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*Original Contribution*

# Increased Morbidity and Mortality in Domestic Animals Eating Dropped and Bitten Fruit in Bangladeshi Villages: Implications for Zoonotic Disease Transmission

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**Abstract:** We used data on feeding practices and domestic animal health gathered from 207 Bangladeshi villages to identify any association between grazing dropped fruit found on the ground or owners directly feeding bat- or bird-bitten fruit and animal health. We compared mortality and morbidity in domestic animals using a mixed effects model controlling for village clustering, herd size, and proxy measures of household wealth. Thirty percent of household heads reported that their animals grazed on dropped fruit and 20% reported that they actively fed bitten fruit to their domestic herds. Household heads allowing their cattle to graze on dropped fruit were more likely to report an illness within their herd (adjusted prevalence ratio 1.17, 95% CI 1.02–1.31). Household heads directly feeding goats bitten fruit were more likely to report illness (adjusted prevalence ratio 1.35, 95% CI 1.16–1.57) and deaths (adjusted prevalence ratio 1.64, 95% CI 1.13–2.4). Reporting of illnesses and deaths among goats rose as the frequency of feeding bitten fruit increased. One possible explanation for this finding is the transmission of bat pathogens to domestic animals via bitten fruit consumption.

**Keywords:** zoonotic disease, domestic animals, morbidity, mortality, bat- and bird-bitten fruit

## Introduction

Most emerging human infectious diseases are zoonoses, infections caused by pathogens of animal origin (Jones et al. 2008). Bats have been identified as an important reservoir for emerging zoonotic diseases (Luis et al. 2013). Their large numbers and species diversity combined with their close interactions with livestock, farms, and humans due to shared habitat and food resources likely facilitate this increasingly recognized role (Olival et al. 2012).

Major drivers of disease emergence such as land-use change, agricultural intensification, wildlife trade, and hunting have been described in the context of significant bat-borne zoonoses such as Nipah virus, severe acute respiratory syndrome coronavirus (SARS CoV), and Ebola virus (Daszak et al. 2001; Patz et al. 2004; Wolfe et al. 2007). The consumption of bat-bitten, saliva-laden fruits

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by domestic animals is a proposed mechanism that may lead to pathogen spillover to livestock, and has been suggested with Nipah virus in Malaysia and Hendra virus in Australia (Marsh and Wang 2012; Field et al. 2001; Mahalingam et al. 2012; Paterson et al. 2011). Because of the close interface between people and their animals, the consumption of bat-bitten fruit by domestic animals may lead to an elevated risk for transmission of zoonotic pathogens from bats into human populations through sick domestic animals.

Bangladesh is located in a geographic area characterized as highly vulnerable to zoonotic disease emergence due to its diversity of wildlife, dense human population, and high level of connectivity between people, domestic animals, and wildlife (Bhatia and Narain 2010). In Bangladesh, there are three species of frugivorous bats that are particularly abundant and known to roost and forage near human settlements: *Pteropus giganteus, Cynopterus sphinx,* and *Rousettus leschenaulti* (Khan 2001). *Pteropus giganteus,* the Indian flying fox, is the natural reservoir for Nipah virus in South Asia, causing human cases annually in Bangladesh (Chakraborty et al. 2015). In addition, *Pteropus* bats in Bangladesh have been associated with more than 55 other recently identified viruses, some of which may have the potential to cause disease in other animal or human hosts (Anthony et al. 2013; Epstein et al. 2008; Yadav et al. 2012; Epstein et al. 2010). Antibodies against an Ebola-like virus have been detected in *Rousettus leschenaulti*, though the virus remains undetected (Olival et al. 2013). All local bat species have been observed feeding on fruits also consumed by people (Sudhakaran and Doss 2012).

We hypothesized that human behaviors putting domestic animals into contact with potentially contaminated bat-bitten fruits are common and that consumption of dropped and bitten fruit could increase morbidity and mortality in domestic animals. In this paper, we use data gathered from Bangladeshi villages to explore the association between consumption of dropped and bitten fruit and animal health.

## Methods

The data used in this analysis were collected as part of a large epidemiological field survey investigating risk factors for Nipah virus infection in Bangladeshi villages. From 2011 to 2013, field teams from the International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b) collected data from 60 villages representing all known Nipah cases between 2001 and 2011, 73 randomly selected villages within the 'high-risk' spillover region colloquially known as the Nipah Belt (Hahn et al. 2014), and 74 villages randomly selected outside of the Nipah Belt area.

