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Refined method of blood collection in chickens using tonic immobility

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Abstract

The domestic fowl (*Gallus gallus domesticus*) is a popular commercial species and is much studied for meat and egg production. Frequent studies on genetics, immunology, diagnostics and therapeutics are conducted in this species. For all these studies frequent blood collection is required. In the present study, blood collection technique in domestic fowl was standardized, wherein a single individual researcher can restrain the bird using the weak hand in the state of tonic immobility and carry out blood collection using the dominant hand, thus precluding the need for additional manpower which enhances the efficiency of collection especially for studies involving large number of birds without exposing them to much stress and discomfort. This fulfils one of the important objectives of refining experimentation by reducing stress on the birds.

Key words: Blood collection, Chicken, Refinement, Tonic immobility

Introduction

The domestic fowl (*Gallus gallus domesticus*) is a popular commercial and laboratory bird with respect to immunological and toxicological studies (Duncan, 2010). The B lymphocytes were first described in fowls with 'B' representing Bursa of Fabricius. Organisation for Economic Co-operation and Development (OECD) has recommended domestic fowl as an animal model for studying certain toxicities (OECD Test No. 418, 1995). Frequently, studies on genetics, diagnostics and therapeutics are conducted in this species because of their commercial importance. Collection of blood is a primary requirement for different immunological, toxicological and genetic researches, diagnostic protocols and various drug screening and pharmacokinetic procedures. In domestic fowl, blood is collected from wing (brachial vein) or leg (medial metatarsal vein), neck (jugular vein) (Aaronn *et al*., 2005) or heart (cardiac puncture). The site chosen is depends on the frequency of blood collection and volume of blood required. For a single collection of 5-6 ml of blood, cardiac puncture is preferred; while veins are preferred for 1-2 ml. Wing vein is preferred for single collection while for multiple collections within a short duration of time, medial metatarsal vein is suitable. Blood collection usually requires two people; still the restraining of birds in these methods is stressful (Grimes, 2002). So, a method of blood collection which can be achieved by a single person without causing much stress to birds was essential to be developed.

Chicken are prone to tonic immobility (birds get immobile and lose righting reflex when placed in some specific positions), which is a fear response elicited by a physical restraint (Gallup *et al*., 1980; Dorothee *et al*., 2008). Birds (Gallup and Gordon, 1979), lizards (Prestrude and Crawford, 1970) and rabbits (Carli, 1974) exhibit this behaviour which is characterized by suppression of the righting reflex lasting from a few seconds to several hours (Jones, 1986). In the present study, the phenomenon of tonic immobility is exploited for blood collection which can be achieved by a single person without any assistance and without causing undue stress to the birds.

Materials and methods

Animals

Broiler birds were procured from commercial broiler shop and housed in the Laboratory animal house facility, Madras Veterinary College in individual cage system under natural day-night cycle. The birds were acclimatised to the laboratory conditions for seven days prior to the experiments. Birds were provided *ad-libitum* feed and water. The study was approved by 'Institutional Animal Ethics Committee' (IAEC) of the institute (Lr. No. 318/DFBS/IAEC/2010, dated 21.09.2010).

Methods

The adult birds (N=210) of about 1-1.5 kg were made to lie on sternal recumbency on the table with the gentle pressure on the back (Fig. 1a). The limb from which blood collection had to be done is called as primary and the other limb was termed as secondary. Secondary limb was kept folded and slid below the wing (Fig. 1b). The primary limb from which blood collection was intended would be free. Bird was turned to lateral recumbency with the primary limb resting on table extending away from the handler. The secondary limb was placed firmly under the wing in such a way that the whole limb including toe nails was covered by wing (Fig. 1c). The handler should keep his forearm on the shaft of tarso-metatarsus of the secondary limb from over the wing and further hold the primary limb at hock joint (Fig. 1d and 2a) thereby occluding the medial metatarsal vein at the joint and raising it across the length of the shaft (Fig. 2b) with the weak hand while the dominant hand was used for blood collection.

Once the bird was comfortable in the restrained position, its neck was placed on the table and extended. In this position, just by rolling the finger away from the tip of beak till 20-25 cm on the table in a straight line will cause tonic immobility in the birds (Fig. 2c). An Alternative method for setting tonic immobility was to keep the head little lower from the level of the table with slight pressure (Fig. 2d) and gently blowing air (breeze) on the face of bird with release of the pressure. Once (hypnotized) immobilized in this position, the bird might rarely move on pricking of needle for blood collection.

Blood can be collected by a single person, provided the person is duly trained. Needle (26 gauge) was placed parallel to the occluded vein and introduced in ascending fashion by holding the syringe (2-5ml) at the plunger and operating the plunger with index and middle figure while the rest of the fingers keep the syringe in place (Fig. 3). The vein can be occluded and bleeding can be stopped by applying pressure or suitable coagulant solutions. The bird is awakened after bleeding is stopped by a gentle push on the back which forces the bird to stand on its leg. For repeated collections, start from the lower most part of the limb and move in ascending fashion for successful collections, thereby improving the chances of successful sampling after hematoma formation (due to improper technique of blood collection in previous samplings).

Other groups with fewer birds of 10 each, blood sampling through restraining by two persons followed by blood collection through wing vein and leg vein was also carried out. These procedures were characterised by violent struggling of bird and attempts to escape the manual restraint making it both difficult for the person as well as the bird for blood sampling. This also resulted in raising alertness in other birds in neighbouring room that are to be experimented upon successively. This further made handling as well as sampling all the more difficult.

