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Short communication

Effects of water source on health and performance of Mongolian free-grazing lambs

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ABSTRACT

Water pollution from animal waste, and its influence on grazing animals, is a current concern regarding Mongolian grazing lands. We allocated 32 free-grazing lambs to four groups and provided each with water from a different source (upper stream, lower stream, well, and pond) for 49 days. We recorded the amount of water consumed by the lambs, as well as their body weight, white blood cell count, acute phase (haptoglobin) protein level, and fecal condition. We measured the chemical and biological qualities of the four types of water, and we detected enteropathogenic and enterohemorrhagic *Escherichia coli* in fecal samples by using a genetic approach. Pond water contained high levels of nitrogen and minerals, and well water contained high levels of bacteria. On day 15 of the experiment, the following parameters were the highest in lambs drinking water from the following sources: water intake (pond or lower stream), body weight gain (pond), WBC count (lower stream), haptoglobin concentration (well), and enteropathogenic *E. coli* infection rate (lower stream). Lambs given upper or lower stream water exhibited more severe diarrhea on day 15 of the experiment than before the experiment. Mongolian sheep seemed to adapt to chemically contaminated water: their productivity benefited the most from pond water, likely owing to its rich mineral content. Lambs that drank lower stream water showed increases in enteropathogenic *E. coli* infection, clinical diarrhea, and WBC count. Water intake was lowest in the lambs given well water, suggesting that they avoided drinking the water because of potential *E. coli* infection; they were thus at increased risk of negative health and production effects. Our study revealed the profound nature of the effects of water quality on livestock health and performance.

1. Implications

Historically, Mongolian pastoralists have not considered water quality and have used easily accessible water points because they have been unaware of any benefits or disadvantages associated with water from different sources. This work may represent a paradigm shift in Mongolian water-use management.

2. Introduction

Water is a major component of the animal body, where it is essential for the transport of nutrients, hormones, and waste products and the regulation of blood osmotic pressure, secretions such as saliva and milk, and body temperature. In grazing animals the amount of wet feces and urine per 1000 kg of live weight per day ranges from 79 to 112 kg; wastes from sheep and dairy cattle contain large amounts of N, P, K, and bacteria and pose a water pollution threat on grazing lands (Hubbard et al., 2004). Water consumption is depressed when manure-contaminated water is provided to cattle. As a result of depressed feed consumption, infection with pathogens and parasites, and less time spent grazing and more time resting, cattle gain less weight when drinking manure-contaminated dirty water than when drinking clean water (Willms et al., 2002; Lardner et al., 2005).

After Mongolia's transition from a planned to a market economy, the total number of livestock there increased from 15 to 30 million between 1930 and 1990, and by 2014 it had reached about 50 million (National Statistical Office of Mongolia, 2013). Coincident with the resulting overgrazing, water pollution is becoming a problem. Concentrations of suspended particles and orthophosphates are increasing in Mongolia's stream systems, and phosphate levels have recently increased in Mongolian lakes (Shinneman et al.,

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2009; Maasri and Gelhaus, 2011). Overgrazing is likely to be an important contributor to the eutrophication of Mongolia's waterbodies and, through this, threatens animal health. Nevertheless, to our knowledge, no study has yet been published on the effect of livestock waste contamination of Mongolia's water on livestock performance and health. Here, we therefore examined the effect of water quality on the health and performance of Mongolian lambs.

3. Materials and methods

The study area was situated in Erdene (47°46′N, 107°27′E), in Mongolia's steppe ecologic zone. The mean annual temperature is 3.5 °C (10–15 °C in summer and −25 to −30 °C in winter), and the mean annual total precipitation is 300 mm. Vegetation in the area is characterized by grasses and a mixture of patchily distributed shrubs and herbs. The study area has a centuries-long history of being grazed by domestic livestock under nomadic patterns of land use. Per-hectare stocking rates in the area are 0.02 (cattle), 0.18 (sheep), 0.13 (goats), and 0.02 (horses). The Tuul River flows through the area and through the capital city, Ulaanbaatar, and downstream the river is more polluted than other rivers in Mongolia (Kelderman and Batima, 2006).

3.1. Water analysis

We sampled water from upper (Tererj, 47°2′N, 107°36′E) and lower (Lun, 47°51′N, 105°12′E) streams of the Tuul River and a representative well and pond close to Erdene. Surface water was sampled with a plastic bucket and stored at 4 °C after filtration through a cellulose acetate filter (pore size, 0.2 μm; Mini Sartorius). Before analysis, each water sample was again filtered with a cellulose acetate sterilized filter (pore size, 0.45 μm; DISMIC, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Inorganic ions (PO_4, NH_4, NO_2, NO_3, Na, Cl) were detected by using ion chromatography (ICS-1000, ICS-2000; Dionex, Osaka, Japan).

