Gastro-intestinal parasites of sympatric red panda and livestock in protected areas of Nepal

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Abstract

Background: Disease transmission among humans, domestic animals and wildlife can have profound consequences in human health, wildlife conservation and maintenance of biodiversity. The issue of disease transmission can be particularly important for threatened wildlife species, yet such information remains scarce due to logistic constraints and government regulation on animal handlings. The red panda (Ailurus fulgens) is one of the globally threatened species challenged with habitat fragmentation and human disturbance. In Nepal, livestock grazing is recognised as one of the major threats to the red panda.

Aim: We aimed to provide the first empirical data on gastro-intestinal parasites for sympatric livestock and red panda from two geographically isolated regions in Nepal.

Methods: In this study, we systematically sampled, and examined the faecal of livestock and red panda in two separate protected areas to provide the first empirical data on their gastro-intestinal parasite, including the prevalence, parasite richness and load.

Results: We documented 11 parasite taxa (7 nematodes, 2 cestodes, 1 trematode and 1 coccidian), of which 8 are shared by both livestock and red panda. Furthermore, parasite prevalence, parasite load and parasite richness were generally higher in the livestock than the red panda.

Conclusion: The data provided from this systematic survey on parasites of sympatric livestock and red panda in wild raises the concern about the potential role of livestock mediating disease dynamics in the red panda. Our study suggests that cross-transmission of parasites between livestock and red panda are likely, and the livestock may be a competent agent bringing disease to both red panda and human. Therefore, managing human-livestock-wildlife contact to reduce disease risk to all groups should be a key component in conservation planning of protected areas.

KEYWORDS
cestode, coccidian, helminth, human-wildlife conflict, nematode, shared host, wildlife disease
INTRODUCTION

Wildlife disease is one of the most important factors to consider in managing endangered species (Eve & Kollogg, 1977; Levin et al., 2009; Wobeser, 2002; Zhang et al., 2008). In particular, parasites and pathogens can influence their hosts’ geographic distribution and population abundance (Albon et al., 2002; Anderson, 1979; Chapman et al., 2006; Dobson & May, 1987; Gulland, 1992, 1995), which are two of the key determinants of population viability for endangered species. However, the role of parasites and pathogens in wildlife conservation is understudied (May, 1988; Tompkins et al., 2011), compared to that of hunting, habitat loss and landscape fragmentation (Johnson & Russell, 2014; Johnson et al., 2015; Kenney et al., 1995; McCallum, 2008; Saunders et al., 1991; Treves & Karanth, 2003). One of the reasons is that parasite studies for wildlife, especially endangered species, are difficult due to logistic constraints and government regulations. Although parasitism data can be obtained opportunistically from dead individuals (e.g., Rodgerws, 1974; Zhang et al., 2008) or zoo populations (e.g., Bertelsen et al., 2010; Lim et al., 2008; Mutani et al., 2003), such data may not constitute an adequate sample of wild populations. Parasitism studies with proper sampling design are urgently needed for many endangered species.

The negative impacts of helminth parasites on mammals have been documented for many species, such as red wolf (Canis rufus), grey wolf (C. lupus), wolverine (Gulo gulo), brown bear (Ursus arctos), primates, and giant panda (Ailuropoda melanoleuca) (Carley, 1975; Chapman et al., 2005; Custer & Pence, 1981; MöRner et al., 2005; Phillips & Scheck, 1991; Zhang et al., 2008). In giant panda, Zhang et al. (2008) pointed to a potential disease (visceral larval migrans, VLM) caused by extraintestinal migration of Ascarid nematodes as the current most significant threat to wild panda survival. Furthermore, while much is yet to be learned about the parasite fauna, their distribution and their mode of transmission in threatened species, parasitism is suspected to be one of the drivers for mammals’ decline worldwide (Leendertz et al., 2006; Pedersen et al., 2007).

