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ORIGINAL ARTICLE

Immune cells in the small intestinal mucosa of newborn yaks

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Background: This study aimed to characterise and evaluate the main markers of T lymphocytes, B lymphocytes, immunoglobulin (Ig) A and IgG plasmocytes, macrophages, and dendritic cells of the intestinal mucosa of newborn yaks.

Materials and methods: Ten newborn yaks (2–4 weeks old) were chosen. Immunohistochemistry and real-time quantitative polymerase chain reaction were used to analyse the immune cell distribution and specific markers at the mRNA expression level in the duodenum, jejunum, and ileum.

Results: The results showed in the epithelium, CD3ε-positive T lymphocyte levels were higher than other immune cell levels (p < 0.05). Additionally, in the lamina propria, the number of cells positive for CD3ε, CD68, and signal inhibitory regulatory protein alpha (SIRPα) were higher in the villi, while CD79α, IgA and IgG cells were more common at the base of the crypt. Moreover, both in the epithelium and lamina propria, the number of CD3ε, CD68 and SIRPα were decreased from the duodenum to the ileum (p < 0.05), additionally the number of CD79α, IgA and IgG positive cells were increased from the duodenum to the ileum of newborn yaks (p < 0.05). Furthermore, the mRNA expression levels of CD3ε, CD68, and SIRPα increased from the duodenum to the ileum (p < 0.05), while the mRNA expression levels of CD79α, IgA and IgG decreased from the duodenum to the ileum.

Conclusions: Immunohistochemical characterisation and expression levels of immune factors in the small intestinal mucosa of newborn yaks suggest that the intestinal mucosa is an important part of the natural barrier and provides useful references for immunity functions of newborn yak intestinal mucosa. (Folia Morphol 2022; 81, 1: 91–100)

Key words: newborn yaks, small intestine, mucosa, immunity cell

INTRODUCTION

Domesticated yaks are a valuable breed resource in the Qinghai-Tibet Plateau. They have unique ecological characteristics and are valued for their meat, draft and milk [19]. Highland pastoral areas are currently dominated by traditional grazing yak breeding; however, due to the harsh plateau environment, extensive management, and the influence of pathogenic factors, the intestinal mucosal immune barrier of yaks is highly susceptible to damage. Therefore,

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the incidence of gastrointestinal function disorder, inflammatory bowel disease, and infectious diarrhoea disease is high. These illnesses increase the length of the growth cycle and, as a result, the yak develops slowly. The mortality rate of newborn yaks is as high as 30% [9], which causes serious damage to the development of agriculture. Therefore, in order to improve yak survival and productivity, it is imperative to understand the characteristics of the small intestinal mucosal immune cells of yaks.

The small intestinal tract contains the largest number of immune cells, comprising a heterogeneous population of T and B lymphocytes, plasmocytes, macrophages, dendritic cells and a variety of non-professional antigen-presenting cells [8, 18]. Appropriate interactions between these different cell types are essential for generating immune responsiveness or tolerance to a large array of environmental antigens. The previous study analysed the distribution and population of immunocompetent cells in the small intestine of sheep, pigs, calves and mice [4, 6, 21, 22]. However, due to the limitation of the global yak distribution, there are few reports on the immune cells of yaks.

The aim of the present study was to provide basic data on the characteristics of immune cells and factors in the small intestine of healthy newborn yaks. We used CD3ε, CD79α, immunoglobulin (Ig) A, IgG, CD68, and signal inhibitory regulatory protein alpha (SIRPα) to characterise T and B lymphocytes, plasmocytes, macrophages, and dendritic cells in the small intestine of newborn yaks, and assayed the mRNA expression levels of immune cell-specific markers.

MATERIALS AND METHODS
Animals and tissues collection

All experimental animals were handled according to the Animal Ethics Procedures and Guidelines of the People's Republic of China and were approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine of Gansu Agricultural University. All yaks were considered healthy based on the results of physical examination and serum biochemical analysis. The animals were euthanased with an intravenous injection of pentobarbital sodium (200 mg/kg). To maintain the original habitat of the animals, the yaks were sacrificed, and samples were collected from local farms.

Ten newborn yaks (2–4 weeks old) were obtained on a local farmer in Xining City, Qinghai Province. The small intestinal regions (duodenum, jejunum, and ileum) were excised from each animal and samples were taken for immunohistochemical and polymerase chain reaction (PCR) analysis. All specimens intended for immunohistochemical analysis were fixed in 4% neutral paraformaldehyde phosphate buffer (pH 7.3). Specimens intended for real-time quantitative PCR (RT-qPCR) were flash frozen and stored in liquid nitrogen until further processing.

