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RESEARCH ARTICLE



Zoonotic Bacteria Harboring in Goat Intestine: A One Health Perspective

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Abstract

The risk of zoonosis transmission when handling livestock or animal products is substantial, 'One Health' interventions should be an effective strategy for the control of many zoonotic bacteria. In this study, 26 fresh fecal samples from 2 clinically healthy goats were collected at different day ages to survey goat-borne zoonotic bacterial infection, and 19 fresh fecal samples from diarrhetic goats were tested to evaluate the possible role of zoonotic pathogens in goat diarrhea. Following all samples were analyzed by Metagenomic Sequencing, a total of 20 kinds of zoonotic bacteria were screened from healthy goats, and 11 (55%) of them were infection mainly during the preweaned period. Of the 19 fresh fecal samples from diarrhetic goats, all were confirmed to be zoonotic bacterial infection positive (range from 11 to 12 species). After comparison with healthy samples of the same or similar day-age goats, it was found that *Lactococcus garvieae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Shigella boydii*, *Campylobacter coli*, *Salmonella enterica*, *Acinetobacter fetus* were highly increased incases in some diarrheic cases, while the remains had no significant change. The results suggest that goats may act as a reservoir for many zoonotic bacteria, and some of them may be associated with goat intestinal inflammation.

Keywords: Goat, Zoonotic, Bacterium, Infection, Reservoir

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INTRODUCTION

Zoonoses, defined by WHO and FAO, refer to "those diseases and infections which are naturally transmitted between vertebrate animals and man".¹ Many organisms can cause zoonotic disease, as long as it can pass from other animals to humans. The numbers of microbial species that can infect human beings are shown to be 1415, of which 868 species (61%) are zoonotic.² The transmission may occur directly or indirectly by means of vectors. The severity of zoonotic diseases in humans varies from mild symptoms to life-threatening conditions.

Zoonoses have a major societal impact on global health security, and timely diagnosis and appropriate management of clinical cases are often challenging. Preventive 'One Health' interventions should be an effective strategy to overcome some of these challenges.³ Both veterinary medicine and public health have roles to play in the surveillance, prevention, and control of this on-going issue.

Meat from goats is very popular in many populations and areas. The exposure in goat raising, slaughtering, and raw meat processing, as well as consumption of meat products has a zoonotic risk. Regarding sheep and goats, the main pathogens are: *Brucella melitensis, Campylobacter* spp., *Listeria* spp., *Salmonella* spp., *Yersinia* spp., Shiga-toxin producing *Escherichia coli, Staphylococcus aureus,* tick-borne encephalitis virus, and *Toxoplasma gondii.*^{4,5}

It is important to have zoonotic bacteria epidemiological data to evaluate the impact on public health and animal diseases, so this work investigated the pathogens in goat intestine and analyzed the possible role of zoonotic pathogens in goat diarrhea.

MATERIALS AND METHODS

Sample Collection

The samples for use in this study were allowed to be collected in a goat farm (the number

Table 1. Number and days old of diarrheal goats (%)

of animals raised was around 1,800) in Jiangsu Province, China. House-feeding mode was used in the farm. All lambs were treated with Tutreuli 1 ml by oral within 24 hours of birth, and vaccinated against infectious pleural pneumonia, clostridial disease, peste des petits ruminants, foot-andmouth disease and goat pox at the age of 15, 20, 30, 40 and 50 days old respectively. Supplementary feeding was started at 15 days old, and weaning was performed at 44 to 45 days old followed by feeding total mixed rations.

To understand the impact of zoonotic pathogens in the intestine of goats, two goats (A and B) were randomly selected from the farm and fresh fecal samples were collected at the age of 1, 3, 5, 10, 20, 30, 40, 45, 50, 60, 70, 80 and 90 days old respectively. To analyze the possible role of zoonotic pathogens in goat diarrhea, fresh fecal samples were collected from nineteen diarrheic goats from the same farm (Table 1). To minimize environmental microbial contamination, approximately 2~3 grams fecal specimen (new excreted and no touching the ground) of each goat was collected by using an alcohol-sterilized scoop and placed in a 5 ml sterile EP tube. Following the EP tubes were stored in the biosafety transport box (UN3373), all samples were sent to Suzhou Genomics Biotech Co., Ltd. (China) for metagenomics sequencing and bioinformatics analysis. The sample collection in the farm occurred under the direction of the farm's usual vet as part of their routine veterinary herd health programme from August to October, 2021.