Gastro-intestinal parasites such as nematodes can negatively affect food intake and nutrient acquisition of host species (Arneberg & Følstad, 1999; Behnke et al., 1992; Galeazzi et al., 2000; Gulland, 1992; Kutz et al., 2004). In humans, gastro-intestinal nematodes such as Ascaris lumbricoides, Trichuris trichiura, Necator americana and Ancylostoma duodenale are responsible for the high mortality in developing countries (WHO, 1999). De Silva et al. (2003) reported that A. lumbricoides can infect over a billion people and T. trichiura 795 million in tropical and subtropical areas of developing countries. Economically, millions of dollars were spent every year on anthelmintic drugs for livestock in Australia to control these parasites (McLeod, 1995). Therefore, the increasing ‘human-domestic animals-wildlife’ contact with potential cross-transmission of gastro-intestinal parasites could pose a threat to both wildlife conservation and human public health as demonstrated in several cases of pathogens (e.g., Cleaveland et al., 2003; Morgan et al., 2006; Wolfe et al., 2005).

The red panda (Ailurus fulgens) is one of the globally threatened species with an estimated > 40% population decline over the past 50 years in its range (Glatston et al., 2015; Wei et al., 1999). One of the threats faced by the red panda is the increasing anthropogenic activities in their natural habitats, such as livestock grazing (Sharma, Belant & Swenson, 2014; Yonzon & Hunter, 1991). Given that livestock and red panda are both herbivores (Panthi et al., 2012; Pradhan et al., 2001; Sharma, Swenson & Belant, 2014), cross-transmission of gastrointestinal parasites between them is likely. As livestock grazing intensifies in red panda habitats, parasitic infection risk could rise, exacerbating the situation for the red panda. However, very little is known about red panda parasitology in wild. To our knowledge, the first and only study on parasites of the red panda was by Bertelsen et al. (2010) for zoo populations. Across 54 European zoos, they found infection of metastrongyloid nematodes in red pandas. In addition to this systematic survey, there were sporadic reports on the death of individual red panda due to Tyzzer’s disease caused by the bacterium (Clostridium piliforme) infection in the United States (Langan et al., 2000) and pneumonia caused by nematode (Angiostrongylus vasorum) infection in the United Kingdom (Patterson-Kane et al., 2009). Despite these efforts, parasitology data from zoo populations do not reflect the situation in wild. Therefore, it is necessary to do a study in the wild population.

Nepal is one of red panda’s range countries and home to Circa 4.2% of the world’s mammal species (Jnawali et al., 2011). However, studies on parasites of wild mammals in Nepal remain very limited. A few surveys have been conducted to date, such as parasite prevalence in red panda (Bista et al., 2017; Lama et al., 2015), rhesus monkey (Macaca mulatta) and Assamese macaque (M. assamensis; Jha et al., 2011; Pokheral & Maharjan, 2014; Tachibana et al., 2013), bats (Adhikari et al., 2020), indigenous pigs (Adhikari et al., 2021) and cross-infection of Schistosoma between elephant and rhinoceros (Devkota et al., 2012). However, these studies are relatively limited in scope, either targeting a particular site or a specific group of parasites. To incorporate parasitology information into the conservation strategy for the red panda, we need a more systematic survey of multiple parasite taxa in sympatric host species across geographic locations. Livestock grazing has been recognised as one of the major threats to red panda populations in Nepal (Sharma, Belant & Swenson, 2014; Yonzon & Hunter, 1991). Nevertheless, the role of livestock in red panda parasitology has not been investigated. At high altitudes in Nepal, livestock is made up of chauri (i.e., hybrids of yak, Bos grunniens, and hill cattle), in which the parasitology information is scarce (but see Byanju et al., 2011; Shrestha & Bindari, 2013). Therefore, the aim of this study is to provide the first empirical data on gastro-intestinal parasites for sympatric livestock and the red panda from two geographically isolated regions in Nepal.

We chose two protected areas for this study: Rara National Park (RNP; 29°30’40″N, 82°03’02″E) in western Nepal and Langtang National Park (LNP; 28°10’25″ N, 85°33’11″E) in central Nepal. These two national parks were both established in 1976 and share similar vegetation and wildlife fauna (Figure 1; Table 1). These two national parks are more than 350 km apart from each other, each representing a unique population of the red panda. Although LNP covers a much wider range of altitudes than RNP, the red panda inhabits similar altitudes at both national parks (LNP: 2400–4000 m; RNP: 3200–3600 m). In spite
of these similarities, the two parks are different in their area size and intensity of human activities, including livestock grazing (Table 1), making them ideal study sites to explore anthropogenic influences on host-parasite dynamics in red panda.

