45 ng/mL in controls, P<0.0001). Parallel, mean serum concentration of Hp (Fig. 2C) were markedly elevated in camel with tick infestation (2.33±0.54mg/L in diseases group versus 0.25±0.26 mg/L in controls, P<0.0001).

Table 1 shows minimum and maximum values and means±SD of hematological parameters in diseased and control camel calves. There were significant increases in total WBCs, lymphocytes and neutrophils in diseased camel calves compared to healthy ones (P≤0.05). Contrary, RBCs, Hg and HCT were significantly lower in diseased group versus healthy calves (P<0.0001). Other hematological variables did not differ significantly (P>0.05).

Differences of blood chemistry are presented in Table 2. Serum concentrations of albumin, calcium, BUN and phosphorus differed significantly between diseased and healthy calves (P<0.05). The serum activity of GGT, CK, ALP and AST were significantly higher in tick-infected camel calves versus healthy animals. Other chemical parameters did not show significance (P>0.05).

Fig. 1: Clinical presentation of camel calves with tick infestation. **A**, upward deviation of the head and neck with stargazing position; **B**, backward deviation of the head and neck with muscular spasm of the extremities; **C**, backward deviation of the head and neck with flaccidity of the extremities; **D**, backward deviation of the head and neck with muscular spasm; **E**, backward deviation of the head and neck with flaccidity of the legs; **F**, flaccidity of the head, neck and extremities.

Table 1: Minimum, maximum and means±SD of hematological variables in camel calves with tick infestation compared to healthy controls.

Parameter	Minimum	Maximum	Diseased (n=21)	Controls (n=10)	P value
WBCs (×10^{9}/L)	3.9	53.2	36.1±20.1^{a}	23.4±8.2^{b}	0.05
Lymphocytes (×10^{9}/L)	0.38	9.85	2.4±2.7^{a}	3.4±1.0^{b}	0.0001
Monocytes (×10^{9}/L)	0.03	4.5	0.7±1.4^{a}	0.6±0.6^{a}	0.8
Neutrophils (×10^{9}/L)	3.4	48.6	34.2±18.0^{a}	18.1±7.1^{b}	0.02
Eosinophils (×10^{9}/L)	0.02	2.16	0.95±0.93^{a}	1.27±1.5^{a}	0.5
Basophils (×10^{9}/L)	0.00	0.78	0.17±0.31^{a}	0.05±0.07^{a}	0.2
RBCs (×10^{12}/L)	1.2	8.9	3.9±2.3^{a}	9.1±0.7^{b}	0.0001
Hemoglobin (g/dL)	2.6	18.5	7.5±5.0^{a}	13.3±1.6^{b}	0.0001
Hematocrit (%)	4.2	21.5	10.3±4.6^{a}	22.6±3.0^{b}	0.0001
MCV (fL)	23.0	49.0	29.0±9.9^{a}	24.9±1.9^{a}	0.1
MCH (pg)	10.9	42.7	17.4±13.3^{a}	14.6±1.2^{a}	0.3
MCHC (g/dL)	47.3	92.3	57.3±15.4^{a}	59.3±5.4^{a}	0.6

WBCs, white blood cells; RBCs, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration. ^{a,b} values with different letters in the same row differ significantly.

Table 2: Minimum, maximum and means±SD of biochemical parameters in camel calves with tick infestation compared to healthy controls.

Parameter	Minimum	Maximum	Diseased (n=21)	Controls (n=10)	P value
Albumin (G/L)	19.0	52.0	40.0±9.6^{a}	50±6.1^{b}	0.0002
ALP (U/L)	20	171	101±57^{a}	86±474^{b}	0.001
AST (U/L)	64	685	330±235^{a}	83±171^{b}	0.01
Calcium (mmol/L)	1.8	2.7	2.3±0.27^{a}	2.6±3.0^{b}	0.04
GGT (U/L)	5.0	41.0	15.7±12.9^{a}	5.0±9.0^{a}	0.5
BUN (mmol/L)	5.3	13.6	8.2±3.1^{a}	3.3±7.7^{b}	0.002
CK (U/L)	163	3754	1630±1217^{a}	170±563^{b}	0.002
Phosphorus (MMOL/L)	1.3	4.1	2.9±1.0^{a}	3.2±4.3^{b}	0.02
Magnesium (MMOL/L)	0.72	1.70	1.02±0.28^{a}	0.91±1.16^{a}	0.9

ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; BUN, blood urea nitrogen; CK, creatine kinase. ^{a,b} values with different letters in the same row differ significantly.

