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The Role of Cryptosporidiosis in Sheep Welfare

María Uxúa Alonso Fresán and Alberto Barbabosa Pliego

Abstract

Welfare in animal production has been defined as the optimal mental and physiological state of the animals. It has been recently redefined according to animals' freedoms. As systems, individual sheep and herds are dynamic with constant interaction with each other and the environment. In this interaction, diseases play a fundamental role in welfare. Parasitism is common in sheep, and several management practices have been established to maintain the herds healthy. *Cryptosporidium* represents a special case, because it is a highly resistant environmental parasite, that can easily infect lambs, producing weakening diarrheas and even death. In this chapter, the role of cryptosporidiosis in sheep welfare and economic loss will be analyzed, as means of providing information on how to minimize and deal with the infection.

Keywords: *Cryptosporidium*, cryptosporidiosis, sheep, lamb, welfare, diarrhea, economic loss, prevention, control, treatment

1. Introduction

Animal welfare and health status in herds go hand in hand. When pathogens are present, disease may develop and will be reflected on decreased productivity. Parasites are pathogens which may be found in the environment as well as in the hosts, some of them as opportunists that can cause disease. Such is the case of *Cryptosporidium spp.*, a ubiquitous parasite with worldwide distribution, which causes diarrhea in newborn lambs that can lead to death or self-limiting diarrheas in immunocompetent hosts. In this chapter, welfare and cryptosporidiosis is discussed in an attempt to provide information on how to prevent and control the disease.

2. Welfare generalities

According to the World Organization for Animal Health (OIE), animal health and welfare is defined as “the physical and mental state of an animal in relation to the conditions in which it lives and dies”, and covers “the five freedoms”: “1) freedom from hunger, malnutrition and thirst; 2) freedom from fear and anxiety; 3) freedom from heat stress or physical discomfort; 4) freedom from pain; and 5) freedom to express normal patterns of behaviour”, [1]. These were developed by the UK Animal Welfare Council (FAWC) in 1979, after researching on farmed animals in intensive systems, [2].

Derived from these freedoms and based on [3], Codes of Best Practices for Welfare Establishments have been developed, such as the one from the Welsh Government [4], in which a series of issues are covered, in order to keep the animals healthy and productive. As an example, this one indicates not only how the animals should be managed, but also how staff and volunteers should be managed too. Regarding animal management, a conscientious record of animal admissions, behavior and assessment, housing and environment, cleaning and hygiene among others are defined. Animal health and disease are also included, as well as rehabilitation, rehoming or release, and transportation, all based on the corresponding legislation.

There is a close relationship between animal health and welfare. If they are optimal, they will promote high productivity and the reduction in the use of antimicrobials, as well as reduced risk for foodborne diseases to humans [5].

3. Welfare and disease

In this sense, the AWIN welfare assessment protocol for sheep [6], defines welfare indicators according to principles and criteria, in which good health is measured by the absence of disease, and according to this protocol and the objective of this chapter, it is indicated by fecal soiling. The European Food Safety Authority (EFSA) has identified gastroenteric disorders among the main welfare consequences in lambs [1]. Regarding gastroenteric disorders, diarrhea is defined as a “complex, multifactorial disease involving the animal, the environment, nutrition, and infectious agents” [7], in which *Cryptosporidium spp.* is among the most common. It is an apicomplexan protozoa that causes profuse diarrhea in neonatal lambs (4–9 days old), with low mortality, increasing concurrent infection and deficiencies in nutrition and husbandry. In well-fed animals, it often persists for 5–7 days, lessens and lambs recover. Diarrhea leads to dehydration, inappetence, abdominal pain and lethargy. Diarrhea is liquid and yellowish, varying from mild and self-limited to severe (when mixed with other pathogens) but relapse is quite common [8]. Cryptosporidiosis produces severe villous atrophy, caused by loss of enterocytes, with crypt hyperplasia by the replacement of epithelial cell loss. This occurs due to the cytotoxic effect of the parasite and apoptosis it causes, resulting in a malabsorptive diarrhea, with prostaglandin-mediated anion secretion [9].

Other pathogens might be present in diarrheas caused by *Cryptosporidium spp.*, such as rotaviruses, *E. coli*, *Salmonella spp.* or coronaviruses. In this case, prognosis degenerates and clinical signs and treatment become complicated [10].

