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Ectoparasite abundance and diversity on farm animals in Monteverde and San Luis along an elevation gradient

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ABSTRACT

Abundance and diversity of arthropods are largely dependent on climate. Elevation gradients provide different microclimates that can support a unique number and variety of species. In order to test the effect of elevation on abundance and diversity of arthropod ectoparasites, I collected samples on four different farms: Life Monteverde and Benito Guindon’s farm in Monteverde at about 1,800 m, and Lelo Mata’s farm and Tim Sayles’ farm in San Luis around 700 m. At each elevation, I compared abundance and diversity of ectoparasites on dogs and cattle. I found a presence of *Ctenocephalides* and *Pulex* fleas on dogs, and 22 different morphospecies of ticks on cattle. Trends in the data show a higher abundance of fleas and a greater diversity of tick morphospecies sampled at low elevation. It is likely that ectoparasites will experience a change in abundance and distribution among and between elevations as global warming continues to adjust microclimates. Therefore, it is important to understand their current abundance and distribution to best prepare for the effects of climate change. Additionally, data from goat samples collected on the farms in Monteverde reveal a presence of *Damalinia caprae* lice. The higher number of adult and total lice on Life Monteverde indicates a more-established population than the nymph-dominated population on goats at Benito Guindon’s farm. This difference in abundance is due to differing ectoparasite control practices and living conditions of animals on each farm. Difference in lice abundance between farms at the same elevation is evidence that ectoparasite abundance can be controlled through best practice management.

Abundancia y diversidad de ectoparásitos en animales de fincas en Monteverde y San Luis

RESUMEN

La abundancia y diversidad de artrópodos dependen mucho del clima. Los gradientes de elevación proveen diferentes microclimas que pueden mantener una única y variada composición de especies. Para evaluar el efecto de la elevación en la abundancia y diversidad de artrópodos ectoparásitos, yo recolecté muestras en cuatro diferentes fincas: Life Monteverde y de Benito Guindon en Monteverde a alrededor de 1800 msnm, y las de Lelo Mata y Tima Sales en el Valle de San Luis, cerca de los 700 msnm. En cada elevación, yo comparé la abundancia y diversidad de ectoparásitos en perros y vacas. Detecté pulgas de *Ctenocephalides* y de *Pulex* en perros, y 22 morfo especies de garrapatas en las vacas. Las tendencias en los datos muestran mayor abundancia de pulgas y mayor variedad en las morfoespecies de garrapatas muestreadas a menor elevación. Es de esperar que los ectoparásitos cambien en abundancia y distribución dentro y entre elevaciones a medida que continúe el calentamiento global. Es importante entender sus distribuciones actuales y prepararse para los efectos de cambio de clima. Adicionalmente, datos de muestreos en cabras en Monteverde reveló la presencia de piojos *Damalinia caprae*. El mayor número de piojos adultos y número total de piojos en Life Monteverde, indica una población mejor establecida que la población dominada por ninfas encontrada en la finca de Benito Guindon. Esta diferencia en abundancia se debe a diferencia en prácticas de control sobre ectoparásitos, así como condiciones generales de los animales. Diferencias en abundancia de piojos entre las fincas a la misma elevación es evidencia que su abundancia puede ser controlada mediante buenas prácticas de manejo.

The rising global temperature is altering the distribution of microclimates, which is in turn affecting the distribution of many organisms. It is predicted that changes in temperature, precipitation, humidity, and other climatic factors will affect the reproduction and population dynamics of parasite arthropod vectors of disease (Gage *et al*, 2008). Increasing temperatures are projected to expand the distribution of these arthropods, and with them, the diseases they carry, which could endanger host communities parasitized by these vectors (Zamora-Vilchis *et al*, 2012). Historically, the low temperature climate of the high elevations helps to reduce the abundance of parasite vectors. Other factors such as reduced habitat, reduced resource diversity, and reduced primary productivity at high elevations also contribute to the lack of arthropods (Zamora-Vilchis *et al*, 2012; McCoy, 1990). In contrast, the higher temperatures and higher humidity in lowland areas provide an ideal environment for reproduction of these disease-carrying parasites. However, the rising global temperatures are causing the disappearance of the factors that have kept ectoparasites out of the lowlands. This could lead to an increased prevalence of parasitic arthropods at higher elevations (Zamora-Vilchis *et al*, 2012).