DNA Extraction

To investigate the microbial species contained in the subcutaneous abscess, genomic DNA from each sample was extracted by using HiPure Stool DNA Kit (Shanghai Magen Biotech Co., Ltd, China). The extracted DNA concentration was tested by using Thermo NanoDrop 2000 Spectrophotometer (America), and the ratio of OD260/280 as well as that of OD260/230 was used to determine the purity of DNA.

Goat Number	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19
Day of Age (d)	19	20	25	25	30	30	35	40	45	60	75	90	90	90	105	105	105	105	210
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Library Preparation and Metagenomic Sequencing

Following 200 µg genomic DNA was randomly fragmented by Covaris S220 Focused ultrasonicator (America) to an average size of 300-350bp, the sheared fragments were treated with End Prep Enzyme Mix (Beijing Biolab Technology Co., Ltd, China) for end repairing, 5' phosphorylation and 3' adenylation, to add adaptors to both ends. Size selection of Adaptorligated DNA was then performed by DNA Cleanup beads. Each sample was then amplified by PCR for 8 cycles using P5 and P7 primers, with both primers carrying sequences which can anneal to Flowcell to perform bridge PCR and P7 primer carrying a six-base index allowing for multiplexing. The PCR products were cleaned up and validated using an Agilent 2100 Bioanalyzer. The qualified libraries were sequenced pair end PE150 on the illumina HiseqXten/Novaseq/MGI2000 System.

Data Analysis

Raw shotgun sequencing reads were trimmed using Cutadapt (v1.9.1). Low-quality reads, N-rich reads and adapter-polluted reads were removed. Then host contamination reads were removed. Samples were each assembled *de novo* to obtain separate assemblies. Whole genome *de novo* assemblies were performed using Megahit (v1.1.3) with different k-mer. The best assembly result of Scaffold, which has the largest N50, was selected for the subsequent analysis.

Prodigal (v3.02) software was to predict coding genes and then integrated the gene sequences of all samples. Use the sequence clustering software MMseq2 for further deredundancy processing. By default, identity 95% and coverage 95% were used for clustering. Using the alignment software SoapAligner (v2.21), we aligned the clean reads to construct nonredundant gene sets and obtained the number of reads aligned by unigene in each sample. Then, based on the number of reads and gene length in each unigene alignment, the abundance information of unigene in each sample was calculated.

Diamond (v0.8.15.77) was used to search the protein sequences of the unigenes with the NR database, CAZy database, eggNOG database, CARD database, and KEGG database with $E<10^{-5}$. The matched result with the best score was selected for annotation. To explore the microbial composition of the samples, we used Diamond to align the unigene sequences with the NR database and obtained the species annotation results of each sequence through the taxonomic annotation information corresponding to each sequence in the NR database. The abundance of a species in one sample equals the sum of the gene abundance annotated for the species.

The samples for this research were analyzed without removal of the host genome, so some genes could be annotated to the host animal or closely related species. The figures of the relative abundance comparison as well as the developmental trends of the 20 zoonotic pathogens are generated by Microsoft Excel.

RESULTS

Zoonotic Pathogens in Clinical Healthy Goats

After removing the host animal and closely related species, about 2,800 kinds of microorganisms were detected in each goat intestine. Out of all microorganisms, 20 kinds of zoonotic bacteria were screened (Table 2), including Lactococcus garvieae, Helicobacter pylori, Klebsiella pneumoniae, Shigella sonnei, Shigella boydii, Campylobacter coli, Salmonella enterica, Acinetobacter baumannii, Shigella flexneri, Shigella dysenteriae, Clostridium perfringens, Campylobacter jejuni, Campylobacter lanienae, Salmonella bongori, Campylobacter fetus, Listeria monocytogenes, Acinetobacter haemolyticus, Mycobacterium tuberculosis, Mycobacterium neglectum and Burkholderia pseudomallei, and

	sonnei
Mycobacterium tuberculosis Campylobacter jejuni Listeria monocytogenes Salmonella	cterium neglectum

