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# Assessing The Impact of Temperature, Volume and Concentration of Serum On Complement Activity in The Red-Eared Slide Turtle, (*Trachemys scripta elegans*)

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**ABSTRACT**

*Serum complement is valuable tool in determining the health status of turtles. Little is known about complement system as a component of innate immunity of the ectothermic vertebrates such as turtles. The research was led to describe the activation of alternative complement pathway of red-eared slide turtle, (Trachemys scripta elegans) Using standard haemolytic assays. The effect of concentration, volume and temperature of serum complement of, the red-eared slide turtle, (Trachemys scripta elegans) on unsensitized rabbit red blood cell hemolysis was measured. Serum concentrations of 12.5% v/v produced 4.2 ±0.17 mm, 25% v/v 4.6 ±0.3mm, 50%, 5.02±0.05mm, and 100%,5.6 ±0.39 mm hemolysis. 10µL volume of serum resulted in 5±0.16 mm, 20µl, 5.7±0.26 mm and 30µL 6.2±0.15mm hemolysis. Incubation of sera at 5-15°C produced 6±0.1 mm, 25°C, 7.9 ±0.27 mm and at 35°C 9.1±0.47 mm hemolysis. The present study is the first to provide unequivocal evidence about the significant effect of concentration, volume and temperature of serum complement on alternative complement pathway activity in turtles. These data suggest that the increased innate immunity induced by high developmental concentration, volume and temperature might increase the resistance of turtles to the outbreak of diseases.*

**Key words***: Trachemys scripta elegans, Hemolytic assay, Serum, Complement, Innate immunity*

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## INTRODUCTION

The natural support answers are nonspecific and begun quickly next appearance. (Medzhitov and Janeway, 2000). The serum complement method, an essential part of the natural immune system, is an old mechanism of innate immunity that could be observed in all vertebrae and several early invertebrates (Dalmasso et al., 1989; Merchant et al., 2006a). The complement method, made of 35 proteins in blood in inactive mode that could be activated by 3 pathways: activated by antigen-antibody complex, classical pathway, alternative pathway, activated by molecules of surface microorganisms; and lectin pathway, activated by bacterial surface carbohydrate (Holland and Lambris, 2002). Any investigations told the presence of complement system parts in a kind of reptiles (Koppenheffer, 1986). Dilemma pathway serum complement in the blood of the American alligator *Alligator Mississippians* is was detected & the same outcomes are detected in freshwater (*Crocodylus Johnston*) & saltwater (*Cro.porosus*) crocodiles (Merchant et al., 2006a, 2005b). Both investigations proposed the life of robust complement methods in the kinds. The RBCs hemolysis test, applied for people in the clinical environment has lately been changed to measure the natural immune action of turtles (Lachmann et al., 1978). The dilemma pathway effect of complement method could be measured, between different methods, with the measurement of serum hemolytic action, if the pathway is activated with foreign red blood cells (Yano, 1992). The investigation could be utilized to assess the impacts of many factors such as diseases, environmental influence and nutrition on the lytic action of complement method (Holland and Lambris, 2002). Tortoises apply to the reptilian level, and 3 kinds of freshwater tortoises relating to the families Emydidae & Trionychidae was located in Iran (Kami et al., 2006). The red-eared slider turtle, *Trachemys Scripta Elegans* has been listed to be presented to the wild outside its native range, containing several countries and regions in Asia, Europe, Australia, & Africa (Newberry, 1984; Uchida, 1989; Da Silva and Blasco, 1995; Ota, 1995; Luiselli, et al. 1997; Chen and Lue, 1998; Cadi et al., 2004). That is the initial report on the natural immune action of *Trachemys Scripta Elegans*. The now research is performed to describe impact of concentration, volume, and temperature of alternative pathway serum complement of *Trachemys Scripta Elegans*, on hemolysis of unsensitized rabbit red blood cells to examine the possible relationships between the results and the mechanisms of immunity available to turtles.

## MATERIALS AND METHODS

### Collection of blood sample

Six adult red-eared slider turtles *Trachemys scripta elegans* were captured from Shoormast Lake, a natural lake located

5.5km far from Pol-Sefid City, located in Mazandaran province of Iran (36º5'16.03"N, 53º2'48.36"E) at an elevation of 950m. Blood was taken and provided to precipitate at room temperature & then centrifuged at 2500×g for 15 min. The serum is removed and combined for after investigation. Whole blood obtained from healthy rabbit is used by citrate sodium to inhibit coagulation. The blood is centrifuged at 3000×g for 10 min & the plasma discarded. The rabbit red blood cells are re suspended in phosphate buffered saline (PBS, pH 7.4) & centrifuged in 3000×g. Next one more PBS centrifugation & re suspension, the RBCs are diluted to 10 % (v/v) with PBS (Lachmann et al., 1978).