Parasites affect all mammal species, and can be especially troublesome for domesticated mammals. Ectoparasites can be present on the surface of the entire body of farm animals such as cattle, goats, and even cats and dogs. Because these animals live in such close quarters, ectoparasites are easily transferred among individuals (Poissant *et al*, 2008). Farmers need to know which species are currently parasitizing their animals, and what species are likely to parasitize them in the future in order to control parasite populations. If not controlled, the ectoparasite load on an animal will begin to affect its health, resulting in losses of livestock production including milk, meat, and pelt sales that exceed $2.26 billion annually (Byford *et al*, 1992). Additionally, this topic is relevant to human health, as some ectoparasites are known to be vectors of disease that affect humans. For example, a specific type of flea called the *Pulex irritans* is able to feed from a variety of mammals, including cats, dogs, goats and humans, and also has been known to transmit plague which is a zoonotic disease that can be transferred to human beings (Bitam *et al*, 2009). Additionally, ticks from the genus *Ixodes*, which are often present on cattle and other livestock, can cause Lyme disease in humans (Kasper, 2015). It is even easier for diseases caused by ectoparasites to be transferred between domesticated animal populations to wild ones, introducing a new source of problems regarding controlling ectoparasites and disease in the wild.

However, farmers have the power to control the transmission of ectoparasites on their farm animals, lessening the impact on wildlife and the chances of transmitting disease. Through certain management practices, such as burning and clearing fields of potential ectoparasite harboring vegetation, creating larger living spaces and grazing areas to limit contact between animals, and regularly grooming and treating animals for ectoparasites ectoparasite populations can better avoided or controlled (Texas A&M Agrilife Extension, 2016; Smith, 2010). A study by Stanko and others (2002) found that host density has a major influence on the species richness of ectoparasite communities. This emphasizes the importance of keeping populations at low density by providing adequate sized living spaces. Additionally, insecticide treatments such as sprays, dips, pour ons, dust, and others are also important for controlling ectoparasite population numbers (Cornell Department of Entomology, 2016). It is important for farmers to become educated about the time of the year that is most effective to administer specific ectoparasite treatments, the safest and most effective dosage for each, and the frequency with which to treat their animals to achieve the most effective results.

As the weather changes and distribution of these ectoparasites adjusts over time, this could result in a newfound struggle to control populations of ectoparasites. There is not much literature on ectoparasite abundance and diversity on farm animals at different elevations. It is important to recognize current abundance and diversity patterns in order to better understand environmental preferences of ectoparasites, and how the changing climate could affect these patterns in the future. Past studies support a correlation between arthropod abundance and elevation, though I could not find any literature about the effect of elevation gradient on ectoparasites specifically. This led me to ask whether ectoparasite abundance and diversity does differ between high and low elevation. Due to the fact that it is more difficult for most arthropods to survive in a high elevation environment with lower humidity, cooler temperatures, and reduced resource diversity (Zamora-Vilchis *et al*, 2012; McCoy, 1990), I predicted that the abundance and diversity of ectoparasites on animals would be lower at high elevation farms, and higher at low elevation farms. Additionally, because previous studies indicate a correlation between host density and ectoparasite diversity and livestock management recommends frequent ectoparasite treatment, I predicted that animals on same elevation farms with larger living spaces more recent ectoparasite treatment would sustain lower ectoparasite abundance and diversity.

MATERIALS AND METHODS

STUDY SITES-- Between the dates of 21 November 2016 to 26 I collected samples on four different farms in Monteverde, Puntarenas, Costa Rica. This includes Life Monteverde (High 1) and Benito Guindon's farm (High 2) in Monteverde at about 1,800 m and Lelo Mata's farm (Low 1) and Tim Sayles's farm (Low 2) in San Luis around 700 m.