4. Species and life cycle

There are more than 38 species of *Cryptosporidium*, but only three main species are reported in small ruminants: *C. parvum*, *C. xiaoi* and *C. ubiquitum* [11]. *Cryptosporidium spp.* oocysts size ranges from 4 to 4.5 μm , with spherical to ovoid shape, and four sporozoites per oocyst. They can be identified through Ziehl-Neelsen stain, where oocysts are shown as bright pink round bodies. The transmission of the infection happens by direct ingestion of oocysts present in food and water. Once in the host, oocysts excyst in the gut and sporozoites are released, entering the intestinal epithelial cells through the brush border [12]. After excystation, sporozoites are found in the extracytoplasmatic

Figure 1.
Cryptosporidium spp. life cycle [14].

parasitophorus vacuole, where the parasite reproduces either asexually or by schizogony, leading to 8 merozoites within a type I meront. Merozoites are capable of invading neighboring epithelial cells and other sites of the intestine, where they either reproduce asexually forming thin-wall oocysts that cause autoinfection or a sexually forming type II meronts differentiating into microgametocytes and macrogametocytes, which will unite to form the zygote. This zygote will form 4 sporozoites in thick or thin-walled oocysts, by a process called sporogony. The ones with the thick wall are released to the environment through feces [13] (**Figure 1**). Up to 1×10^6 - 2×10^6 oocysts/g of feces can be excreted by the host (which are immediately infectious) becoming a source of infection to susceptible lambs [15]. Oocysts can survive in water and in the environment several months, as soon as cool temperatures and suitable moisture exists, and can be easily transported through air and water [16].

5. Prevalence

Cryptosporidium was incidentally discovered by Tyzzer in 1907, but until 1978 oocyst identification in feces samples was confirmed through microscopy, making it the diagnostic method. Several stains have been proven, but the most common is modified Ziehl-Neelsen. Next came immunological tests which are more sensitive and specific for the detection of antigens, giving rise to immunofluorescent tests. These are specially used in water samples. Finally, genetic tools such as PCR were developed to identify the species [17, 18]. Nevertheless, microscopy using stains still remains as the simplest, cheapest and the most common way for detection.

Ever since it was first detected and confirmed as pathogen, there have been many studies regarding prevalence in sheep from different geographical regions, in which its presence may be attributed to either infectivity or the contamination of the environment [19]. In an attempt trying to explain the wide variation in prevalence, it is shown in tables according to Koeppen's climate classification (**Tables 1-5**).

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[20]	1990	Guaiba District, Brasil	Z-N	10 lambs	20	Aw
[21]	1993	Trinidad and Tobago	Z-N	51 diarrheic lambs 31 healthy lambs	24.5 10	Am
[22]	1995	Western Malaysia	Z-N	25 lambs	36.0	Af
[23]	1996	Morogoro Region, Tanzania	Z-N	121 lambs	97.5	Aw
[24]	2001	Ibadan, Nigeria	Z-N		43.3	Aw
[25]	2003	Aba, Nigeria	Z-N	29 sheep	2.1	Am
[26]	2014	Papua New Guinea	PCR	276 adult sheep	2.2	Aw
[27]	2015	India	IF	20 (5 sheep/pool)	45	Aw
[28]	2017	Sinaloa, Mexico	Z-N	1144 lambs (1–90 days old)	41.58	As
[29]	2018	Northern Veracruz, Mexico	Kinyoun	210 healthy lambs	19.5	Aw

Af: Tropical wet; Am: Tropical monsoon; As: Tropical dry savanna; Aw: Tropical savanna.

Table 1.
Cryptosporidium spp. prevalences reported in climate a (tropical).