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pacteriuri	Goat	1 d	3 d	5 d	10 d	20 d	30 d	40 d	45 d	50 d	60 d	70 d	80 d	90 d	Average
actococcus garvieae	A	0.77	1.77	1.22	3.25	3.73	0.22	1.08	0.35	0.26	0.02	0.01	0	0.03	0.978
	в	8.27	2.48	5.06	4.5	0.56	1.62	0.66	0.04	0	0.11	0	0.01	0.06	1.798
Helicobacter pylori	A	0.58	1.28	1.41	2.39	2.74	0.17	0.81	0.27	0.21	0.02	0	0	0.03	0.762
	в	1.49	1.86	3.86	3.39	0.45	1.23	0.51	0.03	0	0.09	0	0.01	0.05	0.998
Klebsiella pneumoniae	A	0.17	0.63	1.49	1.38	1.31	0.06	0.30	0.11	0.08	0.03	0.04	0.04	0.05	0.438
	в	0.19	1.11	1.92	1.51	0.19	0.55	0.20	0.04	0.02	0.05	0.03	0.03	0.04	0.452
Shigella sonnei	A	0.15	0.43	0.36	0.73	0.73	0.06	0.20	0.07	0.06	0.01	0.02	0	0.01	0.218
	в	1.48	0.7	1.2	0.91	0.14	0.31	0.14	0.02	0	0.03	0.01	0.01	0.02	0.382
Shigella boydii	A	0.10	0.26	0.21	0.46	0.49	0.04	0.14	0.05	0.04	0	0.01	0	0	0.138
	В	1.07	0.40	0.72	0.58	0.08	0.21	0.08	0.01	0	0.02	0	0	0.01	0.245
Campylobacter coli	A	0.04	0.12	0.08	0.28	0.29	0.03	0.09	0.04	0.03	0.01	0.01	0.01	0.01	0.080
	в	0.72	0.18	0.34	0.33	0.03	0.13	0.06	0.01	0	0.02	0.01	0.01	0.01	0.142
Salmonella enterica	A	0.01	0.2	0.24	0.23	0.09	0.03	0.01	0.03	0.02	0.02	0.03	0.02	0.02	0.073
	в	0.01	0.45	0.39	0.12	0.05	0.02	0.02	0.03	0.01	0.01	0.07	0.01	0.01	0.092
Acinetobacter baumannii	A	0.03	0.07	0.04	0.16	0.17	0.01	0.04	0.02	0.01	0	0.01	0.01	0.01	0.045
	в	0.43	0.11	0.20	0.19	0.01	0.06	0.02	0.02	0	0.01	0.03	0.01	0.02	0.085
Shigella flexneri	A	0	0.10	0.11	0.10	0.03	0.01	0	0	0.01	0	0.01	0	0	0.028
	в	0	0.22	0.19	0.05	0.02	0.01	0	0.01	0	0	0.01	0	0	0.039
Shigella dysenteriae	A	0	0.07	0.08	0.08	0.03	0.01	0	0	0	0	0.01	0	0	0.022
	в	0	0.12	0.16	0.04	0.02	0	0	0	0	0	0	0	0	0.026
Clostridium perfringens	A	0.02	0.12	0	0	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.020
	в	0	0.09	0.02	0	0.01	0	0.02	0.02	0	0.01	0.02	0.03	0.01	0.018
Campylobacter jejuni	A	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0	0	0	0.01	0.008
	в	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0	0	0	0.01	0	0.008
Campylobacter lanienae	A	0.03	0	0	0	0	0	0	0.06	0	0	0	0	0.03	0.00
	в	0	0	0	0	0	0	0.21	0	0	0	0	0	0.01	0.017
Salmonella bongori	A	0	0.02	0.02	0.02	0.01	0	0	0	0	0	0	0	0	0.005
	в	0	0.04	0.03	0.01	0	0	0	0	0	0	0	0	0	0.006
Campylobacter fetus	A	0.01	0	0	0	0	0	0	0.04	0	0	0.01	0.01	0.01	0.006
	в	0	0	0	0	0	0	0.05	0	0	0	0	0	0.01	0.005
Listeria monocytogenes	A	0.02	0.01	0	0	0	0	0	0.01	0	0.01	0	0.01	0.02	0.006
	в	0	0.01	0.01	0.01	0.01	0	0	0.01	0.01	0	0.01	0.01	0.01	0.007
Acinetobacter haemolyticus	A	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0.001
	в	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0.001
Mycobacterium tuberculosis	A	0.01	0.01	0	0.01	0.02	0	0	0.01	0	0	0.01	0.02	0.01	0.008
	в	0.04	0.01	0.02	0.01	0	0.01	0.01	0.01	0.01	0	0.01	0.01	0.01	0.012
<i>Wycobacterium neglectum</i>	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0.000
	в	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0.001
Burkholderia pseudomallei	A	0.01	0	0	0.01	0.01	0	0.01	0.01	0	0	0	0.01	0	0.005