STUDY SUBJECTS--

	Dogs	Goats	Cattle
Life Monteverde (High 1)	3	5	
Benito Guindon (High 2)	1	5	4
Lelo Mata (Low 1)	4		5
Tim Sayles (Low 2)	2		6

SAMPLING METHODS--

DOGS-- I ran a thin-pronged lice brush through the hair on the rear end of the dog, and placed my finger on any fleas on the comb. I then pinched the fleas and placed them in a small plastic tube filled with alcohol to kill and preserve them. I repeated this process a total of 5 times on the rear end of the dog, and 3 times near the head and collar area. If the dog hair was too long to pass through the comb, I would spend no more than 5 minutes searching through the hair near the rear end and another 5 minutes searching near the head and collar area for a total of 10 minutes per dog.

CATTLE-- To sample the coat, I ran the thin-pronged lice comb through one side of the cattle's back, and used a plastic Ziploc bag to collect the sample of hair and ectoparasites off the brush. I repeated this process on the other side of the cattle's back, and once on each side of the neck. This process resulted in a total of 4 brush samples from each cattle. In order to remove ticks embedded in the skin, I used tweezers to collect ticks off the entirety of the body, focusing on the most areas with the highest concentration of ticks: on the udder and the rear end underneath the tail. I then placed the ticks in and small plastic tubes filled with alcohol.

GOATS—I used the same method used to sample the coat of the cattle.

IDENTIFYING/CATEGORIZING ECTOPARASITES-- I viewed all of the ectoparasites in a Petri dish with alcohol under a dissecting microscope from 10x to 40x magnification. I identified fleas and lice to genus level using identification charts and descriptions from the Chittagong Veterinary School, and Cornell University Veterinary Entomology websites (Cornell Department of Entomology, 2016; Texas A&M Agrilife Extension, 2016). I categorized ticks into morphospecies using shape, design, and coloration.

RESULTS

DOGS

Both higher elevation farms had a smaller average number of fleas per dog than the lower elevation farms. The averages show a difference of 5.83 more fleas per dog at low elevation farms (Figure 1). Both *Ctenocephalides* and *Pulex* genera of fleas were present at high and low elevation, although the *Ctenocephalides* genus was absent at a high elevation farm, and the *Pulex* genus was absent at a low elevation farm. There were more individuals of both species found at low elevation compared to high elevation, and about twice as many *Ctenocephalides* than *Pulex* individuals (Table 1). There was an average of 11 fleas per dog across the entire sample size and a single dog sampled on Low 1 with a flea abundance value of 33; three times the average flea abundance (Figure 2). This individual accounted for 30% of the total fleas collected.

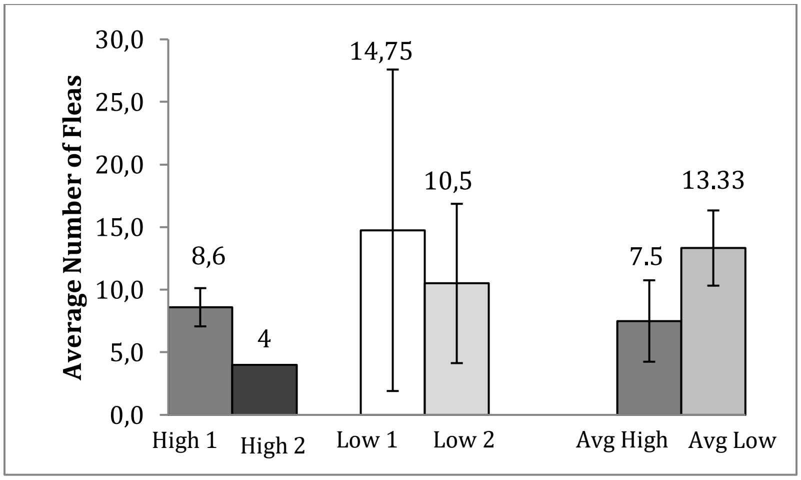

Figure 1. Dog Flea Abundance. Shows the average number of fleas collected off each dog at each farm. High 1 and High 2 are shown first followed by Low 1 and Low 2 averages. Finally, the total flea per dog average of high elevation and low elevation farms is presented.

Table 1: Dog Flea Species Diversity. Shows the number of *Ctenocephalides* and *Pulex* individuals on each farm.