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[30]	1994	Ismalia, Egypt	—	100 lambs	24	BWh
[31]	1998	Gharbia province, Egypt	—	52 s lamb	7.69	BWh
[32]	2002	Tehran, Iran		87 sheep	Lambs (less than a month old): 14.28 Ewes (3 years or more): 17.69	BSk
[33]	2002	Basrah, Iraq	Z-N	45 sheep	13.3	BWh
[34]	2002	Dakahlia Governorate, Egypt	Z-N	200 lambs	13.3	BWh
[35]	2002	Zaragoza, Spain	Z-N	344 lambs	59	BSk
[36]	2004	Sharkia and Dakahlyia Provinces, Egypt		470 lambs and kids (310 diarrheic y 160 healthy)	16.8 in diarrheic animals	BWh
[37]	2007	Aguascalientes, Mexico	Kinyoun	40 sheep	100	Bsh
[38]	2014	Kafr El Sheikh, Egypt	Z-N	45 lambs 75 sheep	4.4 1.3	BWh
[39]	2015	Lahore, Pakistan	Z-N	150 diarrheic and healthy lambs: 0–3 months 4–6 months 7–12 months 1 year	40 22.5 12.5 6.67	Bsh
[40]	2019	Yazd Province, Iran	PCR	192 slaughtered adult sheep	5.7	Bwk

BWh: Tropical and subtropical desert; Bsh: Mid-latitude steppe and desert; BSk: Tropical and subtropical steppe.

Table 2.
Cryptosporidium spp. prevalences reported in climate B (dry).

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[41]	1990	V, VI and Metropolitan regions, Chile	Z-N	57 diarrheic lambs 114 healthy lambs	3.5 (diarrheic) 7.9 (healthy)	Csa/Csb
[42]	1990	Elazig, Turkey	—	267 lambs	12	Csa
[43]	1991	Lorestan, Iran	Z-N	215 healthy lambs	17.2	Csa
[44]	1991	Valdivia, Chile	Z-N	196 dead lambs (0–28 days of age)	7.7	Cfb
[45]	1991	Galicia, Spain	Safranin methylene blue and Köster stain	69 lambs (6 days-6 months of age)	1.45	Csb
[46]	1994	Larissa, Greece	Z-N	65 diarrheic lambs	4.61	Csa
[47]	1996	Castille and Leon, Spain	Z-N	183 diarrheic lambs	45	Csb
[48]	1999	China	—	3250 lambs	23.08	Cfa Cwa
[49]	2000	Izmir, Turkey	—	150 diarrheic lambs 50 healthy lambs	23.3 2	Csa
[50]	2000	Poznan, Poland	Z-N	205 lambs	10.1	Cfb
[51]	2001	Poland	Coproantigen	17 lambs	11.76	Cfb
[52]	2004	São Paolo, Brasil	Z-N	184 lambs in rainy season 179 lambs in dry season	55.4 17.3	Cfa
[53]	2004	Aydin Province, Turkey	Heine	67 diarrheic lambs 77 healthy lambs	79.1 18.2	Csa
[54]	2004	Guangdong, China	Fenol, auramine and Z-N	Sheep	21.7	Cwa
[55]	2005	São Paolo, Brasil	Auramine O and Z-N	20 sheep 20 lambs	26.7 31.9	Cfa

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[56]	2005	Central Mexico	Z-N	1200 (559 lambs and 641 ewes)	General: 34.3 Lambs: 32.5 Ewes: 35.9	Cwb
[57]	2005	Konya, Turkey	Z-N	471 lambs (1–60 days of age)	2.97	Csa
[58]	2006	Hirta, St. Kilda, Scotland	Z-N	Lambs and sheep	2001–2028.6 2002–9.0 2003–2011.9	Csa
[59]	2006	Serbia	Z-N and Kinyoun	126 lambs (1–90 days of age)	42.1	Cfa
[60]	2007	Maryland, USA	IF	32 ewes 31 lambs	9.4 32.5	Cfa
[61]	2007	Galicia, Spain	IF	446 adult sheep	5.3	Csb
[62]	2007	Tunisia	PCR	89 sheep (healthy lambs and adults)	11.2	Csa
[63]	2007	Eastern England	FAT	80 asymptomatic lambs	9.7	Cfb
[64]	2008	Central Macedonia, Greece	Z-N	207 diarrheic lambs 79 healthy lambs 237 healthy ewes	55.07 15.18 10.97	Csa
[65]	2008	East Flanders, Belgium	IF and PCR	137 lambs (1–10 weeks old)	13.1	Cfb
[66]	2009	Australia	PCR	477 pre-weaned sheep	24.5	Csb
[67]	2014	Crete, Greece	IF	425 lambs	5.1	Csa
[68]	2015	Jammu District, India	Z-N	55 diarrheic lambs	45	Cfa
[69]	2016	Veracruz, Mexico	Z-N	80 sheep	70	Cfb