2 | MATERIALS AND METHODS

A total of 60 faecal samples, 15 from livestock (cow/chauri/yak) and 15 from red panda at each of the two sites, were non-invasively collected between May and June 2014. Despite the modest sample size of 15 for the red panda, we believe that they represented a high percentage of the population. Yonzon, Jones and Fox (1991) estimated that ca. 24 red pandas inhabited in LNP, and Sharma (2008) and Sharma, Swenson & Belant (2014) suggested that ca. 11–12 red pandas inhabited in RNP.

Each faecal sample comprised ca. 20 g of faeces taken from either a pellet group of a red panda individual or a pile of livestock dung. Livestock samples were collected immediately after they were defaecated, and each sample was visually confirmed to be from different individuals. Although we did not have direct sightings of the red panda, and therefore cannot be sure whether each faecal sample was from a unique individual, we applied the rule of a minimum distance (ca. 300–400 m, taken into account the estimated home range size of the red panda).
TABLE 2  Parasite prevalence in the livestock and red panda at RNP and LNP

| Parasite taxa                  | RNP  | LNP  |
|-------------------------------|------|------|
|                               | Livestock (N = 15) | Red panda (N = 15) | Livestock (chauri) (N = 15) | Red panda (N = 15) |
| Nematode                      | 0.93 | 1.00 | 0.93 | 0.93 |
| Ascarid nematodes             | 0.47 | 0.53 | 0.87 | 0.77 |
| Ancylostoma spp.              | 0.00 | 0.00 | 0.20 | 0.00 |
| Capillaria spp.               | 0.00 | 0.00 | 0.13 | 0.07 |
| Spiruid nematodes             | 0.00 | 0.07 | 0.13 | 0.00 |
| Strongyle nematodes           | 0.33 | 0.20 | 0.53 | 0.73 |
| Strongylodes spp.             | 0.40 | 0.73 | 0.67 | 0.80 |
| Trichuris spp.                | 0.53 | 0.20 | 0.53 | 0.27 |
| Cestode                       | 0.53 | 0.33 | 0.87 | 0.07 |
| Moniezia spp.                 | 0.33 | 0.00 | 0.27 | 0.00 |
| Taenia spp.                   | 0.33 | 0.33 | 0.87 | 0.07 |
| Trematode                     | 0.27 | 0.00 | 0.00 | 0.20 |
| Fasciola sp.                  | 0.00 | 0.00 | 0.00 | 0.13 |
| Coccidian                     | 1.00 | 0.93 | 0.67 | 0.80 |
| Eimeria spp.                  | 0.40 | 0.27 | 0.67 | 0.40 |

The nematodes include seven taxa, the cestodes include two taxa and the trematodes and coccidians each includes one identified taxon, plus an unidentified group.

panda at ca. 2.9–3.5 km²; Yonzon, 1989; Yonzon et al., 1991) between samples to reduce the chance of duplicate samples from the same individuals. We collected red panda faecal samples at RNP using eight standardised transect lines established by Sharma, Swenson and Belant (2014). The beginning point of each transect was within 50 m of the park road and transects continued uphill. The average transect length was 1.5 km, ranging from 0.6 to 2.5 km. Each day between 07:00 AM and 06:00 PM, we walked the transect lines and collected only fresh faecal samples. To increase sample size, we also collected faeces that were less than 300–400 m apart if the pellet sizes were visually different (different pellet sizes are likely from different individuals), and we collected samples opportunistically while walking from one transect line to another provided that they met the minimum-distance/pellet-size criteria. A similar protocol was applied to LNP, except that the transect lines followed altitudinal contours at an interval of ca. 200 m. A total of four transect lines between 3000 and 3600 m were used at LNP.