Farm	***Ctenocephalides***	***Pulex***
High 1	10	8
High 2	0	2
Low 1	33	20
Low 2	17	0

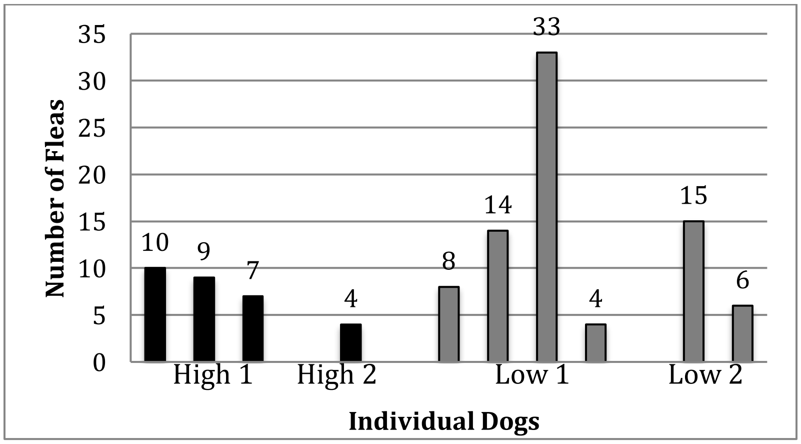

Figure 2. Distribution of Fleas on Dogs. Shows the total number of fleas sampled from each dog on each farm.

CATTLE

There was a lower number of tick morphospecies present at high elevation than at low elevation. There were also fewer unique morphospecies found on the high elevation farm than the low elevation farms (Table 2). There were only 2 overlapping morphospecies of tick between the low elevation farms. There were 7 morphospecies total found at high elevation with 3 species only found at this elevation, and 19 morphospecies total found at low elevation with 15 species only found at this elevation with an overlap of 4 species between the two elevations. The average number of tick species at each per cattle was 1.72 at low elevation, and 1.75 at high elevation, resulting in .03 more at low elevation. The average number of unique tick species per cattle was 1.36 at low elevation and .75 at high elevation, resulting in 0.61 more at low elevation (Table 3). Of the 22 morphospecies collected, only 9% of the species were present on all three farms, 14% were present on two farms, and 77% were present on only one farm.

Table 2: Number of Morphospecies of Ticks on Cattle Per Farm. Shows number of different morphospecies and unique morphospecies of ticks collected off cattle on each farm.

Farm	**# Species**	**#Unique Species**
High 1	7	3
Low 1	8	4
Low 2	14	11

Table 3: Tick Species Presence. Shows the number of species sampled off cattle at each elevation, the number of species found at both and the number of unique species found at each, and their averages for each elevation.

Elevation	**# Species**	**Average # Species**	**# Unique Species**	**Average # Unique Species**
High	7	1.75	3	0.75
Low	19	1.72	15	1.36

GOATS

There were approximately twice as many lice collected on High 1 than on High 2 with an average number of 10.6 lice per goat on High 1 and 5.4 lice per goat on High 2. There were about 4.5 times more adult *Damalinia caprae* lice on High 1 than High 2, and a similar number of *Damalinia caprae* nymphs present on High 2 and High 1. On High 1, the ratio of adults to nymphs was about 2:1, and on High 2, the ratio was about 1:2 (Figure 3). The distribution of lice on goats shows an average of 8 lice per goat. There were three individuals on High 1 with higher than average lice abundances of 10, 18, and 20. There was one individual on High 2 with an above average lice abundance value, 18. There was only one goat with a lice abundance value of 0, sampled on High 2 (Figure 4).