Relative RT-qPCR

Total RNA was isolated from the small intestine using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed to single-stranded cDNA using a reverse transcription kit (MBI Fermentas, Burlington, ON, Canada) according to the manufacturer's instructions. The RT-qPCR primers were designed according to the *Bos grunniens* CD3ε, CD79α, IgA, IgG, CD68, SIRPα, and β-actin gene sequences (GenBank accession numbers: KY911279, KY911280, MG432919, MF099643, KY921959, MH347358, and DQ838049, respectively) using Primer 5 software and synthesised by the Beijing Genomics Institute BGI Company (China). The RT-qPCR primer sequences are presented in Table 1. RT-qPCR was conducted using a Light-Cycler480 thermocycler (Roche, Manheim, Germany) in 20-μL reaction volumes consisting of 1 μL cDNA, 1 μL forward primer, 1 μL reverse primer, 10 μL 2$\times$ SYBR Green II PCR Master Mix (TaKaRa, Shiga, Japan), 0.4 μL Rox, and 6.4 μL nuclease-free H$_2$O. Four replicates were set for each sample to ensure the accuracy of the relative expression of the target genes in the sample. After amplification, according to the system-generated Ct value, the $2^{-\Delta\Delta Ct}$ method was used with β-actin as an internal standard to obtain the relative expression of CD3ε, CD79α, IgA, IgG, CD68 and SIRPα mRNA.

Immunohistochemical examination

The spatial distribution of cells positive for CD3ε, CD79α, IgA, IgG, CD68 and SIRPα in the small intestine of newborn yaks was evaluated by immunohistochemical staining. Fixed tissue sections were mounted on microscope slides in a routine manner and exposed to primary antibodies against CD3ε (monoclonal rabbit anti-cow CD3ε, Abcam, Cambridge, UK; ab16669, 1:200 dilution), CD79α (monoclonal mouse anti-cow CD79α, Abcam; ab199001, 1:100 dilution), IgA (polyclonal rabbit anti-cow IgA, Abcam; ab112630, 1:100 dilution), IgG (polyclonal rabbit anti-cow IgG, Abcam; ab6692, 1:100 dilution), SIRPα (polyclonal rabbit an-

Table 1. Primers used in this study

Genes	Primer names	Primer sequences (5'-3')	Length (bp)	Annealing (℃)
CD3ε	P1	F: GGGCTCATAGTCTGGATTGG	155	57
	P2	R: TGTGTCACTCTGGGCTTGC		
CD79α	P3	F: ACGGCAAGAAGATTCAGCG	182	60
	P4	R: CCAAGGAGGCAATAGGAG		
IgA	P5	F: GGTTCACAGGACCCAGA	227	57
	P6	R: AGCACCTAGTGAAGCCC		
IgG	P7	F: AACCAACACCACAGGAAC	208	60
	P8	R: AGTGTAGTCTCCTATTGCCT		
CD68	P9	F: TGAGAGGAGCAAGTGGGA	194	56
	P10	R: GTGGACATCATCCTGGCTGG		
SIRPα	P11	F: ATCCTGCTGCCCGCTGTA	215	60
	P12	R: AACAGTTGGGCGGCGAG		
β-actin	P13	F: AGGCTGTGCTGTCCCTGTATG	207	60
	P14	R: GCTCGGCTGTGGTGGTAAA		

ti-cow SIRPα, Abcam; ab116254, 1:100 dilution), and CD68 (polyclonal rabbit anti-cow CD68, Abbiotec, San Diego, USA; No:252281, 1:100 dilution) for 2 h at 37℃ in a moist chamber. Biotinylated secondary antibodies were applied for 10 min. Streptavidin-conjugated peroxidase was then applied to the slides for 10 min. The reaction products were formed using 3,3-diaminobenzidine tetrahydrochloride. The sections were lightly counterstained with haematoxylin. The negative control for each sample was created by replacing the primary antibody with serum albumin; all other steps and conditions remained the same.

Examination of sections

The sections were examined under a light microscope with image analysis software (Image-Pro Plus, Media Cybemetics, Silver Spring, MD, USA). Cells positive for CD3ε, CD79α, IgA, IgG, SIRPα and CD68 in the small intestine of newborn yaks were evaluated. Subjective analysis of the distribution of positively labelled cells in the lamina propria and epithelial compartments was performed using a ×40 objective.

In each tissue sample, the number of positively labelled cells was determined in three standard areas (Fig. 1) of the lamina propria: the villi, the upper crypt, and the base of the crypt. Five images were randomly chosen for each of these areas. Results were expressed as cells per 10000 μm^2 of lamina propria tissue [7]. Intraepithelial immune cells were assessed by counting positively labelled cells in five areas (each of 100 enterocytes) of the epithelium, and the results were
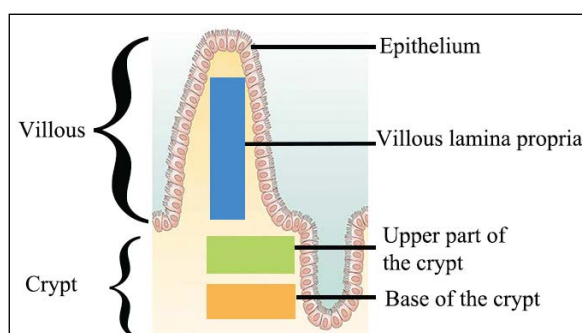

Figure 1. Diagram of small intestinal epithelium and lamina propria to demonstrate the areas used for counting.

expressed as the mean number of cells/100 enterocytes. Repeated independent counts were performed on five serial sections from the same tissue block to assess the precision of both the lamina propria and epithelial cell counting techniques.

