A new technique for worm burden assessment of Angiostrongylus vasorum in experimentally infected foxes (Vulpes vulpes)

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Introduction
Assessing the Angiostrongylus vasorum worm burden of a host animal is very complicated due to its very delicate location in the peripheral branches of the pulmonary arteries. So, in field surveys approximate quantification is usually done by dissecting the pulmonary arteries and lung tissue, resulting in a high degree of worm damage and subsequent identification problems.

Perfusion technique
In a large-scale angiostrongylosis study including 75 experimentally infected foxes, a novel technique for reverse perfusion of the cardio-pulmonary vascular system was developed in order to recover as many intact worms as possible. Anaesthetized foxes were given heparin i.v. (350 IU/kg) in order to prevent blood clotting during post mortem. Three minutes later a lethal pentobarbital dose (100 mg/kg) was given, the thorax was opened and the thoracic organs were perfused in situ. The aorta, vena azygos and both venae cavae were clamped off. About three litres of isotonic perfusion liquid (sodium citrate, 15 g/l + NaCl, 8.6 g/l dissolved in tap water) were pumped via a 16G needle into the left auricle, through the pulmonary veins, the lung capillaries and the pulmonary arteries to the pulmonary trunc from which it was led via a plastic pipe (5 mm diameter) onto a fine sieve (200 µm aperture) for collection of worms. This procedure was followed by removal of the lungs, dissection of the pulmonary arteries and subsequent baermannization of the chopped lung tissue in normal saline. The detailed procedures are shown in figs. 1-10.

Results
By estimating the total worm burden as the sum of recoveries made by perfusion, dissection and baermannization, respectively, 59%, 28% and 13% of the total worm burden (3,311 worms) were recovered by each of these three methods, respectively (see fig. 11). In contrast to dissection and baermannization, however, close to 100% of the worms recovered by perfusion were intact.

Conclusion
There is no golden standard technique for quantification of adult A. vasorum worm burdens. Thus, despite the new perfusion technique is superior for isolating intact worms, optimal worm burden assessment may only be achieved in combination with the two traditional (and laborious) techniques, i.e. comprehensive dissection of the pulmonary arterial branches followed by chopping and Baermannisation of the remaining lung tissue.

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