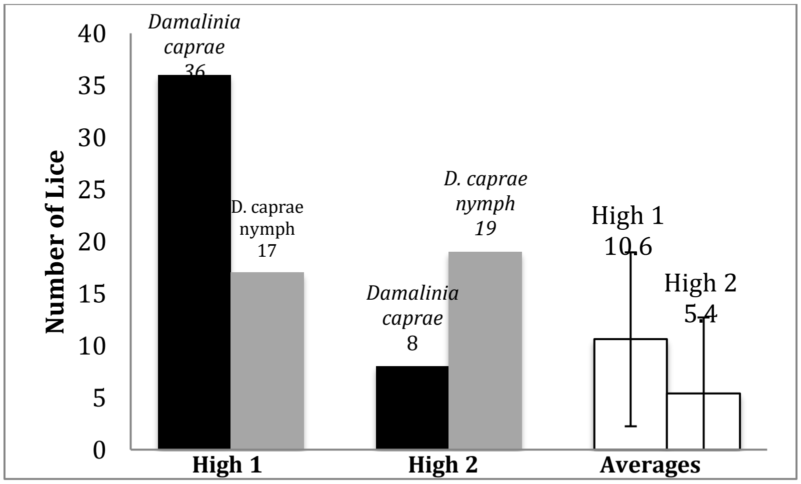

Figure 3. Goat Lice Abundance and Diversity. Shows the total number of lice sampled at each life stage on goats at each farm, both at high elevation. The graph also shows the total average lice per goat on each farm.

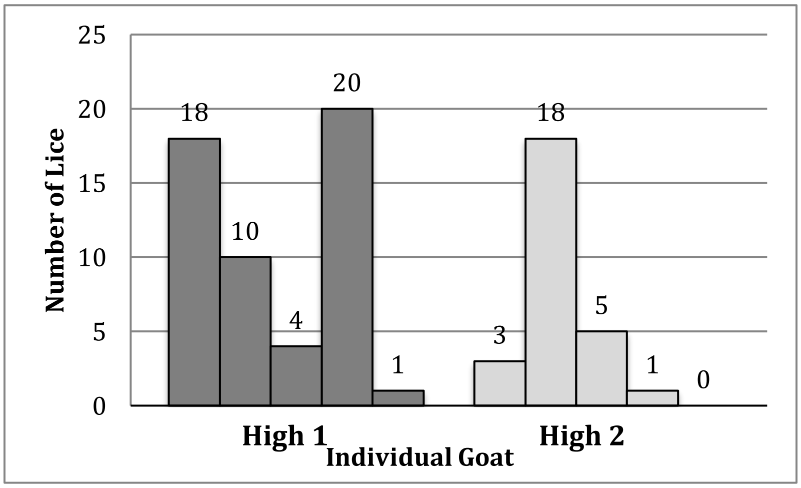

Figure 4. Distribution of Lice on Goats. Shows the total number of lice sampled from each goat on each farm at high elevation.

DISCUSSION

DOGS

The general trend of higher abundance of fleas sampled on the lower elevation farms supports my prediction, and could be due to a difference in environmental factors. Higher elevations are cooler, and experience more fluctuations in temperature than the warmer, and more stable temperatures found at lower environments (McCoy, 1990). According to Haas (1965), fleas experience the highest proliferation rates in locations with a warm, stable climate, and high percent relatively humidity. These environmental aspects are more consistent with the climate at lower elevations, which is likely why there was a higher abundance of fleas at low elevation in this study.

Though both species of fleas are present at both elevations, one high elevation farm only had *Pulex* fleas present, while another farm only had *Ctenocephalides* present (Table 1). This shows a slight preference for survival of one species over the other on different farms. A previous study by Duyck and collaborators (2006) about climatic niche partitioning of fruit flies found that some climatic niches were able to promote coexistence between four fly species. Researchers discovered that each fly species exhibited a clear preference for a specific climate based on the tolerances limits of each species. This ultimately led to niche segregation at each environment, allowing the four species to share a general area with unique preference for specific microclimates. This same pattern of niche partitioning could be occurring between *Ctenocephalides* and *Pulex* fleas along the elevation gradient, resulting in a different abundance of each at different elevations. The data also shows that the total species abundance between the two species was about the same at high elevation, but that *Ctenocephalides* fleas greatly outnumbered *Pulex* fleas at low elevation. In a previous study on flea species infestations on dogs, it was found that 65.1% of the dogs were infested with *Ctenocephalides* alone, while only 18.6% of dogs were infested by *Pulex* alone. An even fewer percentage of dogs were infested with both species of flea (Yore *et al*, 2013). This also found that *Ctenocephalides* were the most abundant species of flea. It is likely that this genus is better equipped to outcompete *Pulex* fleas, which is why my data shows twice as many *Ctenocephalides* individuals than *Pulex* individuals found on dogs (Table 1). This emphasizes the importance of understanding diversity of ectoparasites in order to best control a specific population.