Statistical analysis

All statistical analyses were performed using SPSS (version 21.0; IBM Corp., Armonk, NY, USA). The relative mRNA levels and positive cell numbers among the study groups are expressed as mean ± standard error. Statistical significance was determined using one-way analysis of variance and was set at $p < 0.05$.

RESULTS

Morphological analysis of the crypt-villus axis

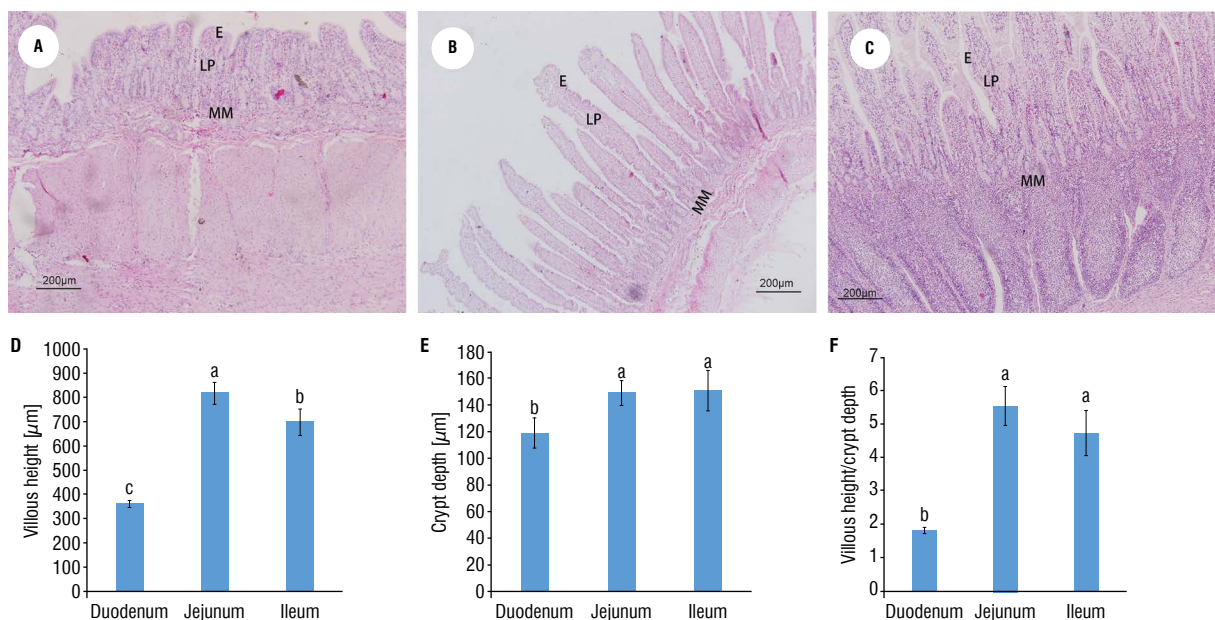The small intestinal mucosa of newborn yaks can be divided into three layers: the epithelium, lamina

Figure 2. Morphological characteristics and analysis (including villous height, crypt depth, and ratio of villous height to crypt depth) of the small intestinal mucosa in newborn yaks; **A–C.** The histological section of the duodenum, jejunum and ileum in newborn yaks (HE, ×100); **D.** The villous height of the duodenum, jejunum and ileum in newborn yaks; **E.** The crypt depth of the duodenum, jejunum and ileum in newborn yaks; **F.** Ratio of villous height to crypt depth of the duodenum, jejunum and ileum in newborn yaks. Bars with different superscripts are significantly different (p < 0.05). The results were presented as mean ± standard error; E — mucosal epithelium; LP — lamina propria; MM — muscularis mucosa.

propria and mucosal muscle. The morphological parameters of the crypt-villus axis in the small intestine include villous height, crypt depth, and the ratio of villous height to crypt depth (Fig. 2A–C). The villous height was highest in the jejunum, followed by the ileum and the duodenum (p < 0.05) (Fig. 2D). Crypt depth was higher in the jejunum and ileum than in the duodenum (p < 0.05) (Fig. 2E). Moreover, there were no differences between the jejunum and ileum (p > 0.05). Furthermore, the ratio of villous height to crypt depth was higher in the jejunum and ileum than in the duodenum (p < 0.05) (Fig. 2F).

