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Differences in Hemoglobin and Packed Cell Volume in Blood Collected from Different Sites in Japanese Quail (*Coturnix japonica*)

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Abstract: Hemoglobin (Hb) and Packed Cell Volume (PCV) values were compared in blood collected from the following four sites of the Japanese quail: jugular, brachial, medial metatarsal veins and the heart. Ten 25-week-old hatch-mate layers of similar body weight having a hard shelled egg in the uterus were used in this study. Hb and PCV values were found to be the highest in the medial metatarsal vein followed by, in descending order, the brachial vein, the jugular vein and the heart. The heart had significantly lower values ($p<0.05$) compared with the jugular, brachial and medial metatarsal veins with the latter two having almost similar values. Blood samples from the jugular vein exhibited the least variability compared to other sites. The brachial vein followed by the jugular vein, were found to be suitable sites for blood sampling as well as for making injections without any significant complication. The heart puncture technique was very risky, traumatic and resulted in many side effects and should not be used, particularly in on-going experiments. Scientists should be aware of possible differences in blood constituents as a result of sampling sites. For obtaining accurate and reliable results, these points should be taken into consideration while designing an experiment or assessing comparative hematological data collected using different collection techniques.

Key words: Bleeding site, blood collection, Japanese quail

INTRODUCTION

The Japanese quail is a very popular animal model for biomedical research covering a wide range of disciplines including growth, reproduction, endocrinology, nutrition, physiology and toxicology. Evaluation of hematological parameters is invariably used as a diagnostic tool to assess the impact of the treatments. A review of literature reflects that a wide variety of blood collection sites are being used for sampling blood from birds (Samour, 2008; Ballard and Cheek, 2010) and this has led to confusion in comparing blood studies reported in the literature. Therefore, the site used for collecting blood as well as handling of birds is a very important variable. It is unlikely that more than one site will be used in the experiment; however, it is necessary to gather information on blood values from various sites that could be used as an alternative source. Similar studies conducted in humans and a variety of animal species have shown that blood sampled from different sites on the same subject differed in hematological and plasma chemical values in rats and mice (Upton and Morgan, 1975; Nemzek *et al*., 2001; Doeing *et al*., 2003; Abatan *et al*., 2008), infant baboons (Berchelmann *et al*.,1973), golden hamsters (Mengebier and Pleasants, 1959), guinea pigs (Roofe *et al*., 1950), dogs (Jensen *et al*., 1994) and in sharks (Mylniczenko *et al*., 2006). Therefore, this study was designed to assess whether similar differences exist with respect to blood parameters (Hb and PCV) in blood sampled from the jugular vein, the brachial vein, the medial metatarsal vein and the heart in Japanese quail.

MATERIALS AND METHODS

Ten 25-wk old Japanese quail layers were used in the present study. The birds were raised in temperature controlled brooders until four weeks of age under continuous lighting, when the females were separated from the males and housed singly in wire cages under 16-h of photoperiod at 23±2°C and provided *ad libitum* access to commercial turkey starter, oyster shells and fresh water (Arora, 1968). In order to minimize the effect of the gender, age, weight and physiological status, only the female birds from the same hatch, of similar body weight (137-140 g) and having a hard-shelled egg in the shell gland were used in this study. Various restraining techniques and the availability of different blood sampling sites in Japanese quail have been reported previously (Arora, 1979). Feed and water were withheld for about 2 h prior to weighing and blood sampling. The birds were handled gently and sedated immediately with Methoxyfluorane to minimize pain, stress and physical movements which may cause damage to blood vessels and serious complications. After sedation, the birds were laid on the table top in lateral recumbency and the legs and wings were secured using adhesive tape and allowed to lie undisturbed for approximately two minutes before blood collection. The feathers on the sites were separated to expose blood vessels with wet swabs dipped in lukewarm saline solution. The sites were cleaned gently, wiped with a cotton swab soaked in 70% Isopropyl alcohol and dried before collection, making the blood vessel more distinctly visible. Blood was collected only one time from four different sites on the same bird

in the following sequence: the medial metatarsal vein, the brachial (basilic) vein, the jugular vein and the heart. Blood from the medial metatarsal vein was collected directly into EDTA-coated vials after pricking the blood vessel with a sterile lancet or sharp needle, whereas, blood from the right jugular vein, the brachial vein and the heart was drawn (0.1-0.2 mL) into a tuberculin syringe using a 1-inch 25-gauge needle and then transferred immediately to EDTA-coated vials. The heart was entered through the lateral thoracic area between the 4^{th} and 5^{th} rib close to the sternum after feeling the heart beat through digital sensation while applying slight negative pressure. The vein was compressed near the elbow joint for brachial and base of neck for the jugular to allow the vein to engorge and then released after the entry of needle, with bevel up, into the veins. This procedure also prevented the vessels from slipping. The whole process of blood sampling was carried out between 9:00 to 11.00 A.M. and took less than 8 min with the assistance of one technician. Bleeding and formation of hematoma was minimized by applying direct pressure against the vessel with a moist cotton swab immediately before the removal of the needle. The blood was processed for Hemoglobin content using the cyanmet-Hb method (Drabkin's solution; Crosby *et al*., 1964) and for PCV using a microhematocrit centrifuge within twenty min of collection. The data were analyzed by one-way analysis of variance to determine the impact of different collection sites and student paired t-tests for comparing group means. The data are given as Mean±SD and $p \leq 0.05$ was considered significant.

