A SIMPLE METHOD FOR ASEPTIC COLLECTION OF BLOOD FROM MINIPIG METATARSAL VEINS

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Abstract

A simple method for aseptic collection of more than 50 ml blood from anaesthesised adult minipigs is described. The blood is collected from *vena metatarsalis plantaris* or from *vena metatarsalis dorsalis* using a standard intravenous catheter.

Pigs and humans share many anatomical and physiological similarities. For this reason, minipigs are highly valuable laboratory models (McAnulty et al., 2011). Minipigs are known for lacking easily accessible blood vessels for venepunction. This can complicate experimental work, especially when relatively high volumes of aseptically collected blood are repeatedly needed, for example in immunoserological or pathogenicty studies.

Besides the milk veins, which are accessible in younger lean animals, the only well visible veins are localized on the external ear (Jackson et Cockroft, 2007). In most cases venepunction of the auricular vessels allows blood easily to be collected, even under aseptic conditions. Nevertheless, the total volume of blood sample is limited by the diameter of the ear veins which are generaly well developed only in adult animals. In addition, repeated access to these veins is not well tolerated and even in some adult individuals, the auricular vessels may be less developed (Fig. 1). The diameter of the vein is a limiting factor also for blood collection from the tail, which is a convenient alternative in adult individuals of normal-sized breeds, but not in minipigs.

Other sites for venepunction of minipigs are veins accessible from the ventral neck area: *v. jugularis*, *anterior v. cava*, *v. brachiocephalica* or *v. carotis* (Boland, 1985; Niiyama et al., 1985; Yen, 2000; Jackson et Cockroft, 2007). Nevertheless, these veins are accessible non-surgically only by blind puncture (Ragan et Gillis, 1975).

The need of >50 ml samples of repeatedly aseptically collected blood from anaesthesised minipig led us to try out an alternative method - venepunction of veins in the metatarsal area.

Material and Methods

Animals, housing diet

The method was verified repeatedly in 5 adult Kostelec minipigs (the Minnesota – type breed) weighing from 35 to 50 kg. The animals received standard diet and were housed individually in mobile cages as described previously (Kučera et al., 2010).

Anaesthesia

The minipigs were anaesthesised by intramuscular injection of azaperone (2 $mg.kg^{-1}$; Stresnil, Jansen Pharmaceutica) and ketamine (16 $mg.kg^{-1}$; Narkamon, Bioveta). The anaesthesia was then maintained by administration of N_2O and oxygen (2:1) with 0.5 – 1.5 % of isoflurane (Aerrane, Baxter). The anaesthetic gases were administered via laryngeal mask (Ambu®, AuraFlex, size 2.5 or 3) using Anestar N7 apparatus (Chirana) equipped with an Isoflurane evaporator (Isotec 3, Ohmeda).

Blood collection

The metatarsal area of the hind leg was clipped and shaved. After thorough washing with surgical soap and brush, the punction site was swabed with 70% ethanol. Than the leg was swathed with a tourniquet placed just above metatarsal joint and the vein was localized by palpation (Fig. 2). After repeated application of ethanol which was allowed to evaporate, the vein was puncted with a 21 gauge i.v. catheter (Venofix, Braun) connected to a syringe (Fig. 3). To demonstrate the possibility of repeated access to the vein the procedure was repeated at least three times within two weeks in each individual.

Haemoculture

Haemoculture was used to prove that blood collection was carried out in aseptic conditions. Using Oxoid Signal Blood Culture System (Oxoid) ten millilitres of blood from each venepuncture were cultured for up to five days at 37 °C. Subsequently, aerobic and anaerobic subcultures were made on Columbia agar with sheep blood, chocolate agar and Schaedler anaerobe agar with sheep blood (all Oxoid). Where appropriate, Anaerogen Compact system (Oxoid) was used for anaerobic cultures.

Figure 1. Poorly developed ear veins in adult minipig allows to insert only 25 – 23 gauge needle and the collection up to 10 ml only.

