

Letter

Blood collection by gingival puncture on hamsters reduces animal number in leptospirosis virulence tests

Leptospirosis is a worldwide zoonotic bacterial disease caused by pathogenic strains of *Leptospira* sp. (Adler, 2015). Infection occurs through direct or indirect contact with contaminated urine from a host, such as domestic animals and rodents. In humans and dogs the infection can result in severe acute disease including renal failure and pulmonary hemorrhagic syndrome, with a high ratio of mortality (Allan et al., 2015). In contrast, livestock presents a reproductive infection, leading to abortions, stillbirth or neonatal mortality. In order to minimize its impact, control includes antibiotic therapy (e.g., dihydrostreptomycin) and vaccination. Commercial vaccines are bacterins of different pathogenic strains of *Leptospira*, but immune response related to vaccination is limited, being short and serovar-specific. Thus, studies regarding the development of new vaccines are needed (Adler, 2015).

Inoculation of hamsters with *Leptospira* is performed for virulence tests as well as for the evaluation of vaccine efficacy (Suepaul et al., 2010). The Golden Syrian hamster (*Mesocricetus auratus*) is considered the best model for experimental infection of leptospirosis. The animals present good susceptibility to various leptospiral pathogenic strains and the resulting clinical disease is largely reproducible (Zuerner and Palmer, 2012). The virulence test identifies virulent strains for which vaccine candidates need to be developed (Adler, 2015). Virulent strains are capable of infecting the animal, surviving its natural defenses, and causing lesion formation.

Two hamsters are used per passage. The first is euthanized at day 4 p.i. for collection of blood samples by cardiac puncture to recover the inoculated strain by bacterial culture and to confirm infection. The other animal remains alive until clinical disease is evident, or until day 21 p.i., for necropsy. Four passages are recommended, totaling eight hamsters per strain (Silva et al., 2008; Suepaul et al., 2010).

Different methods have been proposed for blood sampling in live hamsters, but many of them present drawbacks. Jugular vein or cardiac puncture require anesthesia, while cardiac puncture causes serious tissue damage and usually death (Hartwig et al., 2013; Lindstrom et al., 2015). Puncture of the retro-orbital venous plexus may lead to blindness. A blood sampling technique not requiring anesthesia could reduce animal use and reduce the stress on animals undergoing this procedure.

Following the concepts of the 3Rs – Replacement, Reduction, and Refinement (NRC, 2011) – our group attempted to collect blood samples by gingival vein puncture. This uses the *labialis mandibularis* vein located in the gingival papillae region just below the pair of mandibular incisors. This technique was originally described for rats and mice (de Oliveira et al., 2009), but had not to our knowledge been applied to hamsters before. Briefly, one hamster aged 6–8 weeks is inoculated intraperitoneally with a 10^8 dose of *Leptospira*. At day 4 p.i., instead of cardiac puncture, the animal is properly restrained in the hand of the experimenter and maintained in a dorsoventral position, while the gingiva is exposed. Taking an angle of 20 to 25° along the line between the pair of incisors, a 13 x 4.5 mm needle is inserted about 2 mm into the gingiva (Fig. 1). This technique allows approximately 100 µl of blood to be sampled over a 1 min period of a single sampling event. The blood samples are immediately seeded in culture medium tubes and incubated.

Up to now, we have applied the technique to 14 animals. From all of them good quality blood samples were successfully obtained, and *Leptospira* were recovered in culture from seven of these. *Leptospira* is very difficult to cultivate, thus a 50% recovery is not surprising. Besides, we have been testing the virulence of leptospiral strains obtained from cattle. Many of the strains are adapted to cattle and it is expected that some of them are not infective to hamsters. Thus, the failure to recover *Leptospira* from the other seven hamsters probably reflects those two bacterium-related points, and does not seem to be affected by the sampling technique. Despite the failure to recover *Leptospira* from blood of these seven hamsters, petechiae in lungs were observed on two of them at day 21.

Besides preventing unnecessary animal suffering, the application of this technique to hamsters for virulence tests of leptospiral strains allows a reduction by 50% of the number of animals, since the animal remains alive and the same animal can be used for the second step of the experiment. It has been stated that the technique presents low levels of pain (de Oliveira et al., 2009). Moreover, it can be conducted without anesthesia, so not interfering with renal and hepatic physiological functions of the animal, which could represent a bias for the second phase of the test. None of the studied animals presented consequences of the sampling. Despite the advantages of this

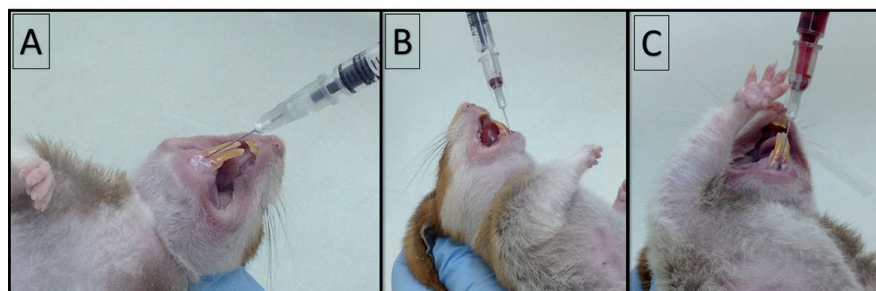


Fig. 1: Three steps for blood collection from labialis mandibularis vein of gingival papillae region in hamster
Animal holding and gingival expose (A), insertion of the needle taking an angle of 20 to 25° (B) and sampling (C).

technique, it has been poorly explored worldwide. We believe that the technique presents an enormous potential, not only for leptospirosis studies, but also in other fields of animal experimentation, such as odontology and immunology, bacteriology (hemoculture), molecular tests (PCR) or any experiment that requires up to 100 µl of blood.

Refinement and rational use of animal models is mandatory for scientific research. Although we cannot yet replace *in vivo* models in leptospirosis laboratories, we can refine their use and reduce the number of necessary animals. It is impossible to estimate the number of hamsters used for experiments on leptospirosis or other diseases worldwide, but only in the USA around 120,000 hamsters were used for research in 2014 (USDA, 2015). Of those, a large percentage was probably sacrificed in order to obtain blood samples.

Applying the technique of gingival collection of blood samples for the first time on hamsters, we could reduce the number of animals without compromising the reliability of our results concerning the virulence tests of leptospiral strains. This represents a remarkable refinement of the test and we believe that this practice should be encouraged.

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