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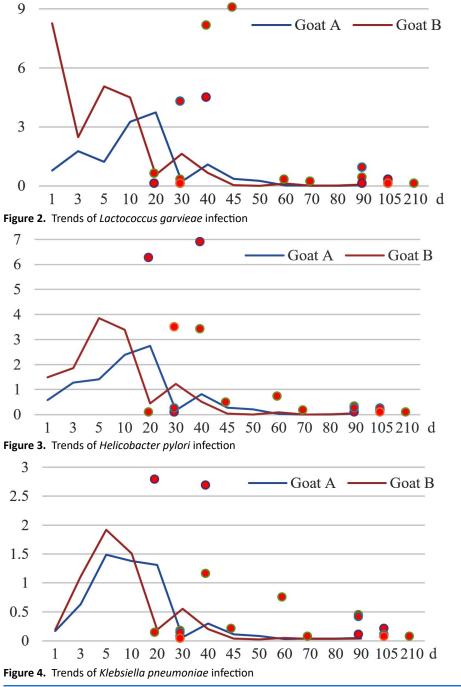
Bacterium								0	Goat Number)er									
	C1	3	ß	C4	CS	CG	C7	C8	60	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19
Lactococcus garvieae	0.51	0.02	0.27	0.06	4.23↑	0	8.03↑	4.45 ↑	4.97↑	0.23	0.10	0.39	0.08	0.82 ↑	0.10	0.27	0.03	0.06	0.01
Helicobacter pylori	0.05	$6.21 \uparrow$	0.02	0	0.22	3.44↑	3.32↑	$6.86 \uparrow$	0.40	0.63↑	0.09	0.28	0.03	0.15	0.08	0.07	0.21	0.06	0.01
Klebsiella pneumoniae	0.12	2.75↑	0.14	0.04	0.12	$1.73 \uparrow$	$1.12 \uparrow$	$2.65 \uparrow$	0.18	0.73	0.04	0.43↑	0.07	0.38个	0.08	0.17	0.08	0.05	0.03
Shigella sonnei	0.05	$1.58 \uparrow$	0.02	0.04	0.08	0.87↑	0.77↑	$1.71 \uparrow$	0.10	0.67↑	0.02	0.20↑	0.02	0.04	0.07	0.23↑	0.05	0.02	0
Shigella boydii	0.02	$1.07 \uparrow$	0.01	0.02	0.04	0.57个	$0.56 \uparrow$	$1.08 \uparrow$	0.07	0.28个	0.01	0.09	0.01	0.03	0.03	0.10	0.04	0.01	0
	0.03	0.63↑	0.03	0.03	0.02	$0.41 \uparrow$	0.26个	0.64↑	0.04	0.04	0.01	0.05	0.01	0.03	0.02	0.03	0.02	0.01	0.02
Salmonella enterica	0.08	0.03	0.03	0.06	0.05	0.01	0.02	0.01	0.07	0.90↑	0.02	0.17个	0.03	0.01	0.07	0.34↑	0.01	0.01	0.01
Acinetobacter baumannii	0.02	0.37个	0	0.01	0.03	0.23个	0.14	0.37个	0.04	0.03	0.01	0.02	0.01	0.03	0.01	0.01	0.02	0.01	0.01
Shigella flexneri	0.03	0.01	0.01	0.03	0.02	0	0.01	0	0	0.42个	0	0.09	0.01	0	0.03	0.17个	0	0	0
Shigella dysenteriae	0.02	0.01	0.01	0.01	0.02	0	0.01	0	0	0.19^{+}	0	0.06	0.01	0	0.02	0.09	0	0	0
Clostridium perfringens	2.00个	0	0.01	0	0.03	0	0	0.06	0.01	0.30个	0.02	0.01	0.01	0.49个	0.02	0.01	0.02	0.01	0.02
Campylobacter jejuni	0	0.01	0.01	0.01	0	0.01	0.01	0.01	0	0	0.01	0.02	0.01	0	0.01	0	0	0.05	0.01
Campylobacter lanienae	0	0	0	0	0	0	0.01	0	0	0	0.01	0	0.01	0	0.01	0.14个	0	0.01	0
Salmonella bongori	0	0	0	0.01	0	0	0	0	0	0.07	0	0.01	0	0	0.01	0.03	0	0	0
Campylobacter fetus	0	0	0	0	0	0.02	0.12个	0	0.01	0	0.01	0.01	0	0	0.01	0.03	0.01	0.01	0
Listeria monocytogenes	0.02	0.01	0	0	0.01	0	0	0	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.01
Acinetobacter haemolyticus	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0.01	0	0
Mycobacterium tuberculosis	0	0.03	0	0	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.01
Mycobacterium neglectum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Burkholderia pseudomallei	0	0.01	0	0	0	0.01	0.01	0.01	0	0	0	0.01	0	0.01	0.01	0	0.01	0	0.01

