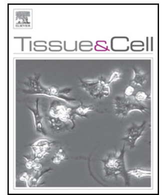
Tissue and Cell 48 (2016) 383–388

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice



Characterization of paneth cells in alpacas (*Vicugna pacos*, Mammalia, Camelidae)

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ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form 20 April 2016
Accepted 25 April 2016
Available online 7 May 2016

Keywords:
Paneth cells
Alpacas
Morphometry
Immunohistochemistry
Lectinhistochemistry
Lactancy

ABSTRACT

Paneth cells are secretory epithelial cells of the innate immune system of the intestine of several mammals, including alpacas. Little is known about the latter; thus, in the present study we described the morphology and histochemical characteristics of Paneth cells in healthy fetuses, and young and adult alpacas. For this purpose, samples of duodenum, jejunum and ileum were taken from 6 fetuses at different days of pregnancy (between days 221–330), 66 offsprings (between 0 and 45-days-old) and 5 adult alpacas (>2-years-old). Samples were fixed in 10% buffered formalin and processed for histological and morphometrical analysis using HE and Masson Trichomicís technique. Immunohistochemistry was used to identify Paneth cells using anti-lysozyme antibody. In addition, the lectinhistochemichal binding-pattern of Paneth cellís granules was evaluated. Lyzozyme was immunohistochemically detected in the granules of Paneth cells from day 283 of pregnancy in all the small intestinal sections of the studied fetuses. In newborn alpacas Paneth cells were initially found in the duodenum, but the following days (days 18–21 after birth) they were also found in the ileum. Their size gradually increased after birth, but then no significant differences were found. In adult alpacas the number was lower than offsprings. We suggest that Paneth cells early differentiate in the small intestine of alpacas, and the increase in their number during the first two weeks of life strongly support their possible involvement in the intestinal defensive functions against the enteric diseases that occur during the lactancy stage.

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1. Introduction

Paneth cells (PC) are one of the secretory epithelial cell lineages of the intestine in mammals. They contribute to the innate immune system (Clevers and Bevins, 2013; Bevins and Salzman, 2011), and play a vital role in regulating intestinal microbiota (Zhang and Liu, 2016) by secreting a great number of antimicrobial substances, such as lysozyme (Otto, 1973; Deckx et al., 1967), defensins (Poindexter et al., 2009; Wehkamp et al., 2006; Ghosh et al., 2002; Porter et al., 1997; Ouellette et al., 1989), trypsin (Ghosh et al., 2002; Bohe et al., 1984), immunoglobulin A (Tang et al., 2006), angiogenins (Eckmann, 2005) and secretory phospholipases A2 (Keshav, 2006). PC degranulation occurs by exocytosis and it is triggered by the increase in cytosolic calcium (Satoh et al., 1995), by neuronal cholinergic stimuli, products derived from bacteria, such as, lipopolysaccharide, lipid A, muramyl dipeptide and lipoteichoic acid (Ayabe et al., 2002), and hormones, such as gastrin and cholecystokinin (Porter et al., 2002; Satoh et al., 1989). In addition, the secretory response also occurs after exposure to macromolecules and signals produced by other epithelial or connective tissue cells (Porter et al., 2002) and vary along the intestine (Karlsson et al., 2008). The secretion of these substances regulates the endogenous microbiota composition, cell proliferation and differentiation, and it also protects the stem cell niche. PC may also contribute to digestive and detoxification processes (Bevins, 2005, 2004; Ouellette, 2005; Porter et al., 2002).

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http://dx.doi.org/10.1016/j.tice.2016.04.003
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Paneth cells were identified in different species, including human being (Ehrmann et al., 1990; Erlandesen et al., 1974), rat (Rodning et al., 1982; Sundstrom and Heleander, 1980; Behnke and Moe, 1964), mouse (Satoh et al., 1992), guinea pig (Vásquez et al., 2014; Satoh et al., 1990), squirrel (Toth, 1980), hamster, monkey (Satoh et al., 1990), rabbit (Zanuzzi et al., 2008; Abdel-Magied and Taha, 1995), horse (Takehana et al., 1998), sheep (Ergün et al., 2003) and camel (Abdel-Magied and Taha, 1995). In most of the species studied, they are located at the bottom of the Lieberkhün glands of the small intestine where they stay for 18–21 days and then they are renewed (Porter et al., 2002). They are easily recognized by their shape and location, and the staining of their granules using different histological techniques (Klaus, 1969a). In general, PC are pyramidal in shape, with an oval or spherical nucleus, basophilic basal cytoplasm and eosinophilic apical granules (Porter et al., 2002; Ouellette et al., 2000).

