Livestock rearing is associated with smaller red blood cells and greater variation in cell size in common vampire bats (*Desmodus rotundus*)

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BACKGROUND

- Novel food provided by human activity can affect wildlife behavior, physiology, and disease spread
- The common vampire bat (*Desmodus rotundus*) is distributed across Latin America and is a reservoir for zoonotic pathogens
- Expansion of livestock rearing may provide blood-feeding bats with accessible and common food
- Frequent feeding could reduce starvation and improve nutrition, affecting survival and immunity
- Nutrition can be hard to measure in vampire bats due to rapid mass loss during starvation and to small blood volumes that can be safely obtained
- Red blood cell (RBC) size has been validated in captivity (e.g., camels) to determine nutritional condition
- Smaller RBCs can indicate dehydration, while greater variation in RBC size may suggest anemia

OBJECTIVES

- Analyze size and variability of RBCs collected from bats across sites of varying livestock density
- Test if livestock expansion is associated with improved nutrition (larger and less variable RBCS)
- Account for factors (e.g., climate) that could also influence RBC size

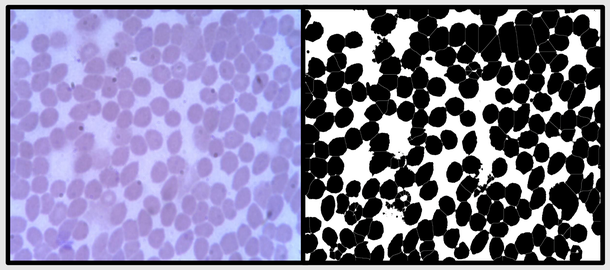
METHODS

Blood collection

- Samples were collected in 2013 to 2014 across three districts of two departments of Peru
- Bats were captured with mist nets and banded
- Blood was collected from the propatagial vein
- Two blood smears were immediately prepared and stained with Camco QuikStain II
- No bats were held for more than two hours

RBC size measurements

- Slides were examined with 100X oil immersion
- Motic Images Plus software was used to take pictures of 10-20 fields of view for each slide
- Images were opened within ImageJ software
- Scale was set to 19.5 pixels to 1 µm
- Images converted to 8 bit grayscale
- Watershed was applied to identify cells (Fig. 1)
- Wand tool was used to manually add individual non-overlapping RBCs to ROI manager
- Area and Feret's diameter measured for 50 cells



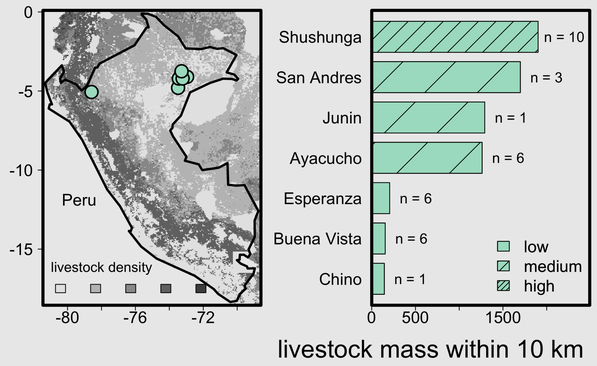
- Fig 1. Example of 100X image from blood smear (right) converted into binary watershed (left).

DATA ANALYSIS

- Two morphology metrics calculated within R:
- Mean RBC diameter (µm)
- RBC diameter coefficient of variation (CV)

- Livestock density was calculated from FAO
- Total cattle, pigs, and chickens within 10 km with each animal weighted by average mass in kg
- Classified into even bins with Jenks breaks

- Linear regression to test relationships between livestock density and RBC metrics
- Also tested relationships with biologically relevant covariates (e.g., latitude, age, sex)
- Used AICc and Akaike weights to assess relative evidence for different hypotheses
- Effects of livestock density on RBC size after adjustment were shown with *visreg* package

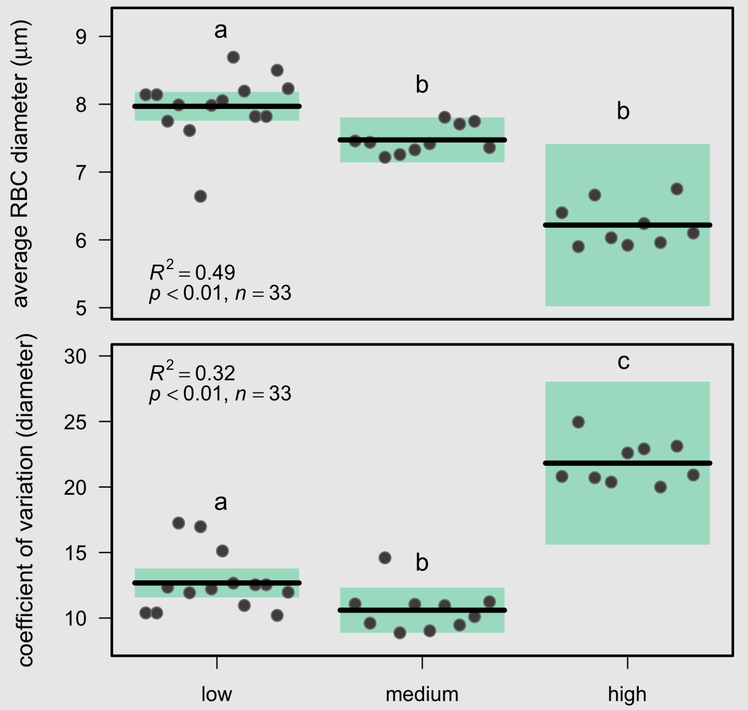


- Figure 2. Study sites across livestock density gradient (grey shading, left). Due to discrete value divisions, livestock density was classed into even categories (black shading, right).

RESULTS

- Slides analyzed from 33 bats, 7 sites (Fig. 2)
- Model ranking with AICc identified livestock density as the best predictor of RBC diameter (w_i = 99%) and size variation (w_i = 95%)
- Absolute latitude (w_i < 5%) was then included in livestock models to adjust for temperature
- After adjusting, livestock mass was correlated with smaller RBCs and larger CV (Fig. 3)

models	ΔAICc	w_i
size ~ livestock density	0.00	99%
size ~ absolute latitude	10.34	0.5%
size ~ age, rep, sex, size	14.4+	0.5%
CV ~ livestock density	0.00	95%
CV ~ absolute latitude	6.17	4%
CV ~ age, rep, sex, size	10.8+	1%



- Figure 3. Model-predicted relationships between livestock density and RBC diameter (top) and variation (bottom) after adjusting for absolute latitude. Plots illustrate the predicted mean (black line), confidence bands (green), and partial residuals. Letters denote significant differences between means after adjusting for latitude and multiple comparisons.

DISCUSSION

- Increasing livestock density was associated with smaller RBCs and more variability in RBC size within individuals
- Suggests that vampire bats living in areas with widespread livestock rearing are more dehydrated and anemic
- Could result from reduced feeding opportunities through habitat overexploitation or greater food/space competition

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