**Serum complement assay**

The tortoise serum is melted at room temperature & utilized for investigation. We assessed the concentration, volume & temperature dependency of tortoise serum to unsensitized RBCs. For the preparation of hemolytic plates, barbital barrier (5.1 ml) is combined by 2% agarose (4 ml) at 56°C. The mix is then cooled to 45°C & mixed by 0.5 ml of a 10% suspension of rabbit erythrocytes that had before been washed by PBS barrier. The last combination (10 ml) was spilled on plates. Wells (diameter, 3 mm) divided with 14 mm are cut in the agarose (Lachmann et al. 1978). Turtle serum was diluted to different titers using PBS (12.5%, 25%, 50%, 100%) then 30 µL of any plasma unit is carried into each well. Incubation was carried out at room temperature (25°C) overnight. A different test is conducted to assess the effect of volume on the complement system of *Trachemys Scripta Elegans* serum, various amounts of plasma (10, 20, 30µL) were used at room temperature (25°C) overnight. To ascertain the temperature dependency of RBC hemolysis, the turtle plasma was incubated at different temperatures (5-35°C). The zones of hemolysis are certainly evident & are aligned manually.

**Statistical analysis**

Any unit is examined in quadruplicate so that accurate analytical outcomes can be achieved. As a positive control, 30µL from a solution containing 1% (v/v) Triton X-100 was added until complete hemolysis had been achieved. For negative control only PBS was used. Those control specimens are utilized as a measuring for whole other specimens. All results represent the mean zone of hemolysis beyond well diameter (3mm) in mm ± standard deviation of four independent determinants. Analytical investigation is managed to utilize SPSS 16.0 for windows package.

**Results**

Incubation of various groups of tortoise serum by RBCs in vitro resulted in hemolytic action in concentrations 12.5% (4.2±0.17mm), 25% (4.6±0.3mm), 50% (5.02±0.05mm) and 100% (v/v) (5.6±0.39mm). In this study, maximal hemolytic activity was exhibited at 100% turtle serum. Hemolysis of RBCs by *Trachemys scripta elegans* serum was concentration dependent ($p<0.05$) **[Fig. 1]**.

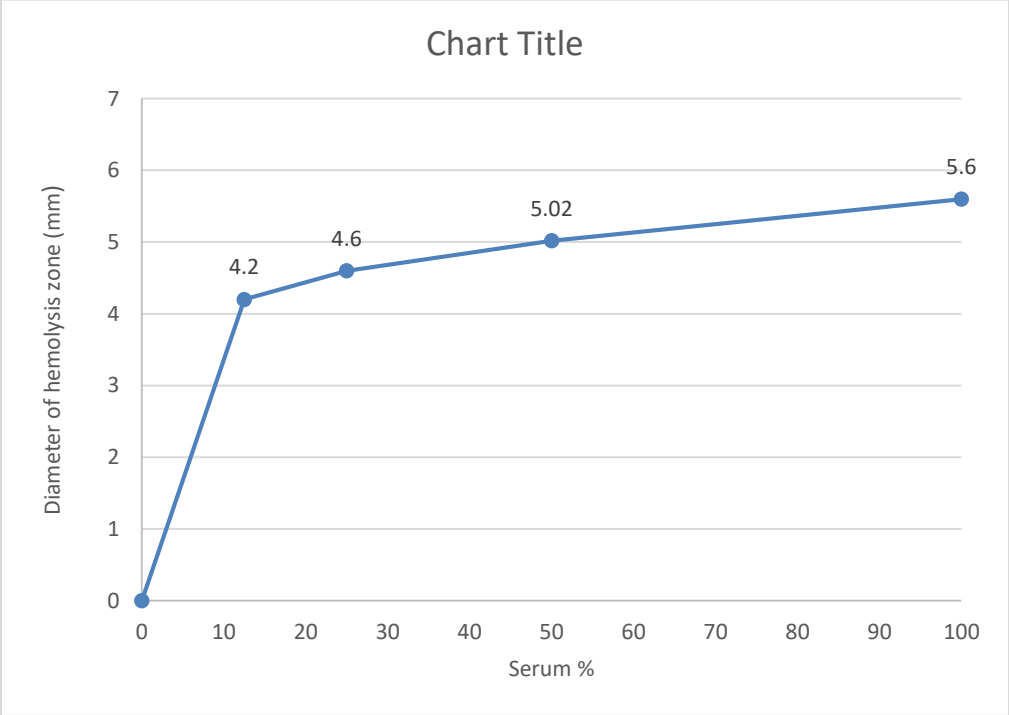


**Fig. 1: Concentration-dependent hemolysis of RBCs by red-eared slider turtle, *Trachemys cripta elegans*. RBCs were incubated with different concentrations of the serum from for *Trachemys scripta elegans*. Each sample was analyzed in quadruplicate and the results represent the mean ± standard deviations.**

