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ORIGINAL PAPER

Viral infection risks for patients using the finished product *Hirudo verbana* (medicinal leech)

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Abstract The virological safety of medicinal leeches has to be ensured prior to their use on patients. While leeches can be kept and bred under standardized conditions, feeding them horse blood adds a non-standardized component, which poses some risk of infection of the treated patients. Here, we investigated the speed at which blood-borne viruses are degraded by the microbial flora in the leech intestine, in order to define the safety of the product and the length of the necessary quarantine period prior to its administration to patients. Feeding blood was spiked with bovine viral diarrhea virus (BVDV), reovirus, and murine parvovirus (10^7 $ID_{50}ml^{-1}$). The virus titer in the intestinal contents of the leeches was determined using permissive cell cultures and compared to that of the original virus titer at the following time points: immediately after feeding; after 3, 14, and 30 days; and monthly thereafter until the 7th month. The BVDV titer was below the detection limit of 10^1 $TCID_{50}ml^{-1}$ after 3 months, while reovirus and murine parvovirus titers were undetectable after 4 months. No positive virus findings were obtained at later time points. Thus, when fed the blood of vertebrates, the finished product "Medicinal leech, *Hirudo verbana*" can be considered virologically safe if the animals are maintained at 20 °C, which corresponds to their natural habitat conditions and ensures a high metabolic rate. Therefore, after the last feeding, a quarantine period of 4–6 months and appropriate care at room temperature, which supports microbial degradation and digestive processes, are recommended.

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Introduction

The use of medical leeches is one of the oldest known natural remedies and has been used for various therapeutic applications for centuries (Kaehler Schweizer & Westendorff, 2013). Under natural conditions, juvenile medical leeches feed on various kinds of aquatic creatures. In addition, older animals also feed on the blood of vertebrates, in particular, that of amphibians and occasionally even warm-blooded animals. Potential hosts include ungulates and other large warm-blooded mammals, such as humans.

The blood obtained by feeding is stored in the intestinal tract of the leech for months. Digestion can take place over a period of more than 1 year, with the help of intestinal bacteria (e.g., *Aeromonas hydrophila veronii*). These bacteria control the degradation process in the intestine and are also responsible for the preservation of the consumed food. For the digestion process, the temperature at which the animals are maintained seems to be particularly important: room temperature (20 °C), which is the water temperature preferred by leeches for their natural habitat, is optimal.

It remains unclear whether zoonotic pathogens, particularly viruses, acquired via the blood and present in the intestinal contents of the leech, remain infectious in the long term. Thus, it is possible that improper handling of the leeches (tearing or bruising during treatment) can pose a residual infection risk for patients. In this study, we investigated the survival of the viruses under the natural intestinal conditions of the leech, in order to clarify the required quarantine period, between the last feeding of the medicinal leech and human use, in order to eliminate infection risks for patients. Moreover, we investigated the optimal temperature at which the animals should be