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each of them is a tiny part of the total community. No Brucelella was detected and potential zoonotic agents were not screened.

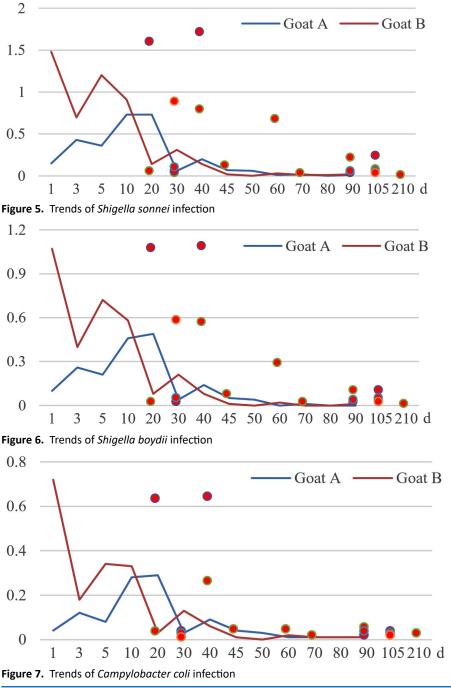
Among the 20 bacterial species, the top five are *Lactococcus garvieae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Shigella sonnei* and Shigella boydii (Figure 1). By analyzing the abundance at different day ages, it was found that 11 (55%) of them, including Lactococcus garvieae, Helicobacter pylori, Klebsiella pneumoniae, Shigella sonnei, Shigella boydii, Campylobacter coli, Salmonella enterica, Acinetobacter



baumannii, Shigella flexneri, Shigella dysenteriae and Salmonella bongori, were infectioned mainly during preweaned period (Figure 2 to 11, and Figure 15, line), while the remaining ones have no obvious regularity(Figure 12 to 14, and Figure 16 to 21, line or column). The abundant presence of 20 bacterial species in the goat intestine suggests an important role of these bacteria as foodborne or waterborne agents and possibly as zoonotic agents.

Zoonotic Pathogens in Clinical Diarrheic Goats

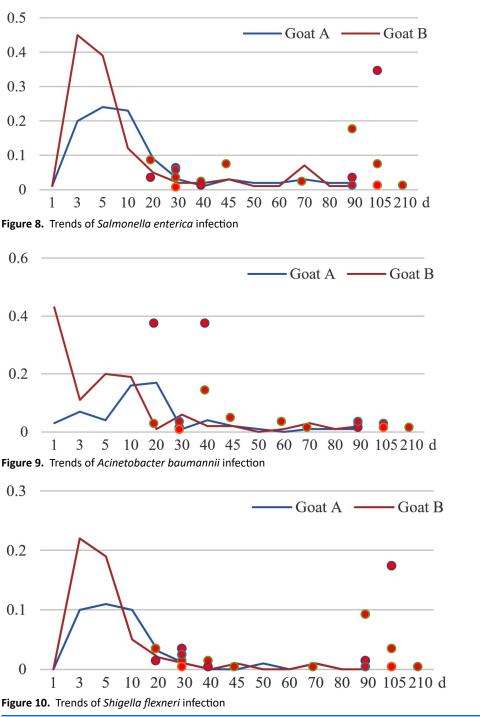
To reveal the possible role of zoonotic pathogens in goat diarrhea, the relative abundance