CD3ε, CD79α, IgA, IgG, CD68 and SIRPα mRNA expression

The relative expression levels of CD3ε, CD79α, IgG, IgA, CD68 and SIRPα mRNA differed between the duodenum, jejunum and ileum of newborn yaks (Fig. 3). Within the same intestinal region, the expression levels of CD3ε, CD68 and SIRPα mRNA were significantly higher than those of CD79α, IgA and IgG. Additionally, in the different intestinal regions, CD3ε, CD68 and SIRPα mRNA expression levels increased from the duodenum to the ileum (p < 0.05), while the mRNA expression levels of CD79α, IgA and IgG decreased from the duodenum to the ileum.
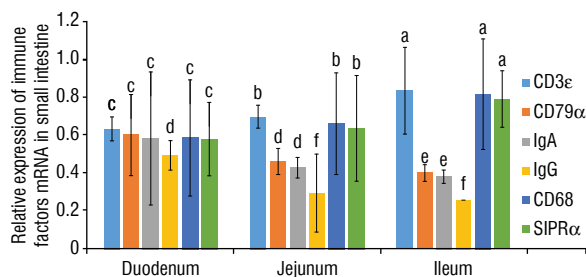

Figure 3. Relative abundance of CD3ε, CD79α, immunoglobulin A (IgA), immunoglobulin G (IgG), CD68 and signal inhibitory regulatory protein alpha (SIRPα) mRNA in the duodenum, jejunum and ileum of newborn yaks. Bars with different superscripts are significantly different (p < 0.05). Data are normalised and presented as ratio mean ± standard error, with β-actin as an internal control.

CD3ε-positive T lymphocytes in the small intestinal mucosa

The membrane staining of CD3ε-positive cells was located in the epithelium and lamina propria of the small intestinal mucosa of newborn yaks (Fig. 4A–C). In the epithelium, the number of CD3ε-positive T lymphocytes increased from the duodenum to the ileum, peaking at the ileum (p < 0.05) (Fig. 5). The difference between the duodenum and jejunum was not significant (p > 0.05). In the lamina propria, CD3-positive T lymphocytes increased from the basal
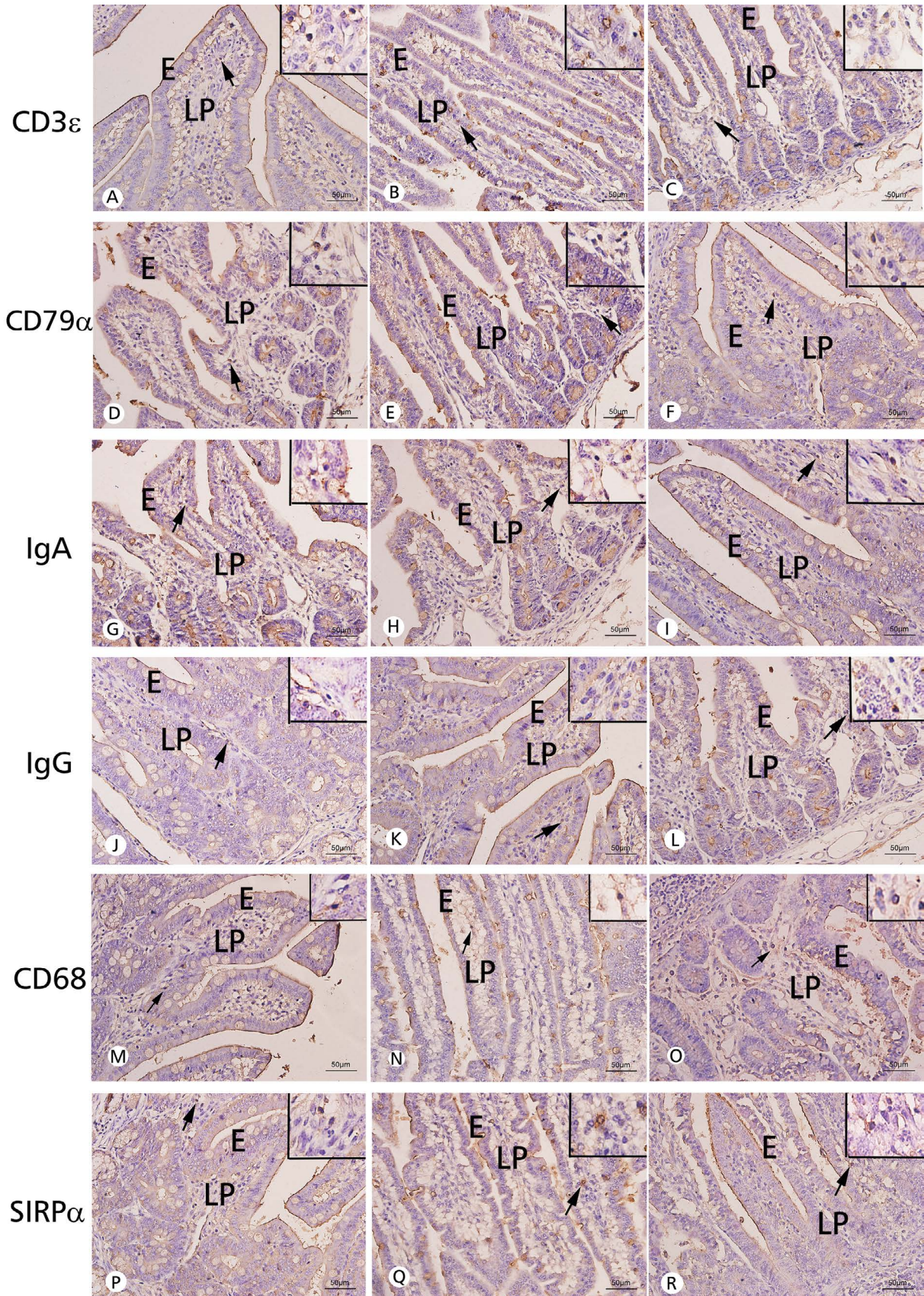
Figure 4. A–R. Immunohistochemical staining of cells expressing CD3ε, CD79α, immunoglobulin A (IgA), immunoglobulin G (IgG), CD68 and signal inhibitory regulatory protein alpha (SIRPα) in the duodenum, jejunum and ileum of newborn yaks; E — mucosal epithelium; LP — lamina propria. Arrows show the positive cells (brown). Bar = 50 μm (low-power lens), bar = 20 μm (high-power lens on the upper right).
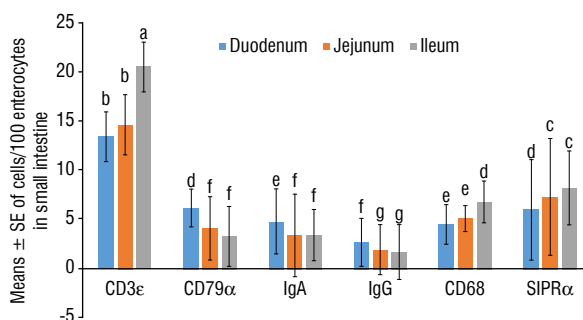
Figure 5. The number of CD3ε, CD79α, immunoglobulin A (IgA), immunoglobulin G (IgG), CD68 and signal inhibitory regulatory protein alpha (SIRPα) positive cells in mucosal epithelium from the duodenum, jejunum and ileum of newborn yaks. Bars with different superscripts are significantly different (p < 0.05). Data are normalised and presented as mean ± standard error (SE) of cells//100 enterocytes.