To detect coliforms in the water samples, we used deoxycholate – hydrogen sulfide – lactose (DHL) agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) plates. Each water sample was diluted with sterile water and then used for smear culture on DHL agar plate. Plates were cultured at 36 °C for 24 h. Red colonies that degraded lactose on the plate were counted; such colonies consist of coliforms, including lactose-degrading *E. coli*.

3.2. Animal analysis

We used 32 spring-born lambs (16 male, 16 female) that were of the same breed and had been managed in the same way drinking water from stream, well and pond water. In August 2014, we randomly allocated the 32 lambs to four groups (eight lambs in each group) and distinguished them by marking them with spray paint. We then freely grazed the mob of 32 lambs on the local grasslands and followed the animals in daytime (from 10.00 to 17.00 h), during which time the lambs were not allowed access to water points. From evening to morning we kept the lambs in a paddock with vacant trough and provided water from a single source (upper stream, lower stream, well, and pond water) to each group in the morning (before grazing) and evening (after grazing) for 49 days from the beginning of August to the end of September. Water was transported from each sampling point by truck and stored in separate storage tanks. For 2 weeks in August, we weighed the drinking water in each tank before and after the morning and evening waterings each day. We also weighed the 32 lambs before the experiment and on days 15, 29, and 49 days of the experiment.

3.3. Blood and fecal analysis

We took blood samples from the 32 lambs into evacuated EDTA tubes before the experiment and on days 15, 29, and 49 of the experiment. The total white blood cell (WBC) count was calculated under a microscope by using disposable microscope slides (C-chip, NanoEnTek). Differential blood counts for WBC (lymphocytes, neutrophils, monocytes, eosinophils, and basophils per 100 WBC) were performed on stained blood smears. Because the acute-phase protein haptoglobin was more sensitive, specific, and efficient than hematological examination (Murata et al., 2004; Skinner and Roberts, 1994), we used haptoglobin as a marker for the immune reaction to bacterial infection in lambs. Serum was separated out from the blood samples, and the haptoglobin concentration was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit in accordance with the manufacturer's instructions (Sheep Haptoglobin ELISA Kit, Cusabio Biotech Co.).

We took fresh fecal samples directly from the 32 lambs before the experiment and on days 15, 29, and 49 days of the experiment. The clinical severity of any diarrhea 15 days after the start of the experiment was scored against that before the experiment. Scores were Level 1: healthy feces (fully formed); Level 2, loss of shape of less than half of the feces; Level 3, loss of shape of more than half of the feces; and Level 4, loss of shape of all of the feces, with watery diarrhea.

3.4. Detecting pathogenic Escherichia coli

Genomic DNA of pathogenic microorganisms was extracted from the fecal samples collected before the experiment and on day 15 of the experiment by using a FavorPrep Stool DNA Isolation Mini Kit (Favorgen Biotech Corporation, Taiwan) in accordance with the manufacturer's instructions. The eluted DNA was stored at −20 °C.

Among the waterborne pathogenic microorganisms, we targeted enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). To amplify the DNA of the pathogenic *E. coli*, we used PCR primer sets specific for genes (*eaeA* and *bfpA*, 454 and 254 bp, respectively) encoding the intimin of EPEC or for Shiga-toxin-producing genes (*stx1* and *stx2*, 350 and 110 bp, respectively) encoding Shiga toxin of EHEC (Pal et al., 1999; Vidal et al., 2004). Each multiplex PCR assay was performed, and the PCR product diluted 50 times was used as the template. Positive and negative (Milli-Q) controls were included in each PCR batch. The PCR products were detected by using microchip electrophoresis (MultiNa, Shimadzu, Tokyo, Japan). Sample reactions in which we detected either *eaeA* or *bfpA* were interpreted as positive for EPEC, and samples in which we detected either *stx1* or *stx2* were interpreted as positive for EHEC.

3.5. Statistical analysis

We analyzed differences in haptoglobin concentration among the water treatments by using univariate ANOVA after confirming the assumption of homogeneity of variance. Water intake and WBC count were analyzed by using repeated-measures ANOVA to examine the interaction terms between treatment and time. Body weight was analyzed by using repeated-measures mixed ANOVA with sex as a random effect to examine the interaction terms between treatment and time. These analyses were performed with Statistica 6.0J software (Systat Inc.).

4. Results

Table 1 shows the quality of the water from the four sources. Pond water contained high levels of phosphate, ammonium and