

Hematologic and Electrolyte Changes in Little Brown Bats Treated for White Nose Syndrome

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Introduction

White Nose Syndrome (WNS) is a devastating fungal disease that can affect whole colonies of hibernating bats. It is caused by *Pseudogymnoascus destructans* (*Pd*), a fungus that invades the epithelium of the bats' wings. The exact mechanism of the disease that causes the death of the animal is unknown, but it has been hypothesized that the fungus causes damage to the wing epidermis resulting in interruption of thermoregulation and water retention, leading to dehydration and an electrolyte imbalance. The disruption of normal physiologic homeostasis during hibernation leads to substantial morbidity and mortality. To date, there are no proven treatments or successful disease control protocols. Recently a potential treatment has been discovered and is currently being tested. The purpose of the present study is to determine the effectiveness of this new potential treatment as it relates to hematologic and electrolyte changes in Little Brown Bats (*Myotis lucifugus*) (Fig. 1).

Materials and Methods

- Six wild caught WNS-positive Little Brown Bats (*Myotis lucifugus*) were placed in hibernaculum chambers (9°C, 90% humidity). Four of these were heavily infected (Fig. 2 & 3) and two were minimally infected, determined by a quantitative PCR method.
- The treatment, a volatile organic compound (VOC), was placed in a petri dish inside the chamber and allowed to aerosolize. All of the bats were exposed to the VOC for 24 hours. Those bats that were heavily infected were exposed to the VOC for another 24 hours one week following the first treatment.
- Ten weeks post-treatment the bats were anesthetized and blood was collected from the interfemoral vein by puncturing the vein with a 26- to 28-gauge needle and collecting the blood in a plastic hematocrit tube (Fig. 4). Another blood sample was taken 8 weeks later using the same technique.
- Complete blood counts (CBC) and plasma electrolytes were analyzed using automated hematology and clinical chemistry platforms. Blood smear examination was performed and white blood cell morphology compared to normal bats (Fig. 5).



Fig. 1 *Myotis lucifugus*



Fig. 2 Gross lesions on the wing of *Myotis lucifugus*. Gross wing lesions can include a multifocal “crepe-like” wrinkling appearance (arrows).

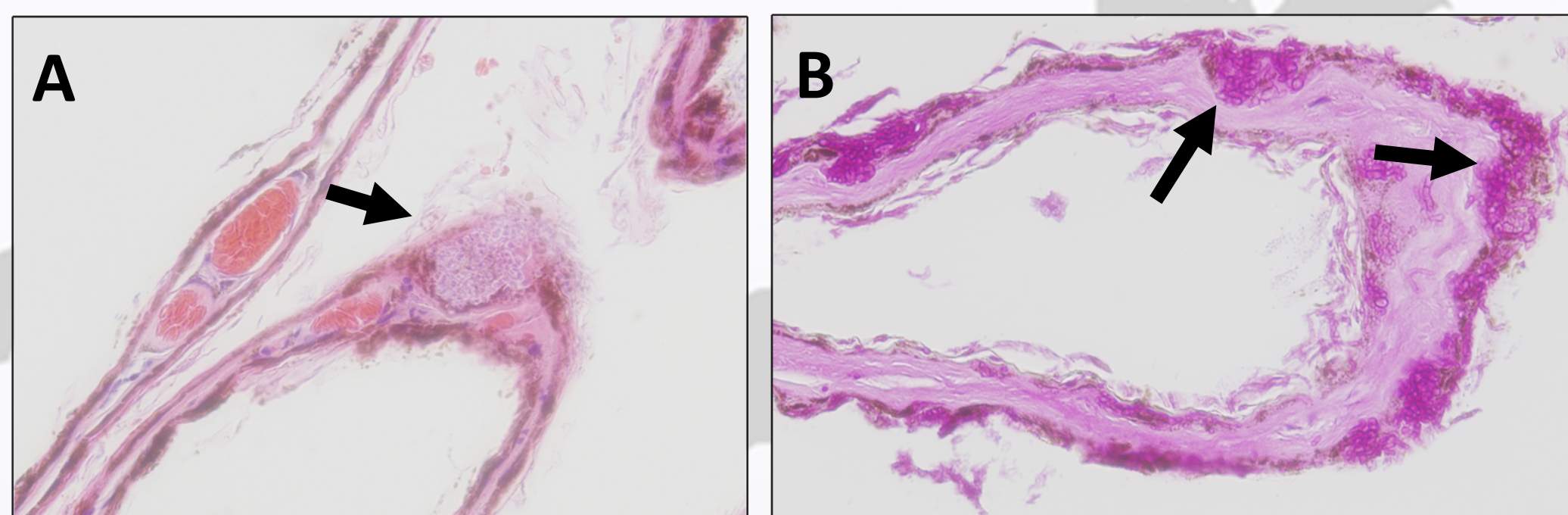


Fig. 3 Histopathology of the wing reveals a heavy growth of fungal hyphae (arrows) on the epidermal surface and invading into the dermal layer (A: H&E, B: PAS stain).