Regarding PC in alpacas little and controversial data is known. Montalvo (1966) did not identify PC in the intestine of adult alpacas, whereas Lira et al. (2012) found them from birth up to the first three weeks of life. Since the number, size, shape and location of PC may change under pathological conditions (Liu et al., 2014; Elphick and Mahida, 2005; Kelly et al., 2004; Lewin, 1969; Klaus, 1969a, 1969b), in the present study we described for the first time the morphology and histochemical characteristics of PC in healthy fetuses, and young and adult alpacas. These data may be useful to understand their possible changes under several enteric neonatal diseases caused by *Escherichia coli*, *Clostridium perfringes*, *Eimeria*, rotavirus and coronavirus, the main infectious agents that frequently affect alpacas (Rosadio et al., 2012; Rosadio et al., 2010; Bustinza, 2001).

2. Materials and methods

2.1. Animals

In the present study five adult alpacas (>2-years-old), 66 offsprings (4 newborns −NB-, 12 offsprings between 1–7-days-old, 9 between 8–15-days-old, 10 between 16–21-days-old, 9 between 22–27-days-old, 9 between 28–36-days-old and 13 between 37–45-days-old) were used, and from pregnant alpacas −according to Olivera et al. (2003) the average gestation period of alpacas is 345 ± 5 days- six fetuses from gestational days 221, 230, 293, 317, 325 and 330-one for each time point- were used. Newborn alpacas did not feed colostrum. Adults and offsprings were sacrificed at slaughterhouse, and fetuses were obtained after the sacrifice of their mother at slaughterhouse. All the animals studied were clinically healthy and none of them showed intestinal lesions at the necropsy.

All the management and procedures were carried out according to the 'Guide for the Care and Use of Laboratory Animals' (National Academy Press, 1996, Washington, DC, USA).

2.2. Histological and histochemical techniques

Animals were carefully necropsied and 2 cm long-samples were taken from the middle of the duodenum, jejunum and ileum of each animal, rinsed in PBS, fixed in 10% neutral buffered formalin and embedded in paraffin (A.F.I.P., 1995). Then, section of 5 μm thickness were cut and stained with Hematoxylin and Eosin (HE) (Stevens, 1990), Masson's trichrome (Bradbury and Gordon, (1990)), Alcian blue/periodic acid–Schiff (AB-PAS) and Floxine Tartrazine (A.F.I.P., 1995).

2.3. Immunohistochemistry

Five μm sections were mounted on slides coated with poli-L-lysin- (P 8920, Sigma-Aldrich), and passed through a decreasing graded alcohol scale, and incubated with 3% H_2O_2 in methanol for 30 min at room temperature to inhibit endogenous peroxidase activity. Antigen retrieval was done twice for 5 min using 750 W microwave irradiation in citrate buffer (pH 6.0). Nonspecific binding sites were blocked with 1% bovine serum albumin (BSA) for 30 min. For PC quantification slides were incubated overnight with the primary biotinylated rabbit polyclonal anti-human lysozyme antibody (Accurate Chemical and Scientific Corporation, Westbury, NY, USA) diluted at 1:50. Then, they were rinsed in PBS three times, and incubated with the detection system Horseradish Peroxidase streptavidin SA 704 (Vector Laboratories, Inc., Burlingame, CA, USA) for 30 min. Lacrymal gland, which contains lysozyme in other species, (Rennie and Parsons, 1981; Pinard et al., 2003) of an adult alpaca was included as a positive control (Fig. 3B). To identify the proliferating cells of the crypt some slides were incubated with the primary antibody anti-PCNA (anti-proliferation cell nuclear antigen, PC10 clon, Santa Cruz Biotechnology, Inc, Ca, USA), diluted at 1:1000, and then they were rinsed in PBS three times, and incubated with Mouse ABC immunostaining detection system (Santa Cruz Biotechnology, Inc, Ca, USA), according to the manufacturerís instructions. Sections were rinsed in PBS, revealed with liquid 3,3-diaminobenzidine tetrahydrochloride as chromogen (Vector Laboratories, Inc., Burlingame, CA, USA) and counterstained with Harris' hematoxylin. The dark, golden brown DAB hydrogen peroxide reaction product showed the positively stained structures. Negative controls included exposure to horseradish-peroxidase and substrate medium without primary antibody.