In rural Bangladesh, families typically share a common courtyard and live together in a compound (*bari*). The survey team selected the first included compound randomly from the compounds on the outer border of the village areas and compounds were then surveyed at regular intervals to ensure even coverage and an enrollment of approximately 25 household compounds over the entire village site. Group meetings were held with village leaders before surveys were conducted to ensure community support, and household refusal rate was estimated at less than 1%.

The survey teams addressed questions to the head of each household compound (referred to as "household head" in this work). The survey collected data on the presence of fruit trees including number and type, frequency of human consumption of intact or bitten dropped fruit, and the perceived likelihood that bats and domestic animals favored specific types of fruit. Household heads were asked to report the number of each type of animal they owned (cattle, buffalo, goats, sheep, pigs, and horses), the presence of sick animals in the year preceding the survey, and the number of each type of animal dying from an illness over a two-month period preceding the survey. The survey did not collect the number of animals that had fallen ill, but reported the presence of ill animals for each species with a binary response. The definition of illness was subjective to the household head, although interviewers provided examples that included fever, lethargy, loss of appetite, vomiting, and foaming at the mouth. To characterize feeding behaviors, household heads were asked if dropped fruits were grazed by domestic animals, if animal owners directly fed "bat or bird bitten fruit" to their domestic animals, and, if so, how frequently. The inclusion of both birds and bats into a single category was necessary as it was very difficult, if not impossible, to differentiate between fruit partially eaten by the two groups.

We conducted an exploratory analysis using logistic mixed effects models to examine the association between grazing dropped fruit and feeding bitten fruit and mortality and morbidity in cattle and goats. We framed a causal diagram to identify associations between variables of interest and to identify confounders (Greenland et al. 1999). We performed separate modeling for each domestic animal type. For each animal type, we examined two separate outcomes, specified as the dependent variable: the reported presence of illness and the reported presence of deaths. We identified two possible feeding behaviors, herd size for the specific animal in question, and two proxies of household per capita wealth as independent variables. The two feeding behaviors identified in our dataset were household heads allowing grazing of dropped fruit and household heads directly feeding domestic animals bitten fruit. As proxies for household wealth (Kumar 1989), we used two non-colinear measures of household per capita wealth: the total number of trees per number of people living in the compound and the total number of domestic animals per number of people living in the compound. We first completed an analysis consisting of the outcome variable (presence of death or illness) and one of the two feeding behaviors adjusting only for village-level clustering as a random effect. We then added in the other independent variables as main effects to create a fully adjusted model. For each animal type we had four separate models for all possible combinations of outcome and feeding behavior. Initial models also accounted for location of villages inside or outside of the Nipah Belt area, but this did not affect the results. Given that this study was not meant to be specific to Nipah virus, this was excluded from the final model.

Adjusted prevalence ratios were calculated using the delta method (Santos et al. 2008). We assessed multicollinearity between independent variables using variance inflation factors. We used the Chi-square test for trend to evaluate for linear trends. Analysis was conducted in R (R Core Team 2014) utilizing the lme4 package (Bates et al. 2014) for logistic mixed effects models.

## Results

### Fruit Tree and Animal Ownership

The field team surveyed a total of 5081 compounds in 207 villages (Figure 1). Household heads reported a range of 0 to 20,015 fruit trees on their property, with a median of 30 and a mean of 78 trees. Ninety-six percent of household heads reported having at least one fruit tree growing on their property and household heads reported a mean of seven different types of fruit tree.