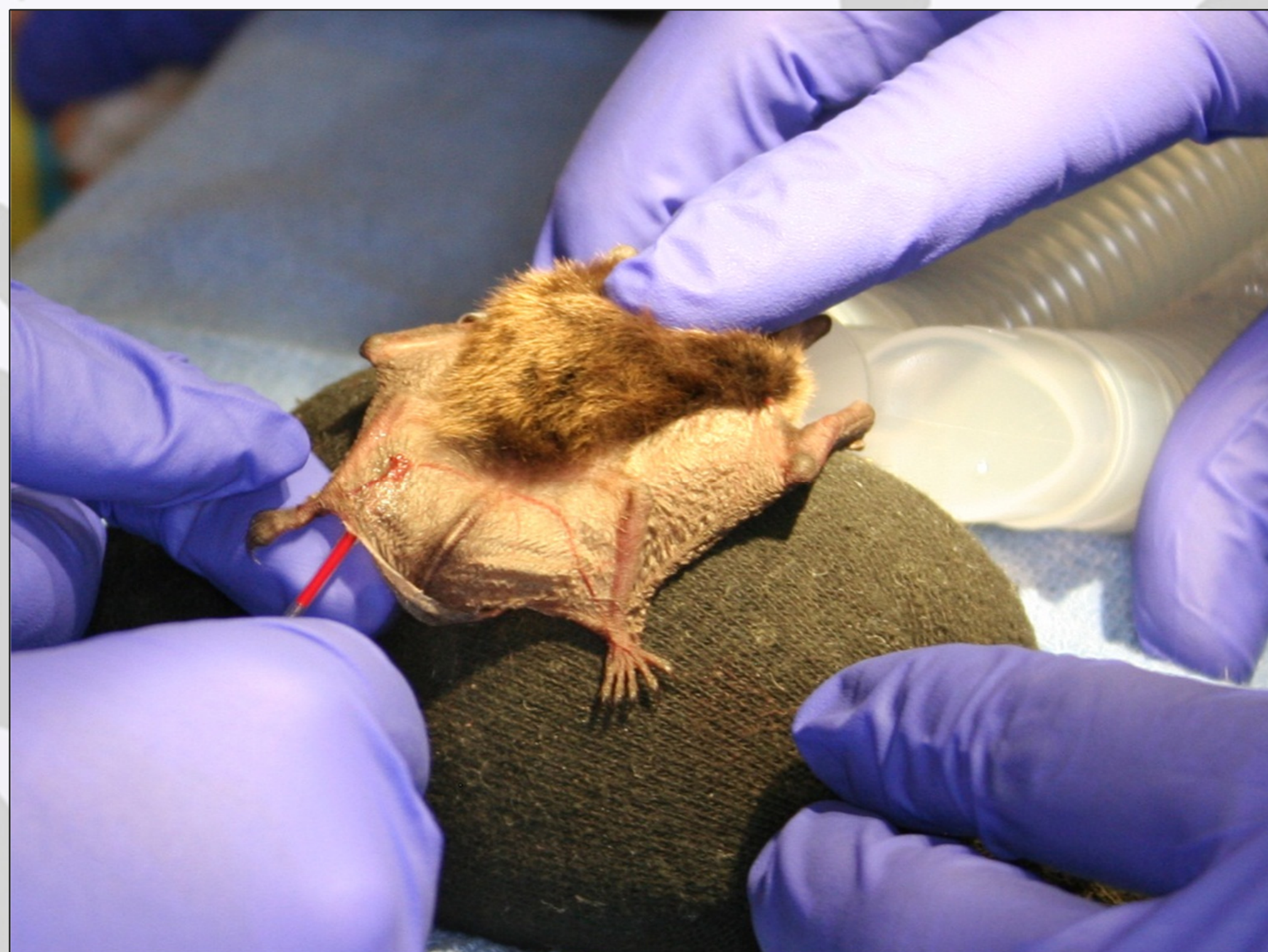


Fig. 4 Blood is collected from the interfemoral vein, found within the uropatagium between the femur and the tail. No more than 10% of the total blood volume was taken from each bat.