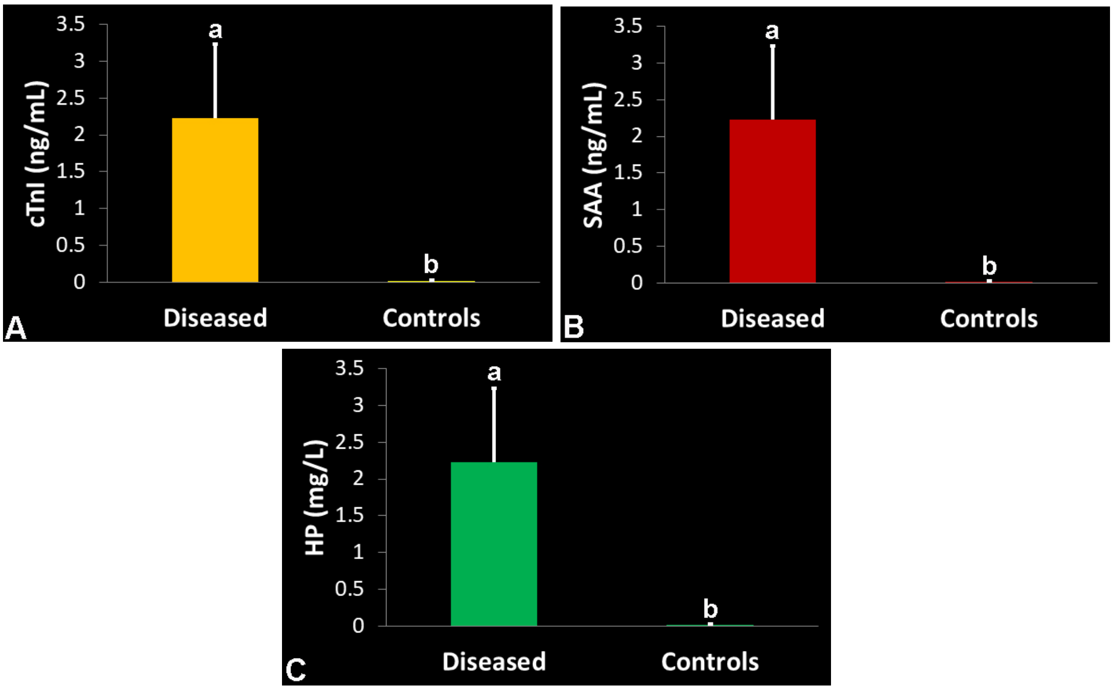

Fig. 2: Serum concentrations of cardiac troponin I (cTnI; **A**), serum amyloid A (SAA; **B**) and haptoglobin (HP; **C**) in camel calves (n=21) with tick infestation compared to healthy controls (n=10). ^{a,b} P<0.0001.

Changes in acid-base balance, blood gases, electrolytes and lactate are summarized in Table 3. The blood pH and AG were different significantly between diseased and healthy group (P=0.05, 0.01, respectively). A highly significant differences were measured for lactate values in tick-infected calves competed to healthy group (P<0.0001). Other tested parameters did not differ significantly (P>0.05).

DISCUSSION

To our knowledge, this research is the first describing the effects of tick infestation in camel calves on the levels of acute phase proteins SAA and haptoglobin, the cardiac biomarker cTnI, hematological and biochemical profiles, acid–base balance and blood gases. *Hyalomma dromedarii* predominated in all the 21 camel calves; a finding agrees

Table 3: Minimum, maximum and means±SD of acid-base balance, blood gases, electrolytes, and lactic acid concentration in camel calves with tick infestation compared to healthy controls.

Variable	Minimum	Maximum	Diseased (n=21)	Controls (n=10)	P value
PH	7.02	7.43	7.23±0.14[a]	7.36±0.01[b]	0.05
PCO_2 (mmHg)	21.0	55.4	41.1±11.8[a]	42.3±1.8[a]	0.7
PO_2 (mmHg)	17.0	66.0	32.3±18.8[a]	24.1±1.1[a]	0.2
BE (mmol/L)	-25.0	3.0	-6.7±10.4[a]	-2.1±1.5[a]	0.2
HCO_3 (mmol/L)	5.8	28.8	20.3±8.2[a]	23.6±1.4[a]	0.3
TCO_2 (mmol/L)	6.0	54.0	23.0±14.2[a]	24.9±1.5[a]	0.7
sO_2 (%)	19.0	91.0	44.5±25.7[a]	39.4±2.6[a]	0.6
Anion Gap (mmol/L)	0.3	32	9.4±12.4[a]	20.9±2.3[b]	0.01
Sodium (mmol/L)	134	177	153±13[a]	159±1.4[a]	0.2
Potassium (mmol/L)	2.5	5.6	3.7±1.0[a]	4.0±0.2[a]	0.3
Chloride (mmol/L)	93	130	113±13[a]	119±2.9[a]	0.3
Lactic acid	0.55	22.0	17.0±8.2[a]	1.2±0.6[b]	0.0001

PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3, bicarbonate; TCO_2, total carbon dioxide; sO2, oxygen saturation. [a,b] values with different letters in the same row differ significantly

with other reports (Alanazi et al. 2020; Zakham et al. 2021; Ali et al. 2023; Aljasham et al. 2023).