crypt to the villi (Fig. 6A). Additionally, the number of CD3-positive cells in the lamina propria was higher in the ileum than in the duodenum and jejunum.

CD79α-positive B lymphocytes in the small intestinal mucosa

The membrane staining of CD79α-positive B lymphocytes was localised to the epithelium and lamina propria of the small intestinal mucosa of newborn yaks (Fig. 3D–F). CD79α-positive B lymphocyte levels were highest in the epithelium of the duodenum and then decreased from the duodenum to the ileum (p < 0.05) (Fig. 5). Additionally, in the lamina propria, CD79α-positive B lymphocytes increased from the villi to the basal crypt. Moreover, the number of CD79α-positive B lymphocytes in the lamina propria was higher in the duodenum than in the ileum and jejunum (p < 0.05) (Fig. 6B).

IgA- and IgG-positive plasmocytes in the small intestinal mucosa

IgA and IgG markers stained the cytoplasm of the plasmocytes. Positive cells appeared in the epithelium and lamina propria of the small intestinal mucosa of newborn yaks (Fig. 3G–L). IgA- and IgG-positive plasmocytes were highest in the epithelium of the duodenum but decreased from the duodenum to the ileum (p < 0.05) (Fig. 5). There were no significant differences between the duodenum and the ileum. Furthermore, in the lamina propria, IgA- and IgG-positive plasmocytes increased from the villi to the basal crypt in all regions of the intestine, peaking in the basal crypt (p < 0.05) (Fig. 6C, D). Moreover,

the number of IgA- and IgG-positive plasmocytes in the lamina propria was higher in the duodenum than in the ileum and jejunum.

CD68-positive macrophages in the small intestinal mucosa

CD68-positive macrophages presented as large, irregular, or elongated cells scattered in the epithelium and lamina propria of the small intestinal mucosa of newborn yaks (Fig. 3M–O). In the epithelium, CD68-positive macrophages increased from the duodenum to the ileum, peaking in the ileum (p < 0.05) (Fig. 5). The difference between the duodenum and jejunum was not significant (p > 0.05). Furthermore, in the lamina propria, CD68-positive macrophages increased from the basal crypt to the villi in all regions of the intestine, peaking in the villi (p < 0.05) (Fig. 6E). Additionally, the number of CD68 positive macrophages in the lamina propria was higher in the ileum than in the duodenum and jejunum (p < 0.05).