Upon collection, we placed the faecal samples immediately into labelled plastic zipper bags with 10% formalin, which were transported back to the laboratory (laboratory location is removed for blind review process) within a week. The samples were stored at 4°C for 1 week prior to further processing. Approximately 10 g of faeces of each sample were processed using standard zinc salt sedimentation-floatation technique (Smith et al., 2007). Sample solution was emulsified with 3–4 ml of saturated zinc salt solution in a 20-ml glass test tube. The sample was centrifuged at 3000 rpm for 5 min, and the top portion of the solution (ca. 20 μl) attached to the cover slip (25 mm) after centrifugation was taken to prepare a slide as a procedure of floatation technique. The specimen was stained with methylene blue. We poured the sediments from the remaining solution into a petri dish and stirred the content gently to mix it. We added ca. 3–4 ml of 33% zinc salt solution, mixed well and the solution was filled the petri dish. One drop from the mixture (ca. 50 μl) was taken to prepare a second slide. The specimen was stained with iodine wetmounts solution. Two slides for each faecal sample, one from floatation and one from sedimentation, were examined under a microscope for eggs of gastrointestinal helminthes and trophozoites or cysts of gastrointestinal coccidians. All helminthes and coccidians were identified to the level of genus or above by morphological characteristics following Foreyt (2001) and Baker (2007).

2.1 Data analysis

The numbers of parasites recovered from floatation and sedimentation were summed to produce total counts of parasites. We estimated the prevalence of 11 most commonly seen parasite taxa, including seven nematodes, two cestodes, one trematode and one coccidian (Table 2), by the proportion of the 15 faecal samples that were infected with a given taxon. We estimated total parasite load (all taxa combined), as well as parasite load for nematodes, cestodes and coccidians, using faecal egg count (the number of parasite eggs or trophozoites or oocyst per gram of faecal materials). We also estimated parasite richness, which is simply the number of parasite taxa found in a faecal sample. We tested the difference in parasite load or richness between livestock and red panda across the two sites using generalised linear model with either a normal distribution (parasite richness) or a negative binomial distribution with a log link function (parasite load). The fixed factors are host species (i.e., livestock or red panda), site (i.e., RNP or LNP) and their interactions. Post hoc contrast comparisons were performed to compare parasite load or richness between host species for each of the two
The prevalence of 11 parasite taxa across host species and study sites. The two host species, the livestock and red panda, as well as the two study sites, RNP and LNP, are all pooled to calculate parasite prevalence (N = 60). The grey bars denote shared parasites, black bars denote the parasites specific to the livestock and the white bar denotes the parasite specific to the red panda.

TABLE 3

| Effect          | DF | Parasite richness | Total parasite load | Nematode load | Cestode load | Coccidian load |
|-----------------|----|-------------------|---------------------|---------------|--------------|---------------|
|                 |    | Chi-square        | Chi-square          | Chi-square    | Chi-square   | Chi-square    | Chi-square   |
| Host            | 1  | 14.22             | 0.0002              | 3.88          | 0.05         | 0.03          | 0.85         | 5.28         | 0.02         | 0.95         | 0.33         |
| Site            | 1  | 8.78              | 0.003               | 3.33          | 0.07         | 4.24          | 0.04         | 0.05         | 0.83         | 0           | 0.95         |
| Host × Site     | 1  | 3.05              | 0.08                | 0.64          | 0.42         | 0.47          | 0.49         | 1.78         | 0.18         | 8.03         | 0.005        |

Parasite load is expressed as faecal egg count (FEC; the number of eggs per gram of faecal sample). Parasite richness is the number of taxa found in each faecal sample. Parasite richness is modelled using normal distribution, and the four parasite load metrics are modelled using negative binomial distribution with the log link function. The deviance/DF = 0.9 − 2.1 across the models, indicating that data dispersion is adequately fitted. The sample size is 15 for the livestock and red panda in each of the two national parks (total sample size = 60). Significant effects are bolded DF, degree of freedom.

sites. Parasite load was not evaluated for trematodes due to their low prevalence. The relationships between total parasite load and richness, as well as between total parasite load and the three taxon-specific parasite loads (i.e., nematode load, cestode load, coccidian load), were evaluated using Spearman rank correlations for livestock and red panda, respectively. The significance level for each Spearman rank correlation was Bonferroni-adjusted (α = 0.05/4 = 0.01), and the adjusted p-values were reported.