2.4. Lectinhistochemistry

The initial steps of the technique were similar to those described for immunohistochemistry. After blocking the nonspecific binding sites with bovine serum albumin (BSA, 1% in PBS) the slides were incubated overnight with the following seven biotinylated lectins (Lectin Kit BK 1000, Vector Laboratories, Inc., Burlingame, CA, USA): Con-A (*Concanavalia ensiformis,* specifically binding α-D-Man and α-D-Glc); DBA (*Dolichus biflorus*, with binding specificity to α-D-GalNAc); SBA (*Glycine maximus*, binding specificity to α-D-GalNAc, β-D-galNAc and α and β-Gal); PNA (*Arachis hypogea*, that specifically binds β-D-Gal and (1–3) GalNAc); RCA-1 (*Ricinus communis*-1, binding specificity β-D-Gal and α-D-Gal); UEA-1 (*Ulex europaeus*-1, binding specificity α-L-Fuc) and WGA (*Triticum vulgaris,* binding specificity α-D GlcNAc and NeuNAc) (Yasui et al., 2006; Goldstein and Hayes, 1978). The optimal lectin concentration was 30 μg/ml in PBS for all lectins, except for PNA (10 μg/ml). The horseradish peroxidase streptavidin SA-5704 (Vector Laboratories, Inc., Burlingame, CA, USA), used as a detection system, was incubated for 30 min. Slides were rinsed three times in PBS for five min each, revealed with liquid 3,3-diaminobenzidine tetrahydrochloride as chromogen (Vector Laboratories, Inc., Burlingame, CA, USA) and counterstained with Harris' hematoxylin. The dark, golden brown DAB hydrogen peroxide reaction product showed the positively stained structures and the results were evaluated according to previous studies (Martínez et al., 2009; Zanuzzi, 2010; Zanuzzi et al., 2008). Negative controls for lectin staining included exposure to horseradish-peroxidase and substrate medium without lectin. Lectin controls were performed by the addition of inhibitory sugars at a final concentration of 0.01 M.

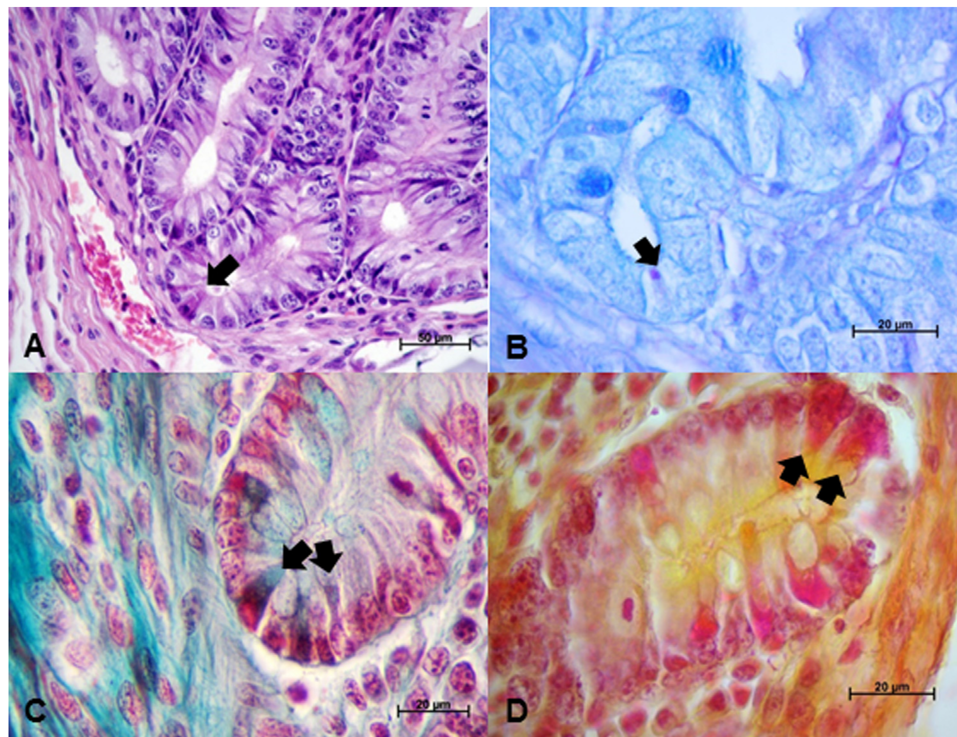

Fig. 1. Paneth cells in the small intestine of alpacas are shown by the arrows. (A) HE staining, Jejunum, 7-days-old, 40×. (B) PAS-alcian blue staining, Jejunum, 1-day-old, 100×. (C) Massonís trichromic staining, Jejunum, 4-days-old, 100×. (D) Floxine Tartrazine staining, Jejunum, 42-days-old, 100×.