SIRPα-positive dendritic cells in the small intestinal mucosa

Strong SIRPα cytoplasmic staining of dendritic cells was observed in the epithelium and lamina propria of the small intestinal mucosa of newborn yaks (Fig. 3P–R). SIRPα-positive dendritic cell numbers were higher in the epithelium of the ileum and jejunum than in the epithelium of the duodenum (p < 0.05) (Fig. 5). Furthermore, in the lamina propria, SIRPα-positive dendritic cells increased from the basal crypt to the villi, peaking in the villi (p < 0.05) (Fig. 6F). Moreover, the number of SIRPα-positive dendritic cells in the lamina propria was higher in the ileum than in the duodenum and jejunum.

DISCUSSION

Many studies have focused on immunocompetent cells in various species, including lambs [4], calves [6], pigs [21], and mice [22]. However, there are limited data available on the small intestine of newborn yaks. To our knowledge, this is the first study to investigate the distribution of immunocompetent cells of the mucosa and to characterise the changes in these cell markers in the small intestine of newborn yak.

The inner surface of the small intestine is covered with finger-like projections called villi that increase the surface area available for the absorption of nutrients from the gut content. The villi increase the length of
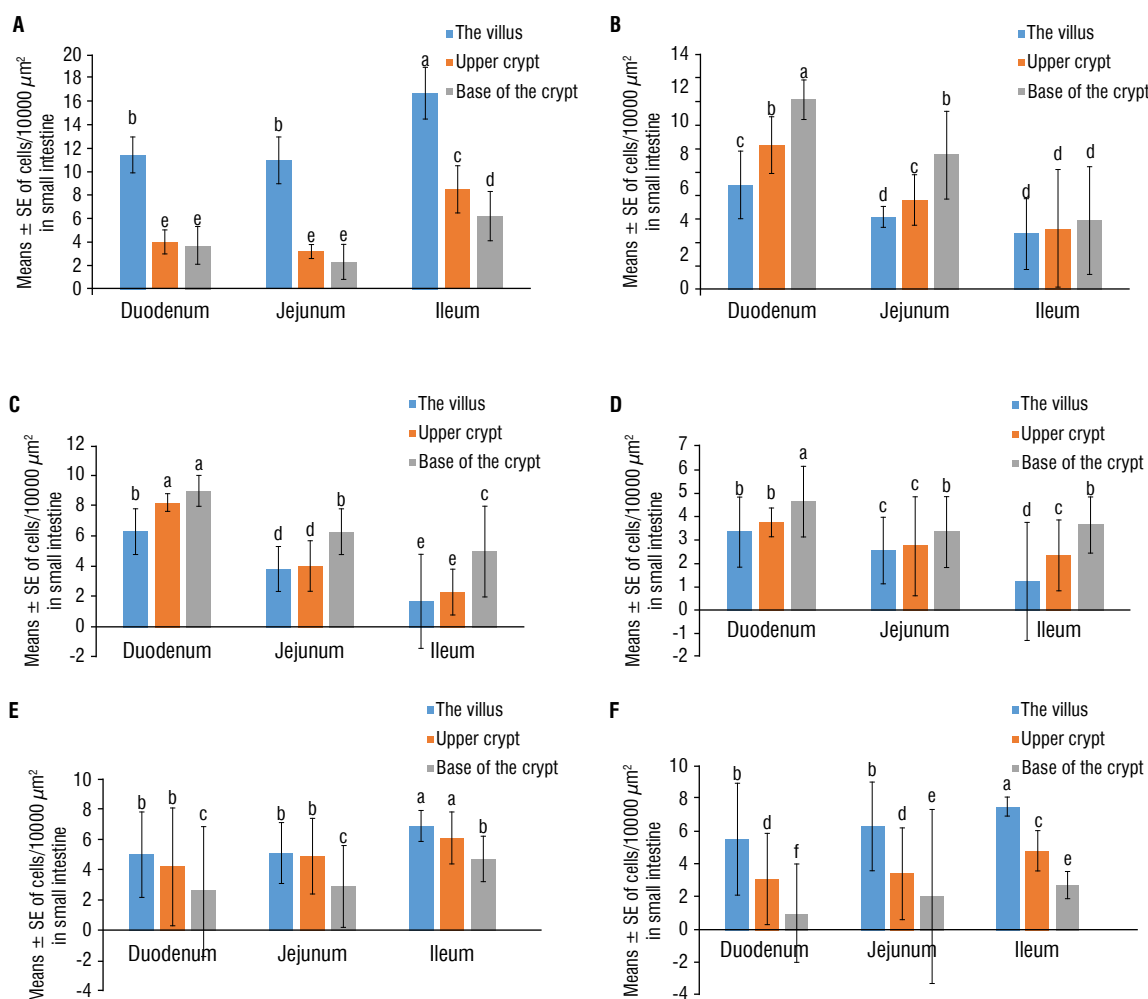
Figure 6. The number of CD3ε (**A**), CD79α (**B**), immunoglobulin A (IgA) (**C**), immunoglobulin G (IgG) (**D**), CD68 (**E**) and signal inhibitory regulatory protein alpha (SIRPα) (**F**) positive cells in mucosal lamina propria from duodenum, jejunum and ileum of newborn yaks. Bars with different superscripts are significantly different (p < 0.05). Data are normalised and presented as mean $\pm$ standard error (SE) of cells/10000 μm^2.