CATTLE

Low elevation farms had a greater number and average number of morphospecies as well as a greater number and average number of unique morphospecies only found at that elevation when compared to the high elevation farms (Table 3). This higher diversity of species supports my prediction and is likely due to the fact that the environment at lower elevations is warmer and more humid (McCoy, 1990). In order to survive, ticks need a humidity levels above their critical equilibrium humidity. They are dependent on moist microenvironments, which help prevent total body water loss and drops in hemolymph volume at low humidity (Hair *et al*, 1975). In a similar study of insects along an elevation gradient, it was found that maximum species richness was most often documented at the lowest elevation sampled (McCoy, 1990). Evidence also suggests that species turnover at relatively high rates is more plausible at lower elevations, further supporting its higher species richness (McCoy, 1990). This could also be because of larger number of herbivorous insect larvae, parasitoids, and insect predators are more abundant at higher elevations (McCoy, 1990). This helps support my data showing that there is higher tick morphospecies abundance at lower elevations where the higher humidity provides a more favorable environment and there are fewer insect predators.

There was a greater percentage of morphospecies that were present at just one farm than those that were present on multiple farms, with an overlap of 2 morphospecies between low elevation farms, and 4 morphospecies between high and low elevation. This lack of overlap between tick morphospecies on different farms is likely due to the fact that cattle from different farm environments are not in direct contact with each other and have limited roaming range, so there is no direct cattle to cattle transmission of ticks between cattle at different farms. They would have to be transmitted by some other source that is able to travel between different farm environments, such as another wild mammal. Even in this case, there are other factors such as climate and predation that could limit a tick's survival during or after transmission. The presence of specific species at each elevation provides further insight into area specific tick diversity. There were only 3 species of tick that were found only at high elevation, in contrast with 15 species found only in low elevation. This supports my prediction based on the hypothesis that the environment at low elevation is much more favorable for tick survival, allowing higher carrying capacity of ticks, more species diversity, and higher rates of species turnover (McCoy, 1990). It is likely that more of these species would be present at high elevation if they were able to survive in that climatic niche.

GOATS

Goats sampled on High 1 had about twice as many adult lice as nymph lice, and goats on High 2 had about twice as many nymphs as adults. These patterns are inverses of each other, indicating that each farm's lice population was at a different point in their life cycle at the time of sampling. The nymphs were between 10-20 days old at the time of sampling and are not able to reproduce, and the adult lice were more than 35 days old with developed ovipositors and are therefore able to reproduce (Murray and Gordon, 1968). The fact that there were more adults on and a greater total number of lice on High 1 indicates that this lice population is older and therefore more established. The large amount of reproductive adults on this farm indicates a mature population with a potential for exponential population growth. Due to the fact that lice reach reproductive maturity after 35 days combined with the amount of mature lice present, it is likely that that population had been reproducing for numerous months. On High 2, the greater presence of lice nymphs than adults indicates a more newly introduced lice population. The presence of more nymph lice under 20 days old than adult lice over 35 days old indicate that this population is most likely a newer population that has not been reproducing for more than a few months.

Though both farms are at a similar elevation with similar climate, there were almost twice as many lice on High 1 than High 2 (Figure 3). This could have been due to a variety of factors, such as size of indoor living space, density of goats, amount of time spent outdoors, and frequency and intensity of ectoparasite treatment (Smith, 2010). The farm, High 1, had 14 goats total that lived in an outdoor enclosure of about 200 square meters. The second farm, High 2, had 24 adult goats that lived in an outdoor area of more than 20,000 square meters. High 2 had 100 times more square meters than High 1 for less than two times the amount of goats. Also, the goats at High 2 are let out to graze all day, and only brought in at night, while the goats at High 1 are let out an average of 6 hours per day. Additionally, the indoor living area was relatively small on High 1, and larger on High 2. Consistent with my prediction, smaller indoor and outdoor areas and less time spent outside likely contribute to why goats on High 1 had higher lice counts than those on High 2. Higher concentrations of animals in smaller conditions allows for more contact, which results in more opportunities for transmission of lice between individuals. Also, less time spent outside means that the goats have less sun exposure. According to Smith (2010), sun exposure can be a method for driving out overheated lice. As supported by my findings, in order to achieve best control of ectoparasite abundance, indoor and outdoor living spaces should be plenty large enough for the population of hosts to avoid transmission by contact.