3 RESULTS

The prevalence of nematodes and coccidians were high for both livestock and red panda across the two national parks (nematode prevalence = 0.93−1; coccidian prevalence = 0.67−1; Table 2). Trematode prevalence was relatively low (0−0.27; Table 2), whereas cestode prevalence was intermediate with large variability across host species and sites (0.07−0.87; Table 2). The prevalence was generally higher in the livestock than in red panda. In RNP, the livestock had a higher prevalence for all taxa except Ascarid nematodes, Spiruid nematodes and Strongyliodes spp., while in LNP, the livestock also had a higher prevalence for all taxa except Strongyle nematodes, Strongyliodes spp. and Fasciola spp. (Table 2). Eight of the 11 parasite taxa examined are shared between the livestock and red panda (Table 2). Ancylostoma spp. and Moniezia spp. are unique to the livestock, and Fasciola sp. is unique to the red panda (Table 2). The shared parasite taxa tend to have a higher prevalence, compared to host-specific taxa (Figure 2), suggesting that cross-transmission of parasites between the livestock and red panda is likely.

The livestock had a higher total parasite load, parasite richness and cestode load than the red panda across the two sites (Table 3). Contrast comparisons between the livestock and red panda for each of the two sites revealed that total parasite load is higher for the livestock only in RNP but not in LNP (Figure 3a), whereas parasite richness and cestode load are higher for the livestock only in LNP but not in RNP (Figures 3b and 4b). There is a significant site by host interaction for coccidian load (Table 3). Specifically, the livestock had a higher coccidian load than the red panda in RNP but not in LNP (Table 3; Figure 4c). The livestock and red panda had a similar level of nematode load (Table 3; Figure 4a). These results suggest that coccidians and cestodes, but not nematodes, are likely responsible for the higher total parasite load in the livestock, compared to the red panda.
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4 DISCUSSION

In this study, we demonstrated that sympatric livestock and the red panda shared gastro-intestinal parasites and that parasite load was consistently higher in the livestock than the red panda across two national parks in Nepal. The fact that the livestock and the red panda shared similar parasite fauna suggests that cross-transmission of parasites is likely. Furthermore, the higher parasite load in the livestock suggests that: (1) livestock may pose a threat to red panda by transmitting parasites into their population, and (2) livestock may serve as an agent that brings parasites from red panda to human settlement.

FIGURE 3 Parasite load and richness of the livestock and red panda at RNP and LNP. (a) Parasite load (total number) is expressed as FEC (the number of eggs per gram of faecal sample). (b) Parasite richness is the number of taxa found in each faecal sample. The unfilled and filled bars denote livestock and red panda, respectively. Asterisks denote significant differences in parasite load or richness between the two host species.

Total parasite load is positively correlated with parasite richness for both livestock ($r_s = 0.68, p < 0.001, N = 30$) and red panda ($r_s = 0.59, p = 0.002, N = 30$). Total parasite load is also positively correlated with nematode load (livestock: $r_s = 0.68, p < 0.001$; red panda: $r_s = 0.74, p < 0.001, N = 30$; Figure 5a) and coccidian load (livestock: $r_s = 0.48, p = 0.04$; red panda: $r_s = 0.62, p = 0.001, N = 30$; Figure 5c). However, total parasite load is not correlated with cestode load (livestock: $p = 0.1$; red panda: $p = 0.8, N = 30$; Figure 5b).

FIGURE 4 Parasite loads of nematodes, cestodes and coccidians of the livestock and red panda at RNP and LNP. Parasite load is expressed as FEC (the number of eggs per gram of faecal sample, but for coccidian, these are the oocysts). The unfilled and filled bars denote livestock and red panda, respectively. Asterisks denote significant differences between the two host species.
Disease transmission associated with ‘spill-over’ and ‘spill-back’ between domestic animals and wildlife can have substantial consequences in human health (Rupprecht et al., 1995), wildlife conservation (Smith et al., 2009) and maintenance of biodiversity (Daszak et al., 2000). Notable examples of cross-transmission of diseases between domestic animals and wildlife include rabies virus and canine distemper virus, which have contributed to the decline of many carnivores including African wild dog (Lycaon pictus; e.g., Ginsberg et al., 1995; Roelke-Parker et al., 1996), Ethiopian wolf (Canis simensis; e.g., Haydon et al., 2002; Laurensen et al., 1998; Randall et al., 2004) and black-footed ferret (Mustela nigripes; e.g., Thorne & Williams, 1988). Although parasites are an integral component of a healthy ecosystem (Hudson et al., 2006) and can help maintain genetic variation in immunity among host species (Smith et al., 2009), a high parasite load or a cross-over to non-natural host species can have undesirable consequences.