Results

Comparison of orthodox sampling using two people through wing vein and leg vein against novel procedure developed in the present study is presented in Table No. 1 The present study, improved upon previously existing procedures of blood sampling in birds. Along with improving chances of multiple sampling in birds, it also helped in sampling in conditions of low man power without any increase in stress either to the birds or the handler. A single handler can collect blood from the bird with minimum stress on the birds. Multiple blood collections can be achieved easily due to the long and clearly visible vein available on both the limbs. The collections through medial tarsal vein can be achieved without any hematoma unlike the other veins. Moreover, immobility of bird further reduces chances of hematomas. This method is suitable for collection of small as well as large quantities of blood. Medial metatarsal vein rarely collapses as compared to other veins such as brachial and jugular vein. No trimming or plucking of feathers is required in this method.

Discussion

In a research setup wherein timely collections of blood are required such as pharmacokinetic studies, timings of blood collection can be accurately maintained during experimentation, besides reducing the chances of stress induction in the bird. The birds are said to be much deeply immobilized if they are pre-exposed to noxious stimuli

(Dorothee *et al.,* 2008). Hence, in case of farm conditions wherein the birds are exposed to loud noise or sudden transport to the laboratory, tonic immobility would work even better for blood collection. Considering the large commercial poultry setup and the large volume of sampling required to be carried out, blood collection achieved by single person using this method reduces manual labour significantly. Hence, this method can be advocated for use in research and commercial poultry units for single as well as multiple collections with minimum stress on the birds associated with other benefits like easy and early clotting and less incidence of formation of hematoma.

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Table No. 1 Comparison of blood collection procedures in chicken

Sl. No	Blood sampling			
	Procedure	Orthodox		Novel
1	Number of birds used for sampling (N)	10	10	210
2	Sampling route	Wing vein	Leg vein	Leg vein
3	Number of persons	2	2	1
4	Recurrent Sampling	Not possible	Possible but with some difficulties	Possible
5	Number of sites for sampling	Two veins	Two veins(Anterograde sampling possible)	Two veins (Anterograde sampling possible)
6	Stress on bird	High	High	Low to Moderate
7	Adverse effects	Bleeding, Struggling	Clot formation, Struggling	Clot formation (Rarely)

Fig. 1

Legends for figures

Fig. 1a : The adult female bird on sternal recumbency on the table with the gentle pressure on the back

Fig. 1b: Secondary limb being folded and slid below the wing

Fig. 1c: Bird turned on lateral recumbency with the primary limb resting on table extended away from the handler. The secondary limb placed firmly under the wing in such a way that the whole limb including toe nails is covered by wing

Fig. 1d: Handler's forearm resting on the tarso-metatarsus shaft of the secondary limb over the wing while holding the primary limb at hock joint, thereby occluding the medial metatarsal vein at the joint

Fig. 2

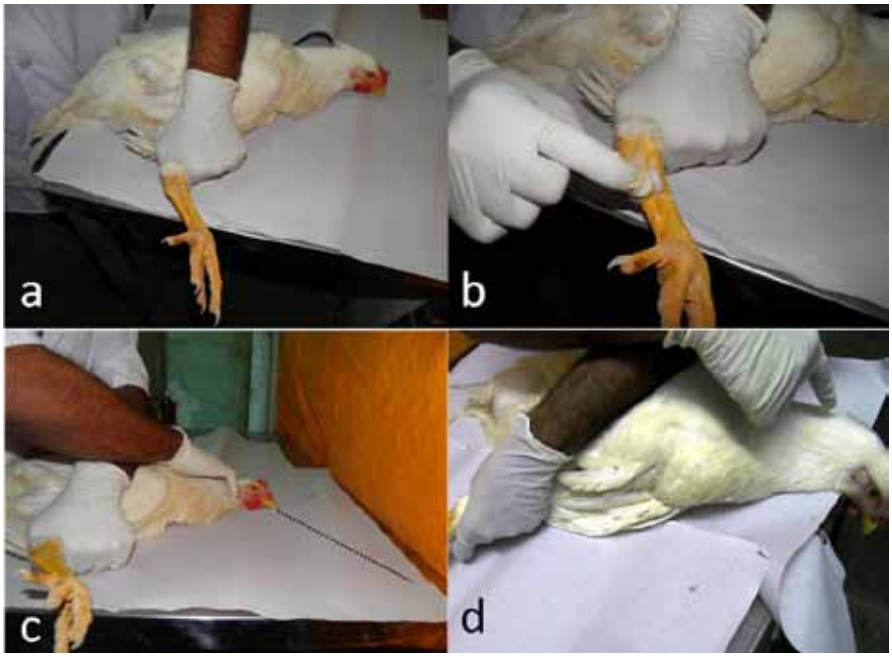

Fig. 2a and b : Raising of metatarsal vein across the length of the shaft

Fig. 2c : Restrained bird with neck placed on the table and extended with dotted line indicating the course of rolling of the finger for setting of tonic immobility.

Fig. 2d : Alternative method for setting tonic immobility - head kept little lower from the table with slight pressure.

Fig. 3

Fig. 3 : Blood collected by a single person using 26 gauge needle placed parallel to the occluded vein and introduced in ascending fashion by holding the syringe at the plunger and operating the plunger with index and middle figure while the rest of the fingers keep the syringe in place