the small intestine and therefore increase the likelihood of a food particle encountering a digestive enzyme and being absorbed across the epithelium and into the bloodstream [13]. In the present study, the largest villous height was observed in the jejunum of newborn yaks; thus, the highest rate of absorption may occur in the jejunum. On the other hand, Zhou [28] reported crypts are formed by secretory epithelial cells. In this study, the deepest crypt depths were located in the jejunum and ileum of the newborn yaks, indicating the highest rate of digestion occurs in the jejunum and ileum of newborn yaks. Furthermore, the ratio of villous height to crypt depth reflected the functional state of the small intestine, with a high ratio indicating a high elimination and absorption function [28]. We also found that the ratio of villous height to crypt depth was higher in the jejunum and ileum than in the duodenum. This may indicate that

the highest rate of absorption and digestion occurs in the jejunum and ileum of newborn yaks.

Possessing the characteristics of both innate and adaptive immunity, T lymphocytes in the mucosa serve as an effective first-line defence against invasive microorganisms [23]. CD3ε is an important differentiation antigen on the surface of the T lymphocyte membrane and a characteristic marker of mature T lymphocytes [10]. We found that the number of CD3ε-positive T lymphocytes was higher in the epithelium and lamina propria of the ileum than those of the duodenum. Consistently, in cats, calves, and goats, the total number of T lymphocytes is greatest in the ileum [4, 6, 24]. Ma reported that CD3ε-positive T lymphocytes in the mucosa of the small intestine can rapidly respond to microbial invasion by activating host defence responses, including the production of mucus and antimicrobial peptides, which

help prevent microbes from reaching the epithelial surface [14]. Additionally, during active infection, T lymphocytes in the mucosa promote epithelial cytolysis and cytokine and chemokine production, which serve to limit pathogen invasion, replication, and dissemination [17]. The distribution characteristics of CD3ε-positive T lymphocytes in the intestinal mucosa in this study suggest that cellular immunity of the intestinal mucosa of newborn yaks mainly occurs in the ileum. Furthermore, we found that the number of CD3ε-positive cells in the lamina propria was higher in the villi than in either of the two crypt regions in newborn yaks. The increase in T lymphocyte density towards the villous tip likely reflects the increased exposure to luminal antigens at this site.

CD79α is a common marker of B lymphocytes that plays a key role in B lymphocyte antigen receptor signal transduction, development, stabilisation, and function [20]. In the present study, a significant number of CD79α-positive cells were observed in the basal crypt area of the lamina propria. Wang reported that B cells of the lamina propria have an increased expression of surface activation markers and exhibit spontaneous immunoglobulin secretion [25]. This indicates that the basal crypt area may be the site of B cell terminal differentiation in the development of immune responses against intestinal antigens. The present study showed a higher number of B lymphocytes in the duodenal epithelium and lamina propria of newborn yaks than in the ileum; similar results have been obtained in lambs and calves [4, 6]. We speculated that the duodenum is likely the first site to come into contact with foreign antigens and activate immune responses. Therefore, the duodenum contains a higher number of B lymphocytes which are stimulated by antigens and involved in humoral immunity.

IgA- and IgG-positive cells are important immunoglobulin secretory cells. Yasuda et al. [27] reported the distribution and quantity of IgA and IgG have been reported to be directly related to the antibody secretion level, and thus reflect the local cellular immune function, of the small intestinal mucosa. In the present study, a greater number of plasmocytes were found in the base of the crypt than in the villi and upper crypt. This indicates that the base of the crypt is a potential site of B lymphocyte terminal differentiation into plasma cells. In both the epithelium and lamina propria, the total number of IgA- and IgG-positive plasma cells was higher in the duodenum than in the jejunum and ileum of newborn yaks. These results

are in general agreement with those found in calves, where the total number of plasmocytes is greatest in the duodenum [27]. Wu et al. [26] reported that, by binding to and entrapping antigens in the mucus layer, IgA and IgG limit their access to the mucosa and promote antigen degradation by enzymes within the lumen. Thus, IgA and IgG may provide a backup system in which antigens that have penetrated the mucosal barrier may be cleared by secretory component-mediated transport in the liver [26]. These findings also reflect that the duodenum is a potential major site of effector function for plasma B lymphocytes. It was likely related to duodenal antigens, bile salts, pancreatic secretions, and other local factors that stimulate the production and maturation of antibody-secreting cells.