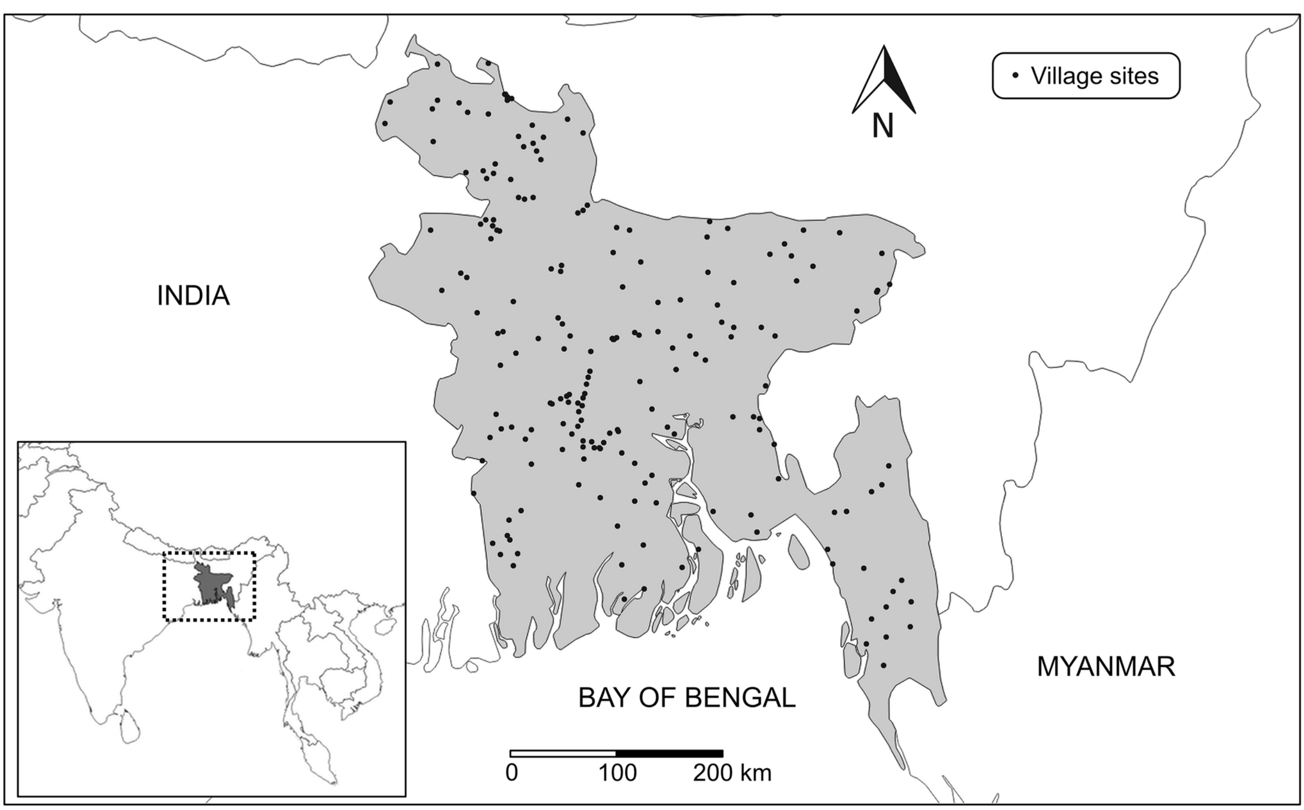


**Figure 1.** Location of all Bangladeshi villages included in the survey, for a total of 5081 household compounds in 207 villages.

**Table 1.** Animal Ownership and Number and Percentages of Households Allowing Animals to Graze on Dropped Fruit and Feeding Bitten Fruit Directly to Animals.

| Animal | % ($n$) households reporting animal | Total animals | % ($n$) households allowing grazing on dropped fruit | % ($n$) households directly feeding bitten fruit | % ($n$) households reporting deaths | % ($n$) households reporting illness |
|---|---|---|---|---|---|---|
| Cattle | 55% (2796) | 9254 | 34% (950) | 25% (702) | 4% (105) | 35% (982) |
| Goats | 30% (1533) | 4265 | 42% (637) | 27% (416) | 10% (151) | 30% (468) |
| Buffalo | 0.7% (37) | 103 | 62% (23) | 30% (11) | None | 16% (6) |
| Sheep | 2% (119) | 436 | 55% (65) | 33% (39) | 14% (17) | 27% (32) |
| Pigs | 0.6% (34) | 99 | 41% (14) | 29% (10) | 12% (4) | 20% (7) |
| Horses | 0.06% (3) | 3 | 33% (1) | 33% (1) | None | None |

Household heads reported having 0 to 80 animals, which, for this study, included cattle, goats, buffalo, pigs, sheep, and horses with a median of 3 animals and a mean of 4. Cattle and goats made up the majority of animals reported (Table 1) with 55% of household heads reporting cattle and 30% reporting goats. Many fewer household heads reported buffalo (0.7%), pigs (0.6%), horses (0.06%), or sheep (2%) ownership. Because so few households raised buffalo, pigs, horses, and sheep, we do not report associations with the health outcome of these animals.