Specifically for the red panda, heavy infection of intestinal coccidians can be problematic. Intestinal coccidians are known to cause severe disease symptoms such as diarrhoea and weight loss, sometimes leading to death, in rabbits (Pakandl, 2009). Due to red panda’s low-quality diet (Wei et al., 2000), symptoms such as diarrhoea may have survival consequences. Furthermore, if the red panda is not a natural host for some of the parasites that are carried by the livestock, spill-over may occur. For instance, the VLM disease that occurs when larval nematodes infect non-natural host and undergo aberrant migration within a host (Beaver, 1969) can cause neurological damages or even death (Sato et al., 2004, 2005; Zhang et al., 2008). This is particularly alarming considering that VLM was found responsible for 50% of the death in a giant panda (A. melanoleuca) population during the period between 2001 and 2005 (Zhang et al., 2008). The red panda and giant panda share many ecological similarities, including overlapped geographic distribution, and similar diets (bamboo specialists) and habitat requirements. Hence, the potential threat of VLM to the red panda is very real. High cub (83%) and adult (47%) mortality of the red panda have been reported (Yonzon & Hunter, 1991). However, the extent to which red panda mortality is related to parasitism and disease has not been studied.

Patterns of parasitism are simultaneously influenced by host characteristics and environmental conditions. Several host characteristics help explain the higher parasite load among the livestock, compared to the red panda in this study. First, the livestock had a larger foraging range that encompasses different habitat types and climatic zones than the red panda. A large foraging range has been shown to favour parasite transmission between Saigas antelope and livestock in Kazakhstan (Morgan et al., 2006). Second, different herds of the livestock frequently come into contact with one another, whereas the red panda is solitary. Third, the livestock are much larger than the red panda (ca. 4 kg; Yonzon, 1989), and larger hosts tend to attract higher parasite richness and parasite load (Arneberg, 2002). On the other hand, environmental conditions may have contributed to the overall higher parasitism in LNP than in RNP (Table 3; Figure 2). The LNP has a larger buffer zone with a human population density twice as high as RNP (Table 1). Furthermore, human settlements including cheese factories and agriculture activities exist inside LNP but not inside RNP. As a result of the cheese factory operation, there are ca. 295 chauri in LNP. However, at our study sites, we observed ca. 150–160 chauri in LNP, compared to ca. 139 cows in RNP (authors’ personal observation). Despite similar numbers of livestock at the two study sites, however, grazing activities do occur more regularly in red panda habitats over...
the years in LNP. The higher human density and more extensive farming activities in LNP made it more susceptible to habitat fragmentation and edge effects, which tend to further increase human-livestock-wildlife contact and disease risk (Hussain et al., 2013).

5 | CONCLUSION

The red panda is a globally threatened species with populations declining throughout its range of countries and are with limited geographic distribution, small population size and slow reproduction that can make their populations extremely sensitive to fluctuation in mortality. Therefore, gastro-intestinal parasites, especially those for which the red panda is not a natural host, can greatly impact their population viability as previously demonstrated for the giant panda. Our study serves as one of the first steps to understand disease dynamics in wild populations of the red panda, particularly on a landscape where humans, domestic animals and wildlife coexist. Globally, many threatened wildlife species share their habitats with livestock, making them vulnerable to spill-over of parasites and pathogens from livestock. Data from our study and future parasite studies can help inform decisions regarding the regulation of livestock grazing (e.g., anthelmintic treatment of the livestock, grazing zonation that avoids red panda habitats) and provide materials for training workshops that bring awareness of human-livestock-wildlife disease transmission to local people and herders.

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AUTHOR CONTRIBUTION

Design, data collection, laboratory work, analysis and writing manuscript: Hari Prasad Sharma. Field data collection, laboratory work and edit manuscript: Bishnu Achhami.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. We did not use the animals in context of this manuscript, therefore no ethical permit was required.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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