CD68 is widely used as a marker for intestinal macrophages [16]. Muller reported that, in non-inflamed intestinal mucosa, the lamina propria extracellular matrix releases transforming growth factor beta and interleukin-8, which then recruit blood monocytes to the lamina propria to become resident macrophages [16]. Farache et al. [5] previously showed that functions of intestinal macrophages include antigen presentation, phagocytosis, and production of immune-regulatory factors. In the present study, CD68-positive macrophages were observed in the epithelium. Mowat reported that the main function of most lamina propria macrophages is to phagocytose bacteria (both commensals and pathogens) crossing the epithelium without evoking a strong inflammatory response [15]. Thus, we speculated that epithelial macrophages in the small intestine of yaks may capture and present antigens to lymphocytes, and may also be engaged in the phagocytosis of senescent intestinal epithelial cells. The number of CD68-positive macrophages in the lamina propria was higher in the villi than in the other regions of newborn yaks. Similarly, Bain and Schridde [1] reported that macrophage concentrations were highest in the villi of the intestinal lamina propria. This result suggests that the villi may play a larger role in antigen stimulation and macrophage recruitment than other areas of the lamina propria. Interestingly, macrophages positive for CD68 were more commonly found in the epithelium and the lamina propria of the ileum than in other regions of the intestine. This suggests that the ileal mucosa of newborn yaks may be more susceptible to antigen capture and processing than the mucosa of the duodenum and jejunum.

Dendritic cells express SIRPα [2]. Mucosal dendritic cells are a key link between innate and acquired

immunity via their roles in antigen presentation and regulation of T cell activation [2]. In the present study, SIRPα-positive dendritic cells were observed in the epithelium of the small intestine of newborn yaks. It has been previously suggested that dendritic cells can extend their dendrites into the lumen between epithelial cells to handle antigens. In this study, we found a higher number of SIRPα-positive cells in the villi of the lamina propria. We believe the appearance of abundant SIRPα-positive cells in the villi of the lamina propria was because the site is easily exposed to lymphocytes and bacterial or dietary antigens. Furthermore, we found that SIRPα-positive cell concentrations were higher in the epithelium and lamina propria of the ileum and jejunum of newborn yaks. The distribution trend was similar to that for T lymphocytes. Coombes and Powrie [3] showed that dendritic cells expressing the chemokine receptor CCR6 could activate T lymphocytes in response to bacterial invasion. Previous reports have indicated that the wide distribution of dendritic cells in the ileum and jejunum play critical roles in the regulation of intestinal immunity, antigen uptake, and T lymphocyte activation [3, 11, 12].

In this study, RT-qPCR was used to detect specific immune cell markers. The mRNA expression levels of CD3ε, CD68, and SIRPα were significantly higher than those of CD79α, IgA, and IgG in the same intestinal regions. Thus, we speculate that in the small intestine of newborn yaks, T lymphocytes, macrophages, and dendritic cells may be more abundant than B lymphocytes and plasmocytes. Additionally, we also found that the mRNA expression levels of CD3ε, CD68, and SIRPα were higher in the ileum, whereas CD79α, IgA, and IgG mRNA expression levels were higher in the duodenum. These observations indicate that local humoral immunity may occur more commonly in the ileum, while cellular immunity may occur more commonly in the duodenum.

CONCLUSIONS

In summary, we immunohistochemically characterised immune cells in the small intestinal mucosa and analysed the mRNA expression level of immune factors in the small intestine of newborn yaks. In the epithelium CD3ε-positive T lymphocytes were more popular than the other immune cells. In the lamina propria, CD3ε-positive T lymphocytes, CD68-positive macrophages and SIRPα-positive dendritic cells were more highly concentrated in the villi, while CD79α-positive B lymphocytes, and IgA- and IgG-pos-

itive plasmocytes were more prevalent in the base of the crypt. Furthermore, a higher number of T lymphocytes, macrophages and dendritic cells were located in the epithelium and lamina propria of the ileum than those of the duodenum and jejunum; B lymphocytes, and IgA and IgG plasmocytes were more likely to be observed in the epithelium and lamina propria of the duodenum of newborn yaks than those of the jejunum and ileum. These findings suggest that cellular immunity and antigen presentation are more readily activated in the epithelium, the villi of the lamina propria, and the ileal mucosa of the small intestine of newborn yaks, while humoral immune cells are mostly concentrated in the base of the crypt of the lamina propria and duodenal mucosa. The results from this study will provide information on the baseline characteristics of immune cells in the small intestine of newborn yaks and serve as a reference for future studies on various immunologic reactions in both healthy yaks and those with digestive diseases.

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