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[70]	2016	Lorestan,Iran	Z-N	345 sheep (lambs and adults, diarrheic and healthy)	5.8	Csa
[71]	2017	Poland	PCR	234 asymptomatic lambs	19.2	Cfb
[72]	2018	Algeria	PCR	62 lambs	14.5	Csa
[73]	2020	French Basque Country, France	Heine	Asymptomatic sheep: 79 lambs 72 ewes	1.3–77.8 1.4–50	Cfb
[74]	2020	Sardinia Provinces, Italy	Z-N	915 lambs (diarrheic and healthy)	10.1	Csa
[75]	2021	Kenya	Z-N	388 sheep	19.6	Csb

Cfa: Humid subtropical; Cfb: Marine West Coast; Csa: Warm summer Mediterranean; Csb: Hot summer Mediterranean; Cwa: Monsoon-influenced humid subtropical.

Table 3.
Cryptosporidium spp. prevalences reported in climate C (temperate).

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[76]	1993	Ohio, U.S.A.	IF	9 newborn lambs (5–10 days of age) 23 lambs (2–3 weeks of age) 23 healthy ewes	100 78.3 17.4	Dfa/Dfb
[77]	1995	Hungary	Z-N	53 lambs	22.64	Dfb
[78]	2009	Kars, Turkey	Z-N	400 diarrheic lambs (up to one month old)	38.8	Dfb
[79]	2013	Iran	Z-N	231 diarrheic and healthy lambs	2.5	Dsa
[80]	2016	Qinghai, China	PCR	350 sheep	12.3	Dwc
[81]	2019	Ladakh, India	Z-N	37 sheep (0–5 years old, diarrheic and asymptomatic animals)	54.0	Dwc
[16]	2020	Azerbaijan	Z-N	1,823 sheep	34.17	Dfb

Dfa/Dfb Humid continental; Dfb Warm-summer humid continental; Dsa Hot summer continental; Dwc Monsoon-influenced subartic.

Table 4.
Cryptosporidium spp. prevalences reported in climate D (continental).

Author	Year	Country	Detection test	Population	Prevalence (%)	Climate
[82]	1997	Different regions in Canada	IF	89 sheep	24	Dfb Dfc Dsb Cfb
[83]	2013	Uttar Pradesh, Uttarakhand, Bihar, Karnataka and Kerala States, India	Z-N	55 lambs	1.8	Csa Cwa Aw Am
[84]	2014	Iran	Z-N	1749 asymptomatic sheep	11.3	BWh Bwk Csa BSk
[85]	2014	Australia	PCR	1182 lambs (12–29 weeks old)	16.9	Cfb Bsh Csa Csb Cfa BWh desert
[86]	2021	Iran	—	3901 sheep Metanalysis	9.9	BWh Bwk Csa BSk

Am: Tropical monsoon; Aw: Tropical wet savanna; Bsh: Mid-latitude steppe and desert; BSk: Cold semiarid; BWh: Tropical and subtropical desert; Bwk: Tropical and subtropical desert; Dfb: Warm-summer humid continental; Cfa: Humid subtropical; Cfb: Marine West Coast; Csa: Warm summer Mediterranean; Csb: Hot summer Mediterranean; Cwa: Monsoon-influenced humid subtropical; Dfc: Subartic; Dsb: Mediterranean-influenced warm-summer humid continental.

Table 5.
Cryptosporidium spp. prevalences reported in studies from countries in which several regions were sampled.