## Fruit Consumption Patterns

Bat visits to fruit trees around household compounds were commonly reported, with 94% of household heads reporting bats visiting fruit trees for food on their property. Sixty percent (2397) reported that human household members ate dropped fruit off the ground and 11% reported that household members ate bat- or bird-bitten fruit on a daily basis. Thirty percent (1265) reported that their domestic animals consumed dropped fruit as they grazed. A smaller 20% (848) of household heads reported that they directly fed bitten fruit to their domestic herds. Of these, 8% (70) fed their animals bitten fruit most days of the week, 7% (58) did so once or twice per week, and 85% (720) did so less than once or twice per week. There was overlap (Figure 2) between the most commonly reported dropped fruits eaten off the ground by humans and the fruits that villagers observed bats and grazing domestic animals consuming.

## Dropped Ground and Bitten Fruit Consumption and Animal Morbidity and Mortality

Household heads allowing grazing on dropped fruit or directly feeding bitten fruit to animals were more likely to report illness within their herd in the year preceding the survey and at least one death in their herd in the 2 months preceding the survey (Table 2). In an unadjusted mixed effects model accounting for village clustering, household heads who directly fed their animals bat- or bird-bitten fruit were more likely to report at least one death in their goats [prevalence ratio (PR) 1.76, 95% Confidence interval (CI) 1.19–2.60] and were more likely to report ill goats (PR 1.45, 95% CI 1.15–1.84). Household heads allowing their animals to graze on dropped fruit were more likely to report deaths in their goats (PR 1.47, 95% CI 1.03–2.11) and ill cattle (PR 1.18, 95% CI 1.03–1.36).

The proportion of household heads reporting both illness and deaths in goats increased with more frequent direct feedings of bitten fruit (Chi-squared test for trend: goat morbidity $\chi^2 = 17.03$, $P = 0.0003$; goat mortality $\chi^2 = 7.02$, $P = 0.008$), suggesting a dose-dependent relationship (Figure 3).

An adjusted mixed effects model controlling for herd size and household per capita wealth while accounting for village clustering yielded similar results to the unadjusted model (Table 2), suggesting that variations in herd size and household per capita wealth did not explain increased mortality and morbidity in the animals. Results were consistent between the unadjusted and adjusted models for the mortality of goats fed bitten fruit (adjusted PR 1.64, 95% CI 1.13–2.4) and morbidity in cattle and goats grazing on dropped fruit (cattle: adjusted PR 1.16, 95% CI 1.02–1.13; goats: adjusted PR 1.38, 95% CI 1.13–1.69). Of the three main fixed effects in our model (herd size and two indicators for household per capita wealth), only herd size had an effect on morbidity and mortality. In all but the cattle mortality models, morbidity and mortality were higher in larger herds (Table 3).

