Brief Original Article

High seroprevalence of pathogenic *Yersinia* spp. in sheep and goats across nine government farms in the Pakistani Punjab

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Abstract

Introduction: Seroprevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* infections in animals and humans is not established in Pakistan. There are only a few reports on the prevalence of pathogenic *Yersinia* spp. and infections in small ruminants, however, the role of sheep and goats in the transmission of pathogenic *Yersinia* remains unclear.

Methodology: A primary survey investigated the presence of anti-*Yersinia* antibodies among a small population of ruminants detected by recombinant antigen targets in nine government farms dispersed throughout the Punjab province of Pakistan.

Results: Antibodies specific for *Y. enterocolitica* were detected in 7/9 sheep flocks and in 4/4 goat flocks. Antibodies specific for *Y. pseudotuberculosis* were detected in 4/9 sheep flocks. Two sheep flocks revealed the presence of both *Y. enterocolitica* and *Y. pseudotuberculosis* specific antibodies.

Conclusion: Due to the high number of the population involved in raising small ruminants the risk to veterinary and public health must be rapidly determined.

Key words: Yersinia, serology; small ruminants; Punjab; Pakistan.


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Introduction

*Yersinia* (*Y.*) *enterocolitica* and *Y. pseudotuberculosis* are the causative agents of yersiniosis, which is mostly a self-limiting gastrointestinal disease in humans and animals. Yersiniosis is one of the most common but largely underdiagnosed bacterial zoonosis observed in all continents, exceeded only by salmonellosis and campylobacteriosis [1-5]. Pigs are considered to be the main reservoir for human pathogenic strains [6]. Pathogenic *Yersinia* (*Y. enterocolitica* *Y. pseudotuberculosis* and *Y. pestis*) species all possess a Yop virulon, which is encoded on the 70 kb *Yersinia* virulence plasmid (pYV). This Yop virulon is made up of Yop effector proteins and other proteins necessary for introducing the virulon into the host cell. Once in the host cell, these virulons interfere with the cytoskeleton, disrupting phagocytosis, and preventing the production of cytokines thereby enabling the survival of the invading pathogen [7]. Further chromosomally encoded virulence factors, such as *ail* and *inv* facilitate invasion, whereas different *Yersinia* stable toxin (*yst*) genes are responsible for the production of heat-stable enterotoxins, found in both enteropathogenic *Y. enterocolitica* biotypes (1B, 2-5) and the allegedly apathogenic *Y. enterocolitica* biotype 1A [8].

There are only a few reports on the prevalence of pathogenic *Yersinia* spp. and infections in small ruminants, however, the role of sheep and goats in the transmission of pathogenic *Yersinia* remains unclear. [9-11]. Sheep and goats are a major source of income in developing countries and transition economies such as Pakistan. The rapid growth of Pakistan’s population has led to a rise in the demand for fruit and vegetables but also for dairy products and meat. According to the Ministry of National Food Security and Research, there are approximately 30 million sheep and 72 million goats in Pakistan and nearly eight million families are...
involved in livestock rearing and production activities (http://www.finance.gov.pk/survey_1617.html, Accessed: 22 September 2019). In 2016/2017, the estimated milk production of sheep and goat origin was approx. 40,000 and 900,000 tons, respectively, and for mutton meat production approx. 700,000 tons (http://www.finance.gov.pk/survey/chapters_17/02-Agriculture.pdf; accessed 19, October 2018). As there are no data available on the prevalence of pathogenic *Yersinia* spp among the population of small ruminants in Pakistan, a primary survey was conducted among nine government farms located in seven districts; Kushab, Bhakkar, Layyah, Rajanpur, Bahawalpur, Khanewal, and Okara of the Pakistani Punjab.

**Methodology**

**Sample collection**

A total of 1000 serum samples from sheep and goats were collected from nine government farms in the Pakistani Punjab stratified by sampling and proportionate allocation [12]. For blood collection, guidelines of the International Animal Care and Use Committee (IACUC) were followed. Approval was given by the Secretary of the Livestock & Dairy Development Department, Punjab, Pakistan (letter No. SO(I&C)/L7DD/2-6/2016). Ethical approval was received from the Ethical Research Board at the University of Agriculture Faisalabad, Pakistan (letter No. ORIC/3253, 16.11.2013).

Blood was collected from the jugular vein using Improvacuter Blood Collection Sets (Improve Medical, Gangzhou, China). Each tube was labelled immediately after collection and placed vertically in a cool box with cool packs. Samples were then transferred to the laboratory in Faisalabad and subjected to centrifugation (4500 rpm/5 minutes/4°C). The sera were transferred to collection tubes (CryoSTM, Greiner Bio-one, Frickenhausen, Germany) and subsequently shipped to the Friedrich-Loeffler-Institut in Jena, Germany. Samples were stored at -20°C until further use.