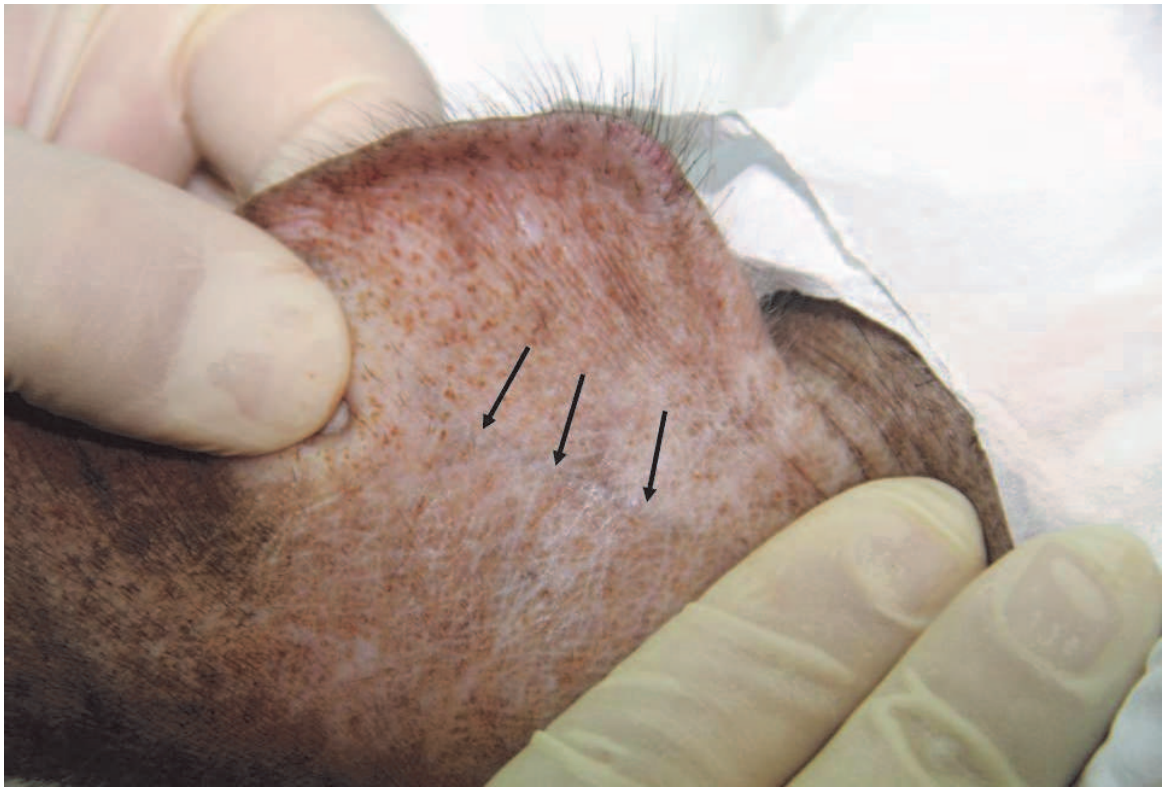


Figure 2. Localization of vena metatarsalis plantaris by palpation

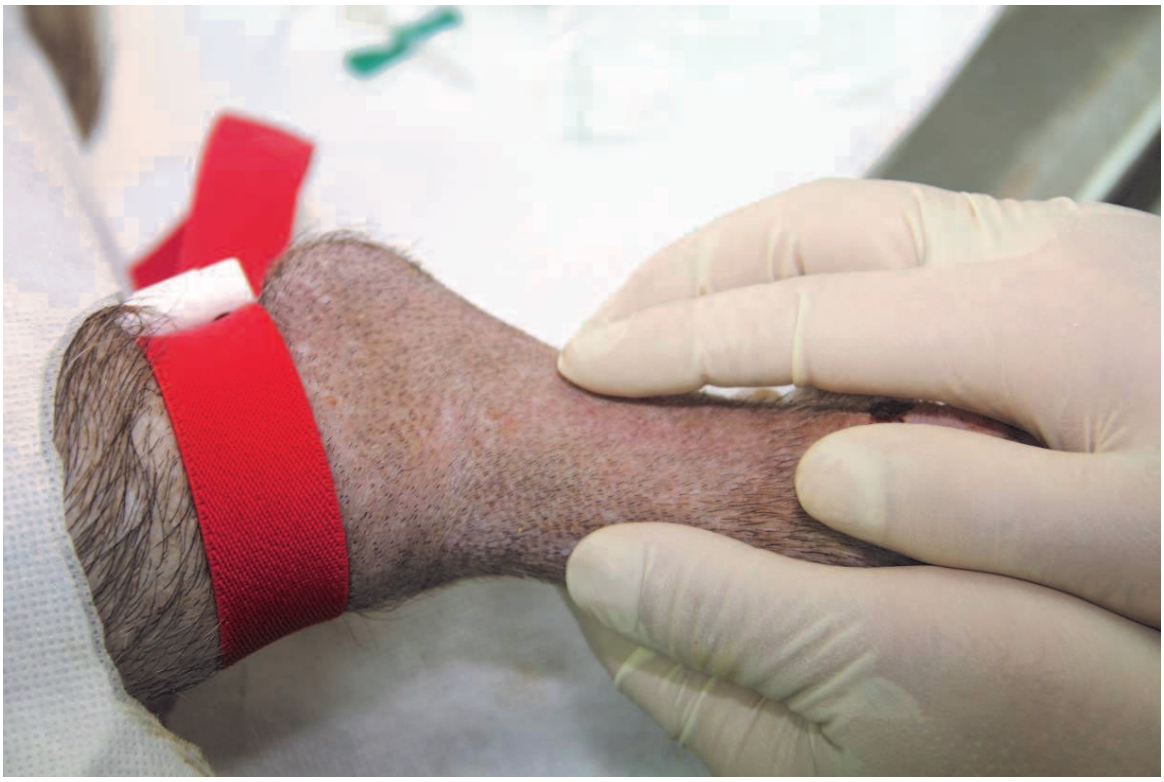
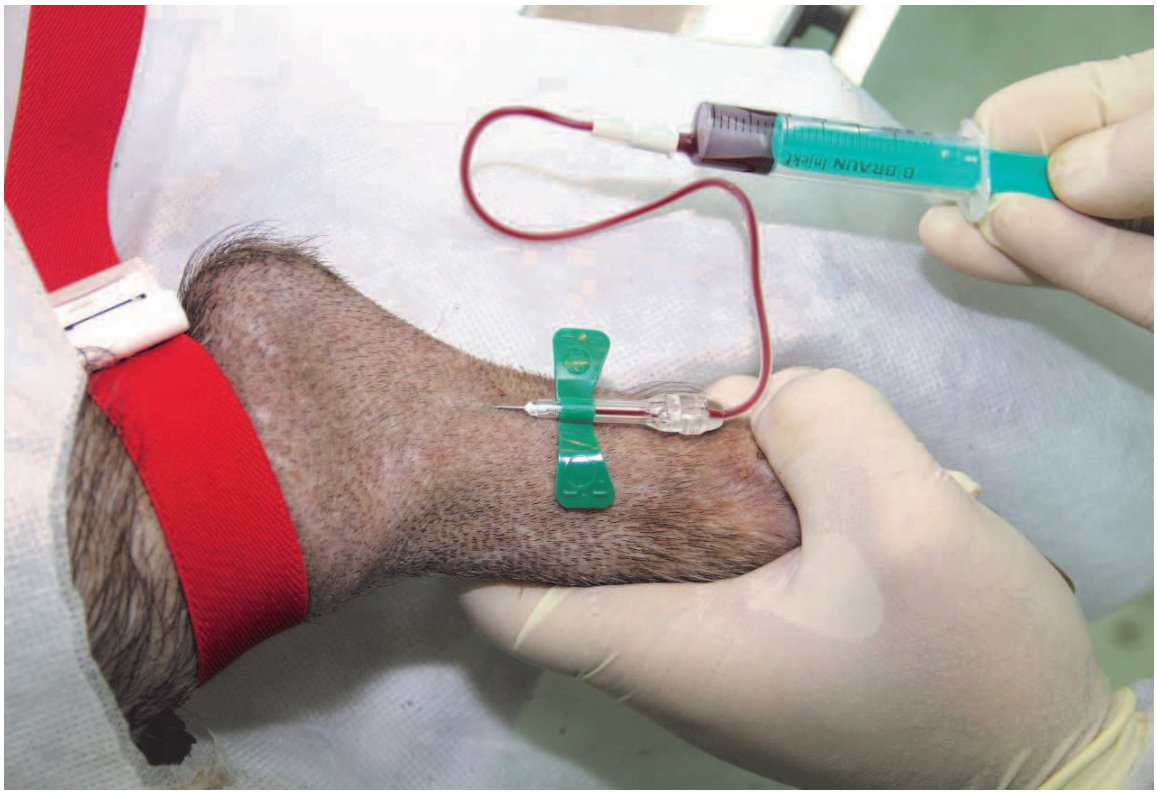


Figure 3. Aseptic bleeding of the vena metatarsalis plantaris for haemoculture



Discussion and Conclusion

Collection of blood from metatarsal veins is a common technique in many species of both wild and laboratory animals, including rodents and birds (Krista et al., 1988; Joslin, 2009). Although catheterisation of *v. saphena* has been described in pigs (Anderson et Jean, 2012), venepunction or catheterisation of metatarsal veins is not commonly recognized as technique for bleeding.

In our experience, in minipigs these veins are not visible, but can be found by careful palpation after swathing the leg with a tourniquet. Nevertheless the metatarsal veins are palpable only in a limited area of the metatarsus. We observed that in most animals, *v. metatarsalis plantaris* was easier to palpable than *v. metatarsalis dorsalis*. The correct localization of the vein is therefore critical for this technique to succeed and requires adequate training.

On the other hand, once mastered this approach allows the insertion of a needle or i.v. catheter at the first attempt. The sterility of all blood samples collected in this manner was confirmed in all the animals included in this trial. The diameter of *v. metatarsalis plantaris* and *v. metatarsalis dorsalis* in all the adult Kostelec minipigs allowed easy collection of more than 50 ml of blood and the repeated access to these veins by 21 gauge catheter was well tolerated.

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Authors wish to thank Jan Kučera, M.Sc. for fotodocumentation of the procedure, and to Jana Matonohová, M.D., Gabriela Kubíčková, M.Sc., and Petr Daněk, D.V.M., Ph.D. for their technical assistance.

The experiment was part of Project 5/2011 approved by The Ethics Committee of the Institute of Animal Science in Praha and by the Central Commission for Animal Welfare at The Ministry of Agriculture of the Czech Republic.