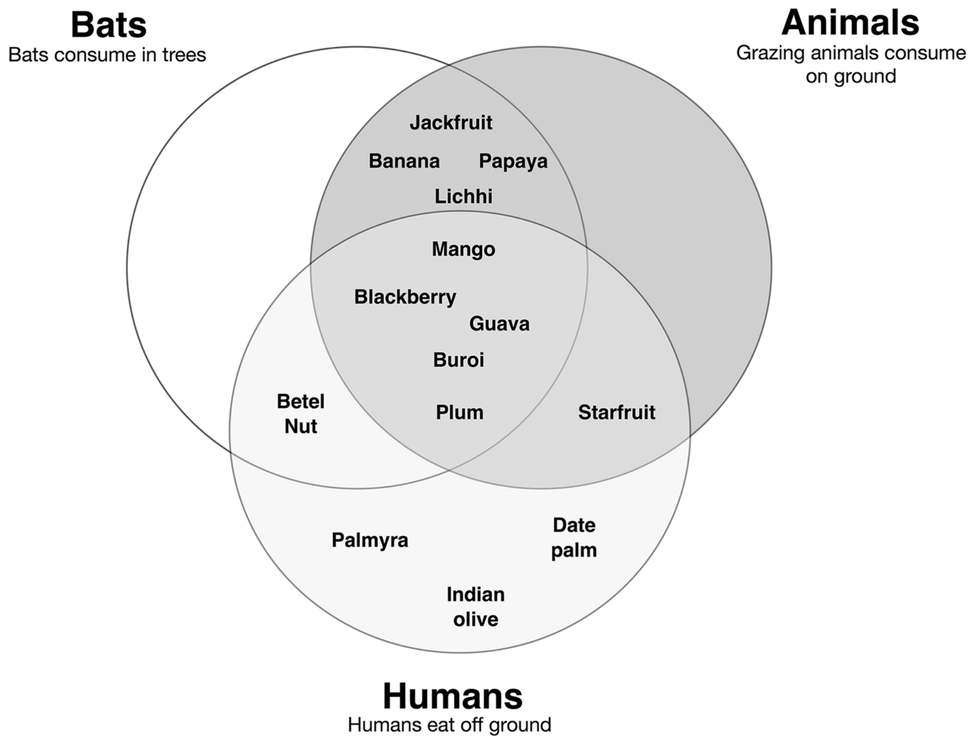


**Figure 2.** Overlaps between fruits consumed by bats, humans, and grazing domestic animals. Household heads identified fruits in each group and the top ten fruits in each group were included in this figure.

**Table 2.** Percentage of Household Heads Reporting at Least One Death in Their Herds 2 Months Preceding the Survey and Illness in Their Herds 1 Year Preceding the Survey by Exposure to Bitten or Dropped Fruit.

| | % (*n*) ≥1 death, consuming | % (*n*) ≥1 death, not consuming | Unadjusted + village clustering (PR, 95% CI) | Adjusted + village clustering (aPR, 95% CI) |
|---|---|---|---|---|
| Domestic animal mortality | | | | |
| Grazing dropped fruit | | | | |
| Cattle | 4% (27) | 4% (78) | 0.94 (0.62–1.44) | 0.92 (0.58–1.45) |
| Goats | 13% (54) | 9% (97) | *1.47 (1.03–2.11)* | 1.41 (0.98–2.02) |
| Directly fed bitten fruit | | | | |
| Cattle | 4% (34) | 4% (71) | 1.05 (0.66–1.68) | 1.06 (0.65–1.72) |
| Goats | 12% (74) | 9% (77) | *1.76 (1.19–2.60)* | *1.64 (1.13–2.4)* |
| | % (*n*) sick, consuming | % (*n*) sick, not consuming | Unadjusted + village clustering (PR, 95% CI) | Adjusted + village clustering (aPR, 95% CI) |
| Domestic animal morbidity | | | | |
| Grazing dropped fruit | | | | |
| Cattle | 42% (378) | 36% (604) | *1.18 (1.03–1.36)* | *1.16 (1.02–1.31)* |
| Goats | 37% (216) | 32% (252) | 1.10 (0.90–1.34) | 1.08 (0.89–1.32) |
| Directly fed bitten fruit | | | | |
| Cattle | 42% (378) | 37% (715) | 1.08 (0.94–1.25) | 1.06 (0.93–1.22) |
| Goats | 42% (157) | 31% (311) | *1.45 (1.15–1.84)* | *1.38 (1.13–1.69)* |

Unadjusted model accounts only for village clustering of households, fully adjusted model controls for herd size and household per capita wealth. Italic cells represent confidence intervals greater than 1, thus indicating statistical significance. *PR* prevalence ratio, *aPR* adjusted prevalence ratio, *95% CI* 95% confidence interval.