**Western Blot**

A total of 388 samples were tested for the presence of anti-**Yersinia** antibodies (IgG) using the recomLine Yersinia IgG 2.0 Kit (Mikrogen Diagnostik, Neuried, Germany) according to the manufacturer’s instructions (Table 1). This test uses recombinantly produced antigen targets, i.e. **Yersinia** outer proteins YOP-M, YOP-D, YOP-E; V-AG, PsaA (pH 6 antigen) and MyfA (Mucoid Yersinia factor A). Briefly, 20 µL of serum was added to test strips placed into incubation trays with 2 mL of wash buffer. The strips were incubated under gentle shaking for one hour at room temperature (RT) and subsequently washed three times with wash buffer for 5 minutes under gentle shaking at RT. Next, the strips were incubated in wash buffer and the corresponding antibody (rabbit-anti-sheep-peroxidase (POD) or rabbit-anti-goat-POD; (Dianova, Hamburg, Germany) for 45 minutes under gentle shaking at RT. After three rinsing steps with wash buffer the strips were incubated in substrate solution. The reaction was stopped after development of bands or after 8 minutes. The strips were washed with distilled water and placed between absorbent tissues and stored in the dark until evaluation. Analysis of the test was carried out after checking for the clearly stained reaction control band, the antibody conjugate control band (here: IgG) and the presence of the cut-off control band. Next, band intensity for the respective antigens was assessed in relation to the cut-off band. Only bands with intensities equivalent or stronger than the cut-off band were considered positive. Tests were considered

<table>
<thead>
<tr>
<th>Farm</th>
<th>Species</th>
<th>Flock size</th>
<th>Samples taken</th>
<th>Samples tested</th>
<th>mean age in months</th>
<th>Females</th>
<th>Males</th>
<th>Yop D</th>
<th>Yop E</th>
<th>Yop M</th>
<th>V-AG</th>
<th>MyfA</th>
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<td>35</td>
<td>51.7 (10-122)</td>
<td>30</td>
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<td>9</td>
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</table>

F: female; m: male.
positive if the anti-Yop D band and one further antigen band (Yop M, V-AG, Yop M) reacted. Differentiation for Y. pseudotuberculosis and Y. enterocolitica in positive tests was done according to the following criteria: For Y. pseudotuberculosis: PsaA band ≥ cut-off and MyfA band < cut-off. For Y. enterocolitica: MyfA band ≥ cut-off and PsaA band < cut-off. If none of the criteria applied, a differentiation between the species was not possible.

Results
In total, 388 samples were tested (248 sera from sheep and a total of 140 sera from goats) for the presence of anti-Yersinia antibodies (Table 1). The flocks had a history of reproductive disorders such as stillbirth, premature delivery, weak lambs/kids and abortion. The age of the sampled animals ranged six months to 11 years and two months to 9 years among sheep and goats, respectively. The youngest positive animal (goat) was 2 months old; the oldest animal (sheep) was 11 years old. Antibodies specific for Y. enterocolitica were detected in 7/9 sheep flocks and in 4/4 goat flocks. Antibodies specific for Y. pseudotuberculosis were detected in 4/9 sheep flocks. Two sheep flocks revealed the presence of both Y. enterocolitica and Y. pseudotuberculosis specific antibodies.

Discussion
Seroprevalence of Y. enterocolitica and Y. pseudotuberculosis infections in animals and humans is not established in Pakistan. Due to the worldwide occurrence of enteropathogenic Yersinia spp. we assumed that yersiniosis should also be present in Pakistan. A large population of small ruminants in Pakistan and a significant number of people involved in livestock rearing and production activities may pose a significant health risk regarding yersiniosis. Moreover, Y. enterocolitica O:9 serovars might also hamper the serological detection of Brucella abortus, the causative agent of the highly contagious zoonosis Brucellosis, due to possible cross-reactions [13,14]. A primary survey was therefore conducted at government farms testing the presence of anti-Yersinia antibodies in goats and sheep using the recomLine Yersinia IgG 2.0 kit. This assay targets antibodies against the Yersinia outer proteins Yop-M, Yop-D, Yop-E as well as V-AG, PsaA and MyfA. Using target proteins specifically associated with Yersinia pathogenicity for serology reduces the number of false positivity in comparison to the lipopolysaccharide (LPS) derived antigens [15]. Only a few studies have been conducted analysing the presence of pathogenic Yersinia spp. in small ruminants. A cohort study from New Zealand analysing goat stool samples revealed that asymptomatic carriage of Yersinia infections was common. Over a one-year study period, 21% of the cultured faecal samples were positive for Y. enterocolitica biotype (bt) 5, O:2,3, 6% positive for Y. enterocolitica bt 1A, 3% positive for Y. enterocolitica bt 3, O:5,27, 14% positive for Y. pseudotuberculosis and 36% positive for Y. frederiksenii [9]. A study from Iran on Yersinia spp. in raw milk from goat and sheep revealed a prevalence of 2.4% and 3% for Y. enterocolitica bt 1A, O:9, respectively [16]. Up to 34% mortality has been reported among sheep due to yersiniosis caused by Y. enterocolitica bt 3 in China [17]. An Australian study on prevalence and faecal shedding of Yersinia spp. in sheep determined point prevalence ranging from 4% to 91% across farms and sampling occasions, with up to 60.7% of lambs shedding Yersinia spp. on at least one sampling occasion. This study detected four species, i.e. Y. enterocolitica, Y. pseudotuberculosis, Y. intermedia and Y. frederiksenii detected by qPCR [11]. In a prospective study conducted between 1996 and 1999 in the Indian Punjab on Y. enterocolitica enteritis, Y. enterocolitica was isolated in 2% of human stool samples, with bt 4, O:3 being the most prevalent [18].

Conclusion
The present primary study has shown for the first time that small ruminants in the Pakistani Punjab are exposed to pathogenic Yersinia spp. Due to a significant number of the people involved in raising small ruminants, further studies are urgently needed to determine the threat of yersiniosis to the veterinary and public health and to characterise the circulating strains with regard to their antibiotic susceptibility.

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References


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