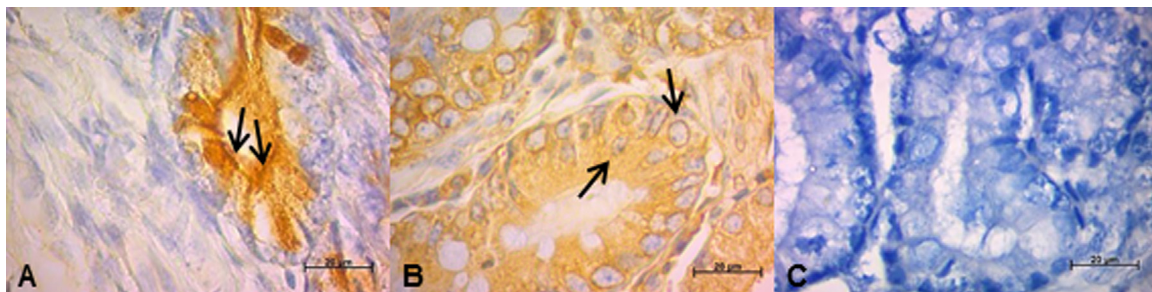

Fig. 2. Lectinhistochemistry. The granules of Paneth cells were strongly stained by SBA lectin (A), and weakly stained by Con-A lectin (B). The remaining lectins did not bind the granules. An example is shown in (C), which corresponds to PNA lectin. Images belong to the jejunum of an animal of 18 days-old. Nuclear counterstaining with Harris Haematoxilin. 100×.

2.5. Image analysis

The histological description and quantification of morphometric parameters were carried out by digital image analysis with the program Axio Vision 4.7 LE Canon. Images from HE staining were captured (10×, 20×, 40× and 100× objectives) and digitized in TIFF format. The area and major and minor axes of 30 PC of each intestinal section and for each animal were measured. Morphometric data were taken only from those cells that showed a delineated shape and a distinguishable nucleus. Similarly, only those cells that were recognized by the image analyzer (Image Pro Plus 6.3), based on the staining or color pattern and on their size and shape, were included in the analysis. In addition, the observations were done by two independent morphologists, in order to obtain a more objective counting and morphometric characterization. The number of PC was estimated in thirty crypts per animal and intestinal section and the average was calculated. Only those crypts fully visible and perpendicular to the muscularis mucosae were considered.

2.6. Statistical analysis

Statistical analysis was performed using SPS 15.0 program. The ANOVA test was used to evaluate differences among groups, followed by multiple comparisons Tukey test. Significant differences were defined as those with $p < 0.05$.

3. Results

3.1. Histology

Using HE staining PC were not easily recognized in any of the intestinal sections studied. In general, PC were arranged in small groups, with pryramidal or columnar shape and showed eosinophilic granules. These granules were best identified by using Massonís trichromic and Floxine Tartrazine techniques from gestational day 330 in all the intestinal sections. In all the animals they were negative to AB-PAS. Representative images for each staining are shown in Fig. 1.

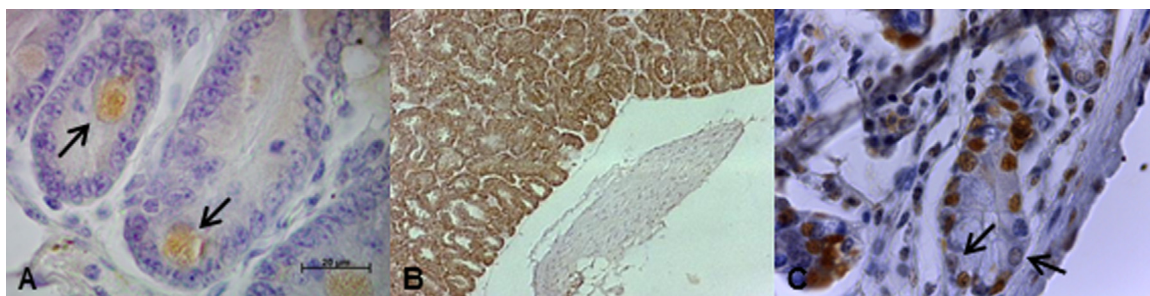

Fig. 3. Immunohistochemistry. (A) Lysozyme immunolabeling. The arrows show positive staining of the granules of Paneth cell in the jejunum of a fetus at gestational day 330, 100×. (B) Lacrymal gland of an adult alpaca. Positive lysozyme immunostaining of the secretory cells. (C) Proliferating cellular nuclear antigen (PCNA) immunolabeling. The arrows show cells with negative immunostaining in the jejunum of an offspring of 27-days-old.

Table 1
PC morphometry in the small intestine of alpacas.