Exposure of different volumes of serum from *Trachemys scripta elegans* to RBCs exhibited volume-dependent hemolysis ($p<0.05$). 10µL of serum resulted in (5±0.16mm) hemolysis. Increased volumes, 20 and 30µL of serum produced (5.7±0.26 mm) and (6.2±0.15mm) hemolysis, respectively **[Fig. 2]**.

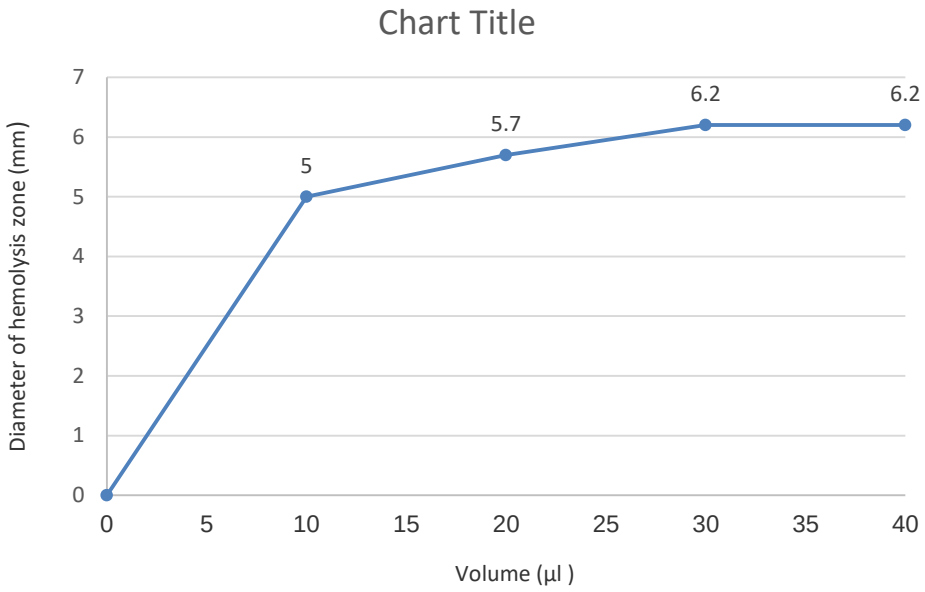


**Fig. 2: Volume-dependent hemolysis of RBCs by serum of red-eared slider turtle, *Trachemys scripta elegans*. RBCs were incubated with different volumes of the serum from for *Trachemys scripta elegans*. Each sample was analyzed in quadruplicate and the results represent the mean ± standard deviations.**

Incubation of serum from *Trachemys scripta elegans* with RBCs at various temperatures (5°C-40°C) occurred in temperature dependent hemolysis ($p<0.05$). Hemolysis zone at 5°C-15°C is exhibited 6. ±0.1mm. This hemolytic action raised at 25°C, 7.9±0.27mm and maximum activity observed at 35°C, 9.1±0.47mm **[Fig. 3]**.