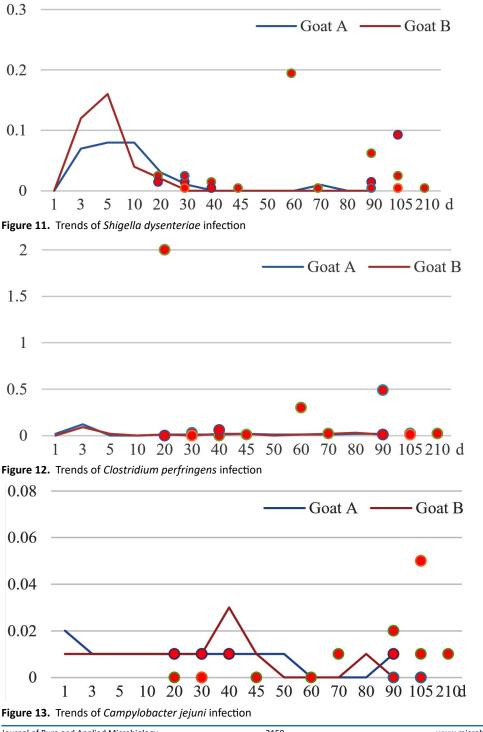
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of 20 zoonotic bacteria were analyzed in the samples from in clinical diarrheic cases (Table 3). Of the 19 diarrhetic goats, all were confirmed to be zoonotic bacterial infection positive (range from 11 to 12 species). After comparison with

healthy samples of the same or similar day-age goats (Table 3, Figures 2 to 21, dot), it was found that *Lactococcus garvieae* was highly increased in 3 preweaned, 1 weaning and 1 postweaned cases, *Helicobacter pylori* in 4 preweaned and



1 postweaned cases, *Klebsiella pneumoniae* in 4 preweaned and 2 postweaned cases, *Shigella sonnei* in 4 preweaned and 3 postweaned cases, *Shigella boydii* in 4 preweaned and 1 postweaned cases, Campylobacter coli in 4 preweaned cases, Salmonella enterica in 3 postweaned cases, Acinetobacter baumannii in 3 preweaned cases, Shigella flexneri in 2 postweaned cases, Shigella



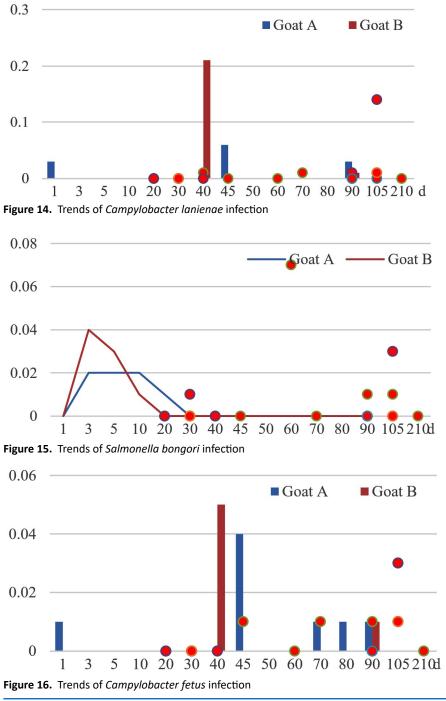


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dysenteriae in 1 postweaned cases, *Clostridium perfringens* in 1 pretweaned and 2 postweaned cases, and *Campylobacter fetus* in 1 pretweaned cases, while the remains no significant change.

DISCUSSION

With the continuous global threat of zoonotic pathogens, effective strategies must be found to control these diseases from the root. Due

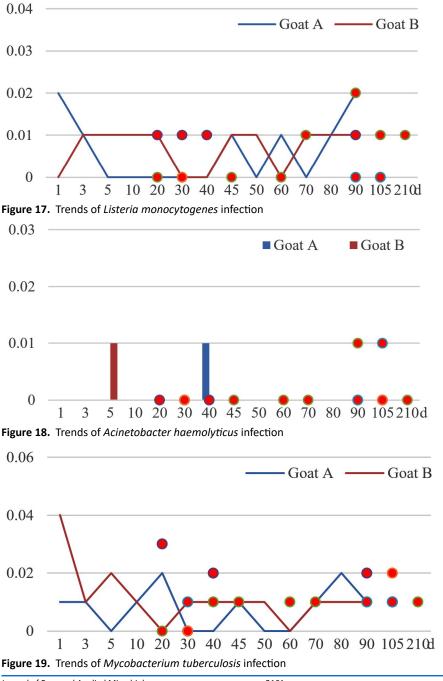




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to the complexity of these pathogens' infection in animals and humans, it should be detected as early as possible and prevent and control the infection of these pathogens in the process of human-animal contact, to achieve the ultimate goal of maintaining human health and ecological environment.^{6,7} The results of this study show us that identifying threat factors from animal sources remains the most effective and economical way to protect human public health, which fits exactly with 'One Health' interventions.³