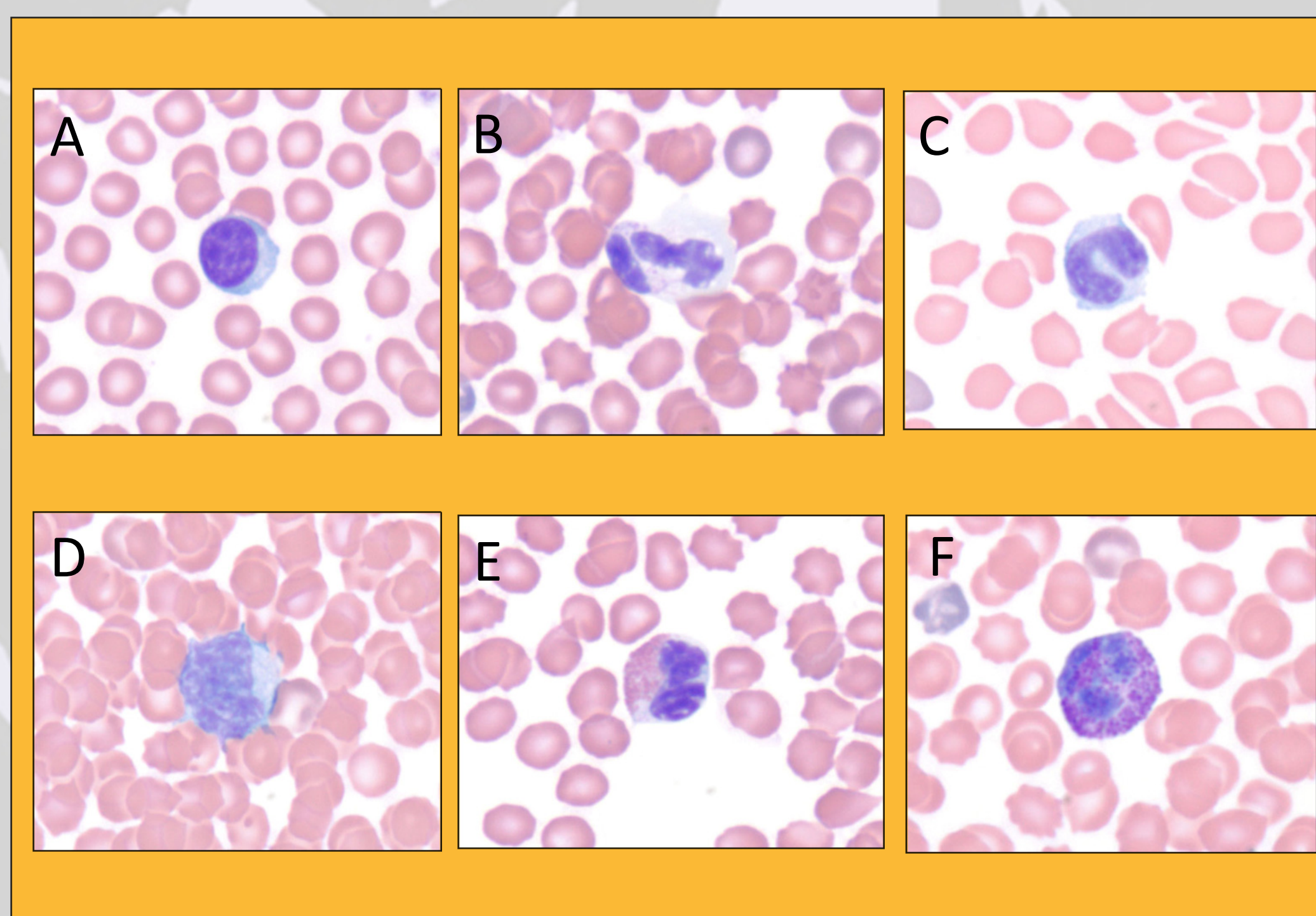


Fig. 5 Examples of (A) lymphocyte, (B) neutrophil, (C) band neutrophil, (D) monocyte, (E) eosinophil, and (F) basophil from a normal *Myotis lucifugus*. White blood cell morphology resembles normal mice.

Results

All the bats tolerated the blood collection well and showed weight gain and improvement in activity level post-treatment. Most of the heavily infected bats revealed marked decreases in plasma sodium and chloride levels at 10 weeks post-treatment compared to the minimally infected bats (Table 1). Additionally, at the 18 week sampling, the heavily infected bats showed marked improvement in plasma sodium and chloride levels (Table 1). No significant changes were noted on the CBC on either sampling day and no significant white blood cell morphologic changes were noted.

Fungal Load	10 wks Na (mEq/L)	18 wks Na(mEq/L)	10 wks Cl (mEq/L)	18 wks Cl (mEq/L)
Minimal	149.6	144	114.1	113
Minimal	151.4	148	121.7	118
Heavy	85	140	67.5	113
Heavy	85	141	68.5	113
Heavy	125.8	147	102.7	117
Heavy	144	146	113.7	113

Table 1 Plasma electrolyte levels from *Myotis lucifugus* 10 and 18 weeks post treatment.

Conclusions

This study supports the hypothesis that the *Pd* fungus on the bats' wings leads to an electrolyte imbalance. It also suggests that, in regards to this imbalance, the VOC may be effective in treating White Nose Syndrome.

Future Directions

Determine the mechanism of action of the treatment as to whether the VOC is killing the fungus or inhibiting its growth. Potential future experiments include obtaining wing biopsy samples and subjecting tissue to live/dead stain protocols to determine fungus viability or obtaining cultures from affected wings to determine growth viability of the fungus following treatment.

References

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- Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G, and Willis CKR. 2013. Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. Biol Lett. 9(4).

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