Results show that it can be found in all different climates. One important factor is the high resistance of the oocysts when shed to the environment. Whenever there are conditions in which the parasite can survive (protection from desiccation or avoidance of direct sunlight) it will be present, even in zones with low humidity [87]. As cryptosporidiosis is multifactorial, it is also very important to take into account the immune status of the host. Immunocompetency varies with age, therefore making lambs the most susceptible. When sheep are immunocompetent, they develop the disease with weakening diarrhea and excrete high loads of the parasite, but it is self-limiting. They remain as healthy carriers, still excreting infective oocysts to the environment. Therefore, it is also important to analyze the prevalence results according to the health status of the host. In some countries, such as Iran, where studies with different climates have been undertaken (Table 5), prevalence has maintained with almost no shifts through time (2014: 11.3%; 2021: 9.9%). Nevertheless, in this same country, in studies where samples were taken in specific zones (dry climate), results show ample variation (2002: 14.28 and 17.69%; 2019: 5.7%), in samples taken from sheep with different ages. In other cases such as Mexico, prevalence was reported in 2005 in a temperate region (34.3%), 2007 in a dry region (100%) and in 2017 (41.58%) and 2018 (19.5%) in a tropical region, where variation in prevalence is wide. India is another example of wide variation in results. In 2013 a prevalence of 1.8% was reported in several climate regions, in 2015, 45% (tropical and temperate climate) and in 2019, 54.05% in continental climate. The studies in Nigeria with only a difference of two years show a prevalence of 43.3% in 2001 and 2.1% in 2003 in tropical zones. Turkey reports in 2004 a prevalence of 79.1% and in 2005 of 2.97% in lambs with wide variation too in the temperate climate zone. With all these examples, it is evident that generalizations cannot be made, neither by country nor continent. These results only prove the parasite's worldwide distribution throughout different climate zones, ages and health status of sheep.

6. Economic loss

Economic loss is reflected not only in mortality, but in retarded growth rates, decreased feed conversion rate, poor carcass quality, veterinary assistance and increased costs due to extra care. Moreover, healthy sheep can shed oocysts, specially periparturient ewes, with the possibility of maintaining the infection within the flock [88] and contaminate the environment [73].

Cryptosporidium causes diarrheas in developing countries, and it has been found that in developed countries it has become a serious problem, due to the limited number of parasitic protozoa in their surveillance programmes, as well as being a neglected disease caused by high confidence in hygiene, municipal sanitization services and good agriculture and livestock practices [18].

The model GloWPa-Crypto was used in [89] and differences were found in oocyst load in extensive and intensive systems, in which intensive ones provided a higher load directly on land.

7. Prevention, control and treatment

Transmission of *Cryptosporidium spp.* is influenced by reservoirs and environmental characteristics. Oocysts are sensible to high temperature and desiccation. Direct sunlight for several hours entirely inactivates oocysts [90]. To prevent and protect lambs from cryptosporidiosis, colostrum is fundamental. Hygiene measures are very important to destroy the parasite and prevent its transmission in between

animals and from the environment. Cleaning and disinfecting pens and buildings, the use of clean straw beds, avoiding high number of ewes in the parturition area and separating healthy from ill animals during diarrhea outbreaks, as well as an appropriate and in time administration of colostrum to lambs, aid in the prevention of cryptosporidial outbreaks and decreases the morbidity and mortality in sheep herds [91].

There are three main ways for treating cryptosporidiosis in farms: using drugs (most of them have been proven at laboratory level), fluid therapy and reducing the quantity of oocysts excreted to the environment [92].

There is no specific drug targeted against cryptosporidiosis, although more than 140 active principles have been tested *in vivo* and *in vitro* but none of them have been useful for eliminating cryptosporidiosis from the infected animal. This can be explained because of its particular intracellular but extracytoplasmatic location in the intestine, making it resistant to antimicrobials as well as a difficult drug target. The first attempts to cure cryptosporidiosis were based on using the same drugs as the ones for genetically related pathogens (Apicomplexa) such as *Toxoplasma* and *Plasmodium*, but are not suitable against *Cryptosporidium* because of differences in its cell biology and biochemistry: it has no apicoplast organelle, neither citric acid cycle nor cytochrome respiratory chain. Due to the fact that cryptosporidiosis has had a higher impact in underdeveloped countries, the strategy for treatment has been the use of existing medications [14].