RESULTS

Statistical analysis of the data reflected that both Hb and PCV values were significantly ($p<0.05$) affected by the collection site. Hb values were the highest in the medial metatarsal (12.80±0.98g) followed by brachial (12.62±1.10g), jugular (10.30±0.94g) and heart (9.82±1.15g) in descending order. Similarly, PCV values were 46.80±2.02%, 46.18±2.07%, 39.66±1.00% and 36.30±1.94% for the medial metatarsal, brachial, right jugular and heart, respectively. Both Hb and PCV values were significantly lower in heart and jugular collections compared with the two peripheral sites. Collection site differences were more pronounced in PCV values as compared to Hb values and the lowest variability among samples was observed in the jugular vein collections.

DISCUSSION

Blood collections from all the four sites, medial metacarpal, brachial, right jugular and heart, used in this study exhibited different Hb and PCV values. Similarly, differences in hematological and plasma chemistry values due to bleeding site have been reported in humans and in various animal species. Nemzek *et al*. (2001) and Doeing *et al*. (2003) collected blood from the same mice from four different sites, heart, tail, foot and saphanous vein and reported significantly lower total leucocytes count in heart blood compared to the other three sources: saphanous, tail and foot. However, there was no significant difference in blood samples collected from the tail, saphenous vein and foot. Lymphocytes were most predominant in the peripheral blood. They also reported that cardiac puncture was the best method of blood collection in mice; it is the fastest and most reliable technique yielding the maximum blood volume with the least amount of stress to the animal. These authors did not report any complications following cardiac puncture. In rats, Upton and Morgan (1975) reported that both Hb and PCV were significantly higher in blood collected from the tail than blood collected from the heart or the abdominal aorta. In humans, blood from ear lobes had higher Hb values than venous blood or finger-tip blood (Bruckmann, 1942). Similarly, blood from the ears of infant baboons had higher hematological values as compared with the femoral vein (Berchelmann *et al*., 1973). In golden hamsters, the platelet counts were significantly higher in tail blood as compared with heart blood (Mengebier and Pleasants, 1959). In guinea pigs, the peripheral blood had higher white blood cell and red blood cell counts as compared with heart blood (Roofe *et al*., 1950). In cows, jugular blood had a higher PCV than mammary vein (Fisher, 1962). In sharks, MyIniczenko *et al*. (2006) reported that PCV was significantly lower in blood samples collected from the cranial dorsal fin sinus compared with caudal tail artery. Our findings corroborate the report of McClure and Cedeno (1955) who used cardiac puncture to collect blood from small birds for serological purposes that cardiac puncture is risky and the right jugular and brachial venipunctures were more reliable. This study supports reports with other animal species that the site of blood collection has a definite effect on blood constituents in Japanese quail. This should be taken into consideration while designing an experiment or evaluating hematological data from other laboratories. In terms of safety, ease of collection, least complications, brachial and jugular are the ideal sites for the Japanese quail. However, one might argue that lower Hb and PCV values from the jugular and heart reported here might have resulted from hemodynamic alteration from handling of birds while collecting blood. This does not seem to be the case because of the fact that the birds were handled gently and then sedated quickly prior to blood collection to minimize stress and pain. The quantity of total blood drawn from the bird was less than one mL (less than one percent of the birds' body weight) which would not have any impact on the hemodynamic of the bird weighing approximately 135 g.

Potential problems and preventive measures: The most common complication from the brachial as well as jugular veinipuncture is the seepage of blood under the skin and formation of hematoma. This can be minimized by applying direct digital pressure over the blood vessel before withdrawing the needle and then applying an antibiotic cream to the affected site to prevent infection. Hemorrhaging from the right jugular vein could complicate the matter further if blood seeps into the air sac. Cardiac puncture, a blind method, is often being used when larger quantities are needed for serology and diagnostic purpose. This method is risky and traumatic and may cause immediate deaths and surviving birds may experience damage to cardiac and adjoining tissues and it may take longer for birds to heal and recover. Also, the blood from Japanese quail tends to clot quickly; therefore, it should be processed as soon as possible. The bird should be handled gently between the palms of both hands and sedated immediately to prevent stress and physical movements. Therefore, researchers must clearly specify the site to be used for collecting blood and that must be kept consistent through out the experiment. This will also enable other investigators to use the same site in similar types of experiments and thus avoid confusion and inconsistencies in the literature.

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