The bacteriological analysis of healthy goats in this study revealed that goats could harbor a variety of zoonotic pathogens in their

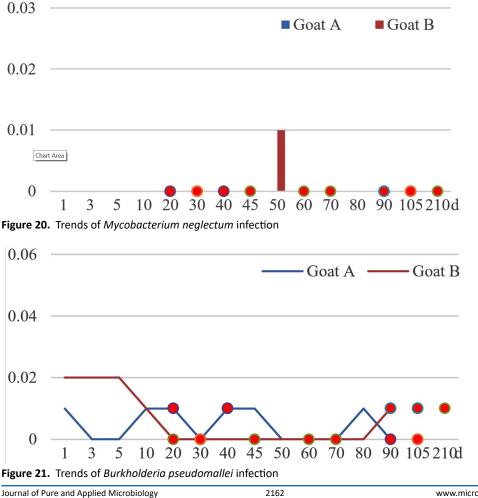


²¹⁶¹

intestines during their growth, especially in the pre-weaning stage. These zoonotic bacteria mainly include the previous reported *Lactococcus* garvieae,⁸ Helicobacter pylori,^{8,9} Klebsiella pneumoniae,¹⁰ Shigella sonnei,¹¹ Shigella boydii,¹² Campylobacter coli,¹³ Salmonella enterica,¹⁴ Acinetobacter baumannii,¹⁵ Shigella flexneri,¹² Shigella dysenteriae,¹² Clostridium perfringens,¹⁶ Campylobacter jejuni,¹³ Campylobacter lanienae,¹⁷ Salmonella bongori,¹⁸ Campylobacter fetus,^{19,20} Listeria monocytogenes,²¹ Acinetobacter haemolyticus,²² Mycobacterium tuberculosis,²³ Mycobacterium neglectum,²⁴ and Burkholderia pseudomallei.²⁵ The results suggest that goats may act as a reservoir for many zoonotic bacteria.

The analysis of diarrheal goats revealed that Lactococcus garvieae, Helicobacter pylori, Klebsiella pneumoniae, Shigella sonnei, Shigella boydii, and Campylobacter coli were highly increased incases in some lambs with diarrhea before weaning. *Salmonella enterica, Acinetobacter baumannii, Shigella flexneri,* and *Shigella dysenteriae* were highly increased incases in some with diarrhea after weaning. *Clostridium perfringens* and *Campylobacter fetus* were highly increased incases in few cases, while the remains had no significant change. The results suggest that some of the zoonotic bacteria may be associated with goat intestinal inflammation.

Whether in healthy or diarrheic goats, the abundance of each zoonotic bacteria in the goat intestine is a tiny part of the total community, but they can spread through shedding in the faeces and subsequent faecal contamination of raw food.²⁶ Therefore, in the process of raising, transportation, slaughter, and processing, it will inevitably bring public health risks to human beings. To take action to prevent this risk, OIE



proposed that the best way to protect human health is to eliminate the pathogens at its source, which requires efforts to establish a multiparty participation and cooperation mechanism around the concept of "one health". Therefore, it is important to have epidemiological data collecting systems and information systems that allow complete diagnostic tracing from herd to slaughterhouse and vice versa. All sides, including research and surveillance, as well as producers are called upon to actively share in protecting the health of consumers as far as it is threatened by latent infections in domestic stock.²⁷

The deficiency of this research is only investigated in the healthy and diarrheic goats of one farm. More farms need further investigation, which is more consistent with the 'One Health' perspective.

CONCLUSION

A total of 20 kinds of zoonotic bacteria were screened from healthy goats, and some of them were highly increased incases in some diarrheic cases. The results suggest that goats may act as a reservoir for many zoonotic bacteria, and some of them may be associated with goat intestinal inflammation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

CC and DC conceived and designed the study, and jointly executed the experiments with GW, ML, SZ and XC. CC, DC and JT co-wrote the paper. All authors read and approved the final manuscript.

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DATA AVAILABILITY

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

ETHICS STATEMENT

This study did not require official or institutional ethical approval. The animals were handled according to high ethical standards and national legislation. The sample collection in these farms occurred under the direction of the farms' usual vets as part of their routine veterinary herd health programme; therefore, no changes in animal treatment or handling occurred as a result of the sample collection required for this study.

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