Significant results have been found when using halofuginone lactate [93], which is the only one licensed treatment for cryptosporidiosis in calves, thought to interfere with the merozoite and sporozoite stages of *Cryptosporidium* and proven effective in the control of oocyst shedding [94]. It is an antiprotozoal drug for *Eimeria* and *Theileria*, derivative of quinazolinone [95], used both for prevention and treatment [96]. Other drugs that have also reduced the excreted number of oocysts [40] are: paramomycin, (an aminoglycoside broad-spectrum antibiotic, poorly absorbed in the gut, remaining active in the lumen [97]), metronidazole (a nitroimidazole used against giardiasis [95]), benzoxaborole (a synthetic boron-heterocyclic compound that inhibits essential enzymes, used as an antimalarial) and occidiofungin (a broad-spectrum glycolipopeptide with antifungal activity) [98, 99]. Nitazoxanide (nitratiazole benzamide derivative, licensed in humans as treatment for cryptosporidiosis [95]), beta-cyclodextrin (a cyclic oligosaccharide made up of glucose residues [100], used as pharmaceutical excipient [101]) as well as colostrum preparations, probiotics and decoquinate (coccidiostat quinolone derivative [102]) have also been used [103]. Therefore, the following sanitary and breeding recommendations are essential: sectioning of age groups, assurance of sufficient colostrum supply and intake, isolation of sick animals from the healthy ones, avoiding over-population, cleanliness and dryness of the environment, disinfection of premises and daily cleaning of equipment [93].

As alternative treatments, experiments in mice using watery and alcoholic extracts from *Curcuma longa* and *Coriandrum sativum* have been effective in reducing the number of excysted oocysts [104]. *Peganum harmala*, *Artemisia herb-alba* and *Olea europea* have shown similar results [105].

The most important measure to lessen the clinical signs of cryptosporidiosis is oral or intravenous fluid therapy. To decrease the spread of the disease, it is imperative to avoid oocyst shedding to the environment. For decontamination of surfaces, over 35 disinfectants have been tested, but only 5 are effective: 50% ammonia, 3% hydrogen peroxide, 10% formalin, Exspor and Oo-cide. Formaldehyde or ammonia gas used for steam heat sterilization and fumigation are also recommended [103]. UV radiation and ethylene oxide have been reported to be effective [106].

Figure 2.
Cryptosporidium spp. in fecal smear (Ziehl-Neelsen stain, oil immersion, 100X). Arrows show deep pink oocysts. (photograph by Alonso-Fresán).

Not only lambs may be infected through the fecal-oral route, but they can also get infected by drinking contaminated water. This may happen due to the high number of oocysts released to the environment and their resistance to chlorine and other conventional disinfectants used in water treatment processes. Oocyst concentrations of more than 150/L of surface water have been found in agricultural run-off water [15]. Therefore, another effective way of preventing *Cryptosporidium* spread into the environment and watersheds is using vegetation buffers, which aid in the retention of oocysts (**Figure 2**) [107].

Proper composting of manure ($>60^{\circ}\text{C}$) inactivates oocysts and reduces the risk of their viability. Slurry storage produces ammonia and low pH, and anaerobic digestion using mesophilic and thermophilic bacteria also helps reducing oocyst viability. Fencing sheep away from water sources prevents water contamination with feces [108].

Even though many antigenic target candidates have been characterized, there is still no vaccine with proven effectiveness or ideal cost–benefit ratio [109].

8. Conclusion

Cryptosporidium spp. is a ubiquitous parasite that directly affects animal welfare by producing self-limiting diarrheas in immunocompetent hosts. In lambs, it is capable of producing a weakening diarrhea which may lead to death. The disease may be complicated by the interaction of other pathogens such as *E. coli* or *Salmonella spp.* Asymptomatic sheep may excrete oocysts, becoming a source of infection and contaminating the environment. Prevalence worldwide in sheep herds may be up to 100%. To prevent the disease, hygiene measures should be taken to avoid environmental contamination. Pregnant ewes should be separated from the herd during and after parturition. If lambs get infected, they should be treated in the same way as with any other diarrheic disease, with fluid therapy.

Many active principles have been tested, as well as alternative natural remedies such as plant extracts in an attempt to cure cryptosporidiosis which have only shown to reduce the number of oocysts excreted, mildening the disease in lambs and reducing environmental contamination, but none of them cures the disease. Vaccines are still in development as means of prevention. Even though there is neither a specific treatment for curing cryptosporidiosis nor a vaccine, manure should be properly composted and to avoid water contamination, vegetation buffers may be used.

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