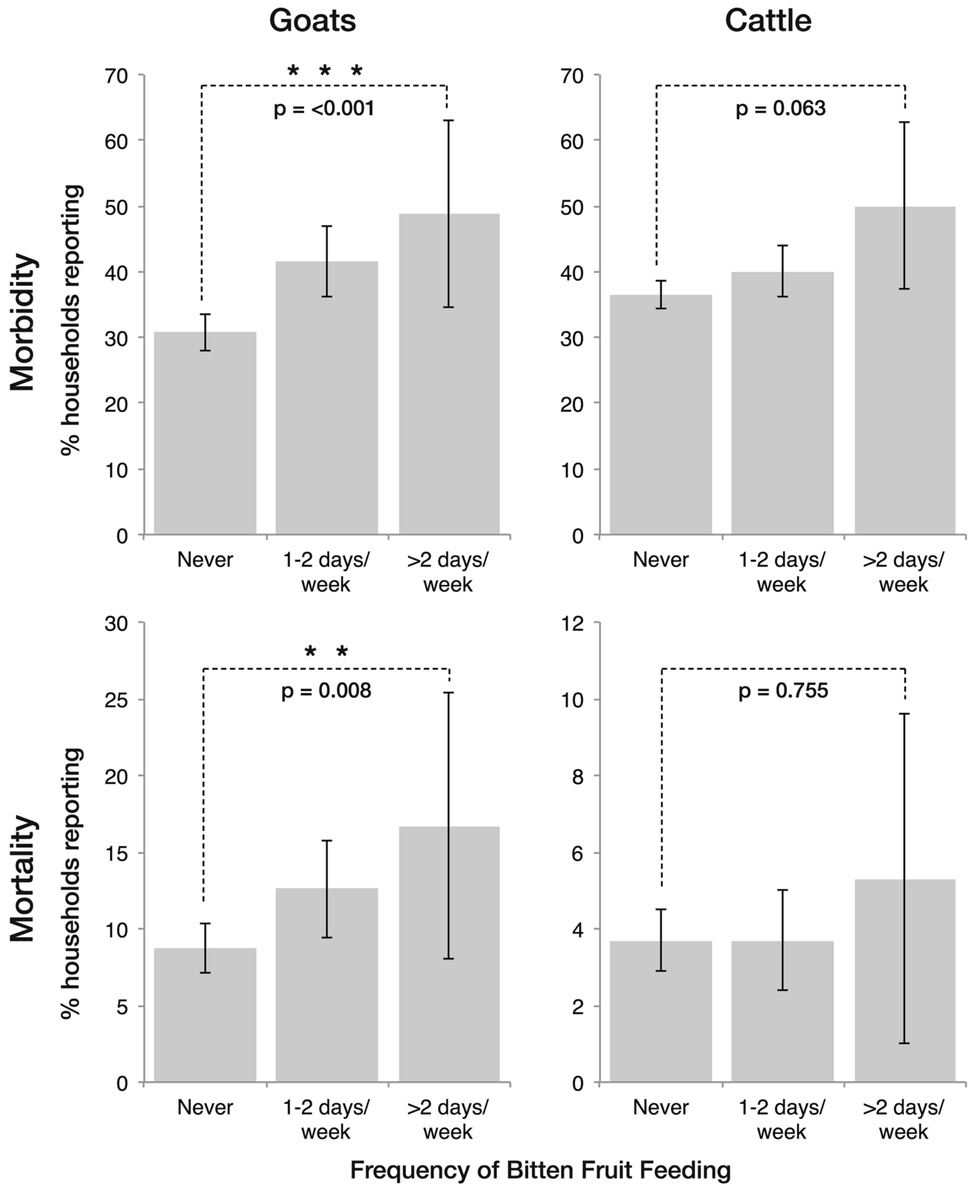


**Figure 3.** Percentages of households reporting illness in the year preceding the survey and deaths due to illness in the 2 months preceding the survey in cattle and goats by increasing frequency of feeding bitten fruit. *P* values calculated using Chi-square for trend. *Error bars* represent 95% confidence intervals.

## Discussion

Villagers in Bangladesh use bitten fruit as a food supply for their domestic animals, both allowing their animals to graze on dropped fruit and directly feeding their animals bitten fruit. In our study, directly feeding bitten fruit was associated with mortality and morbidity in goats and allowing grazing on dropped fruit was associated with morbidity in cattle. Household heads actively feeding their goats bat- and bird-bitten fruit were more likely to report both deaths and illnesses among their goats. The relationship between morbidity and mortality and the consumption of bitten fruit was dose-dependent for goats: the more frequent the feeding of bitten fruit, the more likely household heads reported illnesses and deaths.

One explanation for our findings that has implications for public health is the transmission of zoonotic pathogens to domestic animals via the consumption of bat-bitten, saliva-laden fruit. Animals are directly fed bitten fruit and also may come in contact with bitten fruit while grazing on dropped fruit. While our study did not allow us to clearly differentiate between bird- and bat-eaten fruit, there is much evidence to suggest that bats pose the larger threat (Luis et al. 2013). Given the inclusion of likely innocuous bird-bitten fruit, we may be under-representing the danger to domestic animals of consuming bat-bitten fruit. Our

**Table 3.** Adjusted Prevalence Ratios and Confidence Intervals for Fixed Effect Terms Used in the Mixed Effects Model for Each Animal Type, Health Outcome, and Fruit Feeding Behavior.