At the time I took the samples, the goats on High 1 had not been treated for lice in over a year, using the injectable deparasitizer, Interex. However, the goats on High 2 had been treated about 2 weeks before I had taken my samples. The internal deparasitizer, BioMac, used by High 2 aims to kill adult lice by putting chemicals in the blood that kill the lice that feed on it. However, the eggs are not killed by this treatment, which is why I saw a higher abundance of nymphs 14 days later. Because lice eggs hatch into nymphs after 10 days, it is important to treat the animal again between 10 and 20 days later so that you make sure to kill all of the nymph lice before they turn into reproductive adults. The difference in abundance of lice and the time since anti-ectoparasite treatments shows the importance in treatment in maintaining ectoparasites at a lower abundance, and provides support to my prediction. In general, treatments should be administered on time and in the correct quantity in accordance with the instructions in order to best control ectoparasites. Lastly, understanding the life cycle of the ectoparasites that inhabit each animal and the way that the medication rids of these pests could reveal additional methods to best control the population, such as administering anti-parasite medication 10-20 days after the initial dose, or allowing goats to graze for longer in the sun to drive out lice (Smith, 2010).

I observed a pattern of individual goats and dogs carrying an exponentially greater ectoparasite load than average. Certain individual goats carried more than twice the average number of lice, and an individual dog carried three times as many fleas than average. This could be explained by the lifecycle and behavior of lice and fleas. Both of these ectoparasites lay eggs on their host, and those eggs hatch and begin feeding off the host. Unless their current host is in contact with a more desirable host or the flea population has reached carrying capacity, it is likely that the ectoparasites would remain concentrated on the single host. Previous research has shown that ectoparasites do also exercise host preference. This usually depends on host temperature conditions, skin thickness and texture, extensiveness of peripheral circulation, consistent hair growth, and well-regulated body temperature (Prasad, 1987; Prasad, 1969). Additional variables, including hormones, sex, and health of a host can also affect ectoparasite preference (Ali *et al*, 1966; Ferrari *et al*, 2003). These factors would allow the population of ectoparasites to proliferate on specific individuals, resulting in much larger abundance than average. It is likely that these desired hosts are vectors for transmission of ectoparasites to other individuals. In a past study of transmission of lice to between goats, Hallam (1985) found that goats could be infested with lice from another individual from as few as 14 days of contact After 13 weeks of contact, there was a relatively similar abundance of lice on each individual within the sample group. This idea of ectoparasites spreading from one heavily infested individual to others likely happens in a similar manner for most ectoparasites. Some, such as fleas that can jump, may be able to spread more quickly and exercise more host preference than those that are largely stationary, such as lice. This emphasizes the importance of administering frequent treatments to all animals to avoid heavily concentrated parasite loads and transmission of lice to other individuals.

GENERAL

As climate change continues to alter and adjust species distribution, ectoparasites will likely begin to move to a higher elevation, similar to the adjustment patterns seen in plants and other animals (Lenour *et al*, 2008). With the changing distribution of these ectoparasites comes a change in distribution of the diseases associated with them. This creates new challenges for farmers, especially those at high elevation in dealing with a higher abundance and new diversity of ectoparasites. This could cause a decline in health of animals caused by ectoparasite load, resulting in livestock profit loss (Byford *et al*, 1992). It also could result in greater presence of disease that affects not only livestock, but also wild animals, and even humans (Bitam *et al*, 2009). However, with the proper knowledge and resources, ectoparasite populations can be efficiently controlled (Smith, 2010). This study provides information regarding the abundance and diversity of fleas, ticks, and lice on farms at two elevations, which can provide insight into how the distribution of ectoparasites may change as a result of climate change causing warming of higher elevations in the future.

Ectoparasites have the potential to be the subject of applicable studies in the future. The correlation between specific microclimates and ectoparasite diversity and abundance could be better established if ectoparasites and temperature, humidity, and climate stability data were collected at farms. Also, it would be insightful to study the impact of ectoparasites on animals by quantifying the number and diversity of ectoparasites on an animal, and measuring its vital signs to determining if there is a correlation between ectoparasite load and general health of the animal.

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