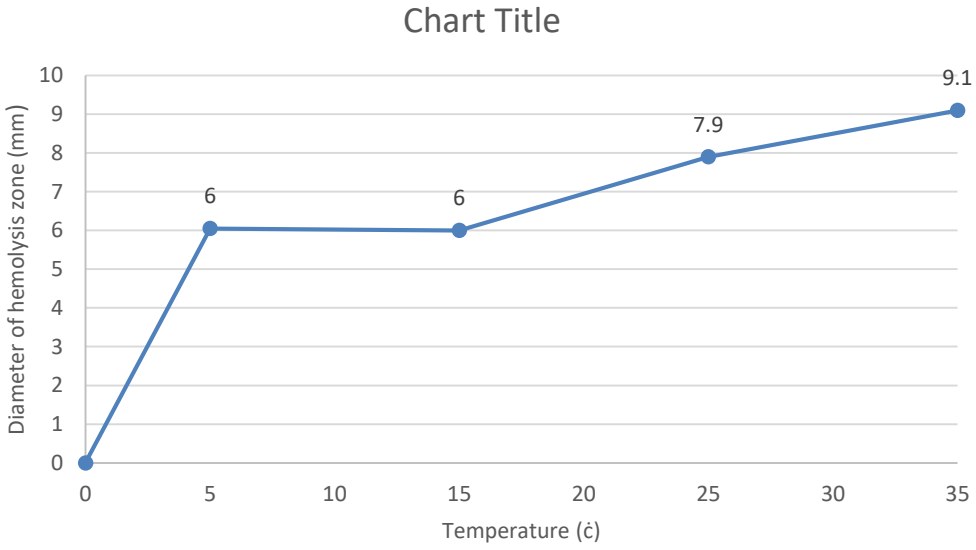


**Fig. 3: Temperature-dependent hemolysis of RBCs by serum of red-eared slider turtle, *Trachemys scripta elegans*. RBCs were incubated with 100% turtle serum at different temperatures. Each sample was analyzed in quadruplicate and the results represent the mean ± standard deviations.**

**Discussion**

Plasma complement is an important part of natural protection could be seen in all vertebral, & several old invertebrates (Smith et al., 1999; Nair et al., 2005). This behavior of complement system plasma has been reported in reptiles (Koppenheffer, 1987; Sunyer et al., 1998) The results of this study show that the hemolysis of RBCs method could be favorably utilized for the evaluation of the natural protected method of tortoises. In this investigation the hemolytic activity of red-eared slide turtle *Trachemys scripta elegans* serum on unsensitized rabbit red blood cell was characterized. We found alternative complement activity of *Trachemys scripta elegans* serum at 12.5% showed some hemolysis increasing in 25% and 50% and maximum at 100%. Expression of various volumes of serum from *Trachemys Scripta Elegans* to RBCs produced in volume dependent hemolysis. The hemolysis of RBCs by *Trachemys Scripta Elegans* serum into vitro depended on the temperature at that it was produced. The greatest hemolytic action happened at 35°C. The activity of *Trachemys Scripta Elegans* serum is alike to that of *Phrynops Geoffroanus* (Ferronato et al., 2009), But, the action is very less than that recognized in crocodilian kinds (Merchant and Britton, 2006a; Merchant et al., 2005b, 2010; Siroski et al., 2010). Investigations of the innate immune system from another reptile such as the Australian freshwater, *Crocodylus Johnston* (Krefft 1873) & saltwater, *Crocodylus porosus* (Schneider 1801), crocodile American alligator, Alligator Mississippians is (Daudin 1801); & the broad-snouted caiman, *Caiman latirostis* (Daudin 1801), showed concentration-dependent action. 20% American alligator plasma occurred in 90.4% hemolysis action (Merchant et al., 2006a). The freshwater turtle, *Phrynops GeoffRoanus* plasma presented fewer than 10% of action by 80% of plasma concentration. But, a little volume of hemolysis is recognized at 90% tortoise plasma that raised to 25% at 100% plasma (Ferronato et al., 2009). The concentration of serum complement proteins is higher in crocodilians than in turtles (Merchant et al., 2005b). The volume -dependent hemolysis was observed in serum of Komodo dragon, (*Varanus komodoensis*), *Amphiuma tridactylum* plasma and serum of *Mecistops cataphractus* and *Osteolaemus tetraspis*. The freshwater turtle *P. geoffroanus* exhibited maximal activity at 35ºC (Ferronato et al., 2009). Komodo dragon plasma showed the highest action at 35°C (Merchant et al., 2012). In another kind of crocodilians analyzed, the peak plasma complement work is recognized at 30°C in the freshwater crocodile, 25°C in the saltwater crocodile (Merchant and Britton, 2006a), 35°C in the American alligator (Merchant and Britton, 2006a), 30°C in the African dwarf crocodile (*Osteolaemus tetraspis*) and 25°C in the African dwarf crocodile *(Mecistops cataphracts*) (Merchant et al., 2013). Hemolysis of RBCs by *Amphiuma tridactyl* um serum is temperature dependent, with maximal action at 30◦C (Major et al., 2011). In conclusion, the alternative complement hemolytic pathway activity of the *Trachemys Scripta Elegans* serum complement may be affected by concentration, volume, and temperature. In the present study, concentration and volume dependent tests are led at room temperature. Future studies should examine level- and volume-dependent examination at different temperatures. These data give new penetrations into the innate immunity of *Trachemys Scripta Elegans* against pathogens and reveal the value of serum complement as a component of innate immunity to control microbial infections.

**Ethical approval**

Any relevant international, national, and institutional guidelines for the care & treatment of animals are observed.

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