| | Adjusted prevalence ratio | 95% confidence interval |
|---|---|---|
| Directly fed bitten fruit | | |
| Goat morbidity | | |
| Herd size | *1.09* | *1.04–1.15* |
| Household animal wealth | 0.98 | 0.84–1.147 |
| Household tree wealth | 0.99 | 0.99–1.00 |
| Goat mortality | | |
| Herd size | *1.17* | *1.09–1.25* |
| Household animal wealth | 0.99 | 0.76–1.29 |
| Household tree wealth | 0.99 | 0.98–1.00 |
| Cattle morbidity | | |
| Herd size | *1.07* | *1.04–1.10* |
| Household animal wealth | 0.96 | 0.86–1.07 |
| Household tree wealth | 1.00 | 0.99–1.00 |
| Cattle mortality | | |
| Herd size | 1.04 | 0.98–1.11 |
| Household animal wealth | 1.04 | 0.74–1.46 |
| Household tree wealth | 0.98 | 0.96–1.00 |
| Grazing on dropped fruit | | |
| Goat morbidity | | |
| Herd size | *1.10* | *1.04–1.15* |
| Household animal wealth | 0.98 | 0.84–1.14 |
| Household tree wealth | 1.00 | 0.99–1.00 |
| Goat mortality | | |
| Herd size | *1.17* | *1.09–1.26* |
| Household animal wealth | 0.99 | 0.76–1.28 |
| Household tree wealth | 1.00 | 0.98–1.00 |
| Cattle morbidity | | |
| Herd size | *1.07* | *1.04–1.10* |
| Household animal wealth | 0.95 | 0.85–1.06 |
| Household tree wealth | 1.00 | 0.99–1.00 |
| Cattle mortality | | |
| Herd size | 1.04 | 0.98–1.11 |
| Household animal wealth | 1.05 | 0.75–1.46 |
| Household tree wealth | 0.98 | 0.96–1.00 |

Italic cells represent confidence intervals greater than 1, thus indicating statistical significance. Of the fixed terms, only herd size was found to be significant. Wealth measures refer to household per capita wealth of tree and animal assets.

finding of a dose-dependent relationship between animal health and frequency of feeding bitten fruit might also be consistent with pathogen transmission, as more frequent feedings would lead to an increased inoculum, making infections more likely.

While it is unclear what pathogen or group of pathogens might be causing the mortality and morbidity seen in this study, previously reported serologic evidence from Bangladesh does suggest consuming bat- or bird-bitten fruit poses an infection risk to domestic animals. Serologic evidence of an uncharacterized non-Nipah, non-Hendra henipavirus was discovered in goats, pigs, and cattle that were raised near bat roosts in Bangladesh, and cattle and goats with antibodies were more likely to have a history of feeding on fruits partially eaten by bats or birds (Chowdhury et al. 2014).

Given the close and frequent interactions between humans and domestic animals, zoonotic pathogens infecting domestic animals could be more likely to spill over into human populations. Although no association between bitten fruit consumption and Nipah virus infection in humans has been uncovered (Hegde et al. 2013; Rahman et al. 2012; Hossain et al. 2008), case reports of human Nipah virus infection in Bangladesh suggest that sick domestic animals may occasionally transmit infections to humans, with human cases occurring in individuals contacting sick cattle, pigs, and goats (Hsu et al. 2004; Luby et al. 2009; ICDDRB 2003).

Outside of Bangladesh, the movement of pathogens from bats to human populations via domestic animals consuming contaminated bitten fruit has been proposed in human cases of Hendra and Nipah viruses. In the Australian emergence of Hendra virus, horses potentially became infected with Hendra virus after consuming spats dropped by *Pteropus* bat species (flying foxes) and then may have infected their human caretakers (Mahalingam et al. 2012). The 1998 Malaysian Nipah virus outbreak, which affected 265 people and led to 105 deaths (Looi and Chua 2007), coincided with a large scale pattern of agricultural intensification, with mango and pig production increasing throughout the Malay peninsula (Pulliam et al. 2012). Pigs raised in fruit orchards had easy access to dropped fruit and masticated fruit fiber spats from overhanging trees (Parashar et al. 2000). Humans then contracted the disease from the handling of sick pigs. The virus remains viable in mangos for up to 30 h (Fogarty et al. 2008), and the fruit likely both attracted fruit bats and provided an effective vehicle for viral transmission to domestic animals.