Ticks represent the second most important prevalent disease vectors after mosquitoes and transmit pathogens to more than 100,000 humans per year (de la Fuente et al. 2008). Dromedary camels are susceptible to numerous ectoparasites. Of these parasites, several species of ticks infest camels in many Arabian countries, including Saudi Arabia (Alanazi et al. 2020; Ali et al. 2023). Ixodid ticks are blood-sucking ectoparasites, and they transmit different pathogens that infect human being and other vertebrates, causing a variety of diseases (Azagi et al. 2017; Coronel-Benedett et al. 2018; Alanazi et al. 2020; Getange et al. 2021; El-Alfy et al. 2024). In comparison with other hematophagous arthropods, hard ticks feed for a longer period (de la Fuente et al. 2017) thus increases the possibility of pathogen transmission (Richards et al. 2017). Tick infestation can initiate different signs as dermatitis, irritation, paralysis, fatigue, anemia, weight loss, and even collapse (Sofizadeh et al. 2013; Mansfield et al. 2017).

The camel calves in this study were presented during the postnatal period (1-4 weeks of age) with different presentations. The calves had either lateral presentation or sternal positions. Backward deviation of the head and neck with spastic muscle contractions of the limbs is one of the presenting forms in calves had lateral position. The second form included backward deviation of the head and neck but with flaccidity of the extremities. The third form showed complete relaxation of the head, neck and for- and hind limbs. On the other side, camel calves with presenting sternal recumbency had only one form comprising of opisthotonos combined with stargazing position. Most of the calves (15 out of 21) did not respond to medical therapy with the remaining 6 did. In our previous reports summarizing adult camels with ticks, similar clinical manifestations were found including complete loss of appetite, ataxia, recumbency and opisthotonos (Tharwat and Al-Sobayil 2014b; Tharwat 2020a).

The cardiac muscle marker cTnI tested significantly elevated in diseased versus age-matched healthy calves. This biomarker has also been reported to be high in several conditions in camels pointing out to the cardiac insult (Tharwat et al. 2014b, 2024; Tharwat 2023). Similar significant increases were found in the serum levels of the acute phase proteins SAA and haptoglobin. The cTnI, SAA and haptoglobin were also found to be high in adult camels having ticks and therefore were used prognostic indicators (Tharwat and Al-Sobayil 2014b; Tharwat 2020a). Saliva containing toxins that are injected by ticks may be the cause behind the toxic myocardial injury in the camel calves as proved also by significant elevations in GGT, ALP, CK and AST activity. The severe anemia encountered also in diseased calves may be a predisposing etiology for myocardial damage (Tharwat and Al-Sobayil 2014b).

Regarding the hematological variables evaluated in this report, leukocytosis due to secondary infection consisting of neutrophilia combined with lymphocytosis was found. In addition, severe anemia as a result of significant decreases in RBCs, Hg and HCT was detected in the camel calves. This pronounced anemia was expected due to the blood sucking nature of the *Hyalomma dromedarii* ticks as found previously in adult camels infested with this blood sucking ectoparasites (Tharwat and Al-Sobayil 2014b).

Concerning the biochemical panel, albumin, calcium, BUN and phosphorus differed significantly in diseased versus healthy calves. The enzymes ALP, AST, GGT and CK were significantly higher in diseased calves compared to healthy ones. Similar results were found in adults infected with ticks (Tharwat and Al-Sobayil 2014b). As a result of tick infestation in the calves, blood pH and AG differed significantly between tick-infested and control group. The most important finding was the significantly high values of lactate in diseased versus control group. The decreased blood pH indicates metabolic acidosis. On the other side, the increased lactate level may be due to the inability for metabolism of all the stored pyruvate that should be changed into lactate (Tharwat and Al-Sobayil 2014b).

In conclusion, this study showed that postnatal camel calves infested with *Hyalomma dromedarii* ticks had different clinical presentations either in lateral or sternal positions. Diseased calves have a prove of cardiac injury as a result of cTnI increases and acute phase reaction documented by significant alterations in SAA and haptoglobin. Unsound camel calves have also leukocytosis, anemia, metabolic acidosis and severe lactic acidemia.

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Conflicts of interest statement

The authors have no conflicts of interest to disclose.

Author contributions

MT: concept, design practical work and writing the manuscript draft. WRE and AAA revised, edited the manuscript draft and prepared the figures and tables. All authors revised and approved the final manuscript for publication.

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