From an evolutionary standpoint, the introduction of a novel pathogen into domestic animals may also represent a change in the selective pressures on the pathogen, and may lead to the selection of new mutations that might make the pathogen more transmissible to and between humans (Antia et al. 2003). While this remains a theoretical possibility, it has not been shown. In the case of Hendra virus, for example, genomic sequencing has demonstrated little variation among horse isolates (Marsh et al. 2010), suggesting that changes in selective pressures as the virus has moved from bats to horses have not led to mutations.

In addition to the active feeding of bitten fruit and grazing on dropped fruit found on the ground, larger herd sizes were associated with increasingly reported morbidity and mortality in goats and cattle. This may be linked to the fact that the survey question was a binary report of the presence of sick animals, and the more animals in a herd, the higher the chances of having at least one animal sick. Additionally, larger herds represent a larger susceptible population and close association between larger numbers of animals likely increases incidence and spread of disease. Having larger herds may also mean that household heads are spreading finite resources, including feed and deworming medicines, among a higher number of animals, leading to poorer health in the herd overall.

Pathogen transmission to domestic animals via bitten fruit is not the only explanation for our results, and while we have attempted to control for other possible causes of morbidity and mortality, we were limited due to the exploratory nature of our survey. Households that rely heavily on dropped fruit as a source of food for animals or feed their animals bitten fruit, for example, may be too impoverished to provide their animals with other sources of food or provide appropriate veterinary care to their animals. Mortality and morbidity therefore could be related to poverty rather than pathogen transmission. While our analysis suggests that proxy measures of household per capita wealth have no bearing on the morbidity and mortality outcomes of herds, we could only use wealth indicators based on the data collected in the survey. Use of two production assets as measures may be insufficient to distinguish gradations of wealth or may inappropriately clump households into a small number of groups (McKenzie 2005). With access to other indicators in our dataset, we might have been able to detect a difference based on wealth.

Unfortunately, we did not have access to any assessment data providing a diagnosis for reported sick livestock or a clear cause of death. Without diagnoses, we cannot be sure that bat-related pathogens were responsible for the mortality and morbidity reported. Illnesses may be transmitted from other animals, or, even more broadly, stem from the possibility that animals allowed to eat dropped fruit may also be more likely to indiscriminately eat other things that are toxic or injurious. Because we do not have data on the cause of death, we cannot verify that deaths were caused by infection. Deaths could, for example, be caused by injury, trauma, or toxic exposure, and these exposures might be more common in animals that are free range and allowed to graze on dropped fruit as these animals may not be protected in enclosures or well attended. Nor did we have seasonality data linking illness or deaths in herds to specific times of year. This might have allowed us to control for known common seasonal illnesses in domestic animal herds.

Our survey, which was not designed primarily for the purpose of analyzing the role of dropped fruit consumption on animal health, was also a source of limitation. The survey only collected data on domestic animal deaths occurring in the 2 months preceding the interview date. Although interviews occurred throughout the year, making seasonal basis less likely to be an issue, we are likely under-reporting domestic animal deaths. We may, therefore, be underestimating the risk of mortality from eating dropped and bitten fruit. In addition, household heads were not asked to distinguish between fruit with bites and masticated fruit ("spats") which bats are known to drop as they feed. Nor is it clear from our data how often animals were fed spats, which may be more laden with saliva than a bitten piece of whole fruit and may increase risk of disease transmission.

Finally, we lacked the statistical power required to adequately analyze the effects of dropped and bitten fruit consumption on all domestic animal types. Because Bangladeshi villagers generally own goats and cattle, we were able to collect sufficient data on these species, but a larger or species-directed study would be needed to explore similar relationships with other domestic animal species. The stronger statistical results in goats might suggest that the pathogens involved are either more pathogenic in goats or that goats ingest a larger inoculum.

Despite the limitations to our scientific inference given the exploratory nature of this study, our analysis suggests an association between consumption of bitten fruit and mortality and morbidity in goats and cattle. This could reflect spillover of pathogens into domestic animals. If this is the case, it raises questions regarding future risk to human populations and the costs, both in lost animals and in treatment, to farmers that rely on these animals for income, food, and productivity. Changing behaviors surrounding the active feeding of bitten fruit to domestic animals may provide a path to decrease mortality and morbidity in domestic animals and mitigate zoonotic disease emergence. Some initial possibilities might include cutting away bitten areas on fruit or washing fruit before feeding to domestic animals. Finally, future serologic studies and pathogen discovery studies aimed at sick domestic animals actively being fed bitten fruit and their human owners may provide an efficient approach to identifying spillover events and detecting pathogens that pose the highest risk to humans.

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