	Fetus	Offsprings										Adults	
	293 days	317 days	325 days	330 days	N	1–7 days	8–15 days	16–21 days	22–27 days	28–36 days	7–45 days		
Major axis(μm)	D	20.11^{a}	18.13^{a}	20.88^{a*}	18.13^{a}	20.21^{a}	18.65^{a*}	19.69^{a}	19.98^{a*}	21.09^{a}	21.34^{a*}	23.15^{b}	16.22^{c}
	Y	17.99^{a}	15.71^{a}	15.76^{a}	17.27^{a}	22.11^{b}	22.02^{b}	22.04^{b}	23.13^{c}	22.29^{b}	24.16^{c}	22.99^{c}	17.17^{a}
	I	14.30^{a*}	16.42^{a}	16.58^{a}	16.79^{a}	17.57^{a*}	21.26^{b}	22.33^{b}	21.69^{b}	24.61^{c*}	25.76^{c}	24.22^{c}	16.38^{a}
Minor axis (μm)	D	6.82^{a}	7.85^{a}	8.99^{b*}	8.52^{b*}	6.91^{a*}	6.62^{a}	7.76^{a}	6.68^{a}	8.87^{b}	7.47^{a*}	8.03^{b}	7.68^{a}
	Y	5.52^{a*}	8.79^{c}	7.08^{b}	7.12^{b*}	8.38^{c*}	7.73^{c*}	7.48^{b}	8.19^{c*}	9.08c	8.28^{c*}	8.51^{2,3}	7.70^{2,3}
	I	6.5^{b}	5.99^{a*}	6.15^{a}	5.67^{a*}	5.36^{a*}	6.18^{a}	6.67^{b}	6.87^{b}	8.02^{c}	6.61^{b*}	7.89^{c}	7.35^{c}
Area(μm^2)	D	135.93^{a*}	138.50^{a}	196.15^{c*}	149.63^{a}	152.94^{b}	138.62^{a}	154.96^{b}	142.99^{a}	202.45^{c}	172.06^{b}	193.06^{c}	135.43^{a}
	Y	91.56^{a}	139.14^{a}	105.99^{a}	130.92^{a}	164.47^{b}	157.08^{b*}	157.02^{b}	179.58^{c*}	194.61^{c}	189.75^{c}	180.22^{c}	119.50^{a}
	I	90.57^{a}	94.04^{a*}	94.81^{a}	89.26^{a*}	88.47^{a*}	136.21^{a}	147.84^{a*}	145.06^{a}	191.66^{b}	167.62^{b}	183.61^{b}	111.90^{a}

N, neonate; D, duodenum; J, jejunum; I, ileum.
^{a,b,c}indicate statistical significance among columns for each age ($p < 0.05$).
* indicate statistical significance among rows for each intestinal section ($p < 0.05$).

Table 2
Number of PC in the small intestine of alpacas.

Intestinal section	Fetus					Offsprings						Adults
	293 days	317 days	325 days	330 days	NB	1–7 days	8–15 days	16–21 days	22–27 days	28–36 days	37–45 days	
Duodenum	0.9 ± 0.7^{1a}	$1.3 \pm 0.7^{1,2a}$	$1.5 \pm 0.7^{1,2a}$	$1.9 \pm 0.9^{1,2,3a}$	2.9 ± 0.8^{4a}	$1.5 \pm 1.1^{1,2a}$	$2.2 \pm 1.3^{2,3,4a}$	$2.2 \pm 1.6^{3,4a}$	$2.6 \pm 1.1^{3,4a}$	3.0 ± 1.5^{4a}	$2.3 \pm 1.2^{2,3,4a}$	1.1 ± 0.5^{1a}
Jejunum	$1.3 \pm 0.9^{1,2a}$	0.8 ± 0.5^{1b}	0.9 ± 0.4^{1b}	1.1 ± 0.7^{1ab}	$1.3 \pm 0.5^{1,2b}$	$1.3 \pm 0.7^{1,2a}$	2.2 ± 1.4^{3a}	2.1 ± 1.1^{3a}	$1.9 \pm 1.2^{2,3b}$	2.2 ± 1.2^{3b}	2.0 ± 1.2^{3ab}	$1.2 \pm 0.9^{1,2a}$
Ileum	1.2 ± 0.8^{1a}	1.1 ± 0.8^{1ab}	1.2 ± 0.7^{1ab}	1.4 ± 0.7^{1b}	1.3 ±n0.5^{1b}	$1.9 \pm 1.0^{1,2,3b}$	$2.0 \pm 1.3^{1,2,3a}$	2.8 ± 1.3^{3b}	$2.6 \pm 1.1^{2,3a}$	$2.7 \pm 0.9^{2,3a}$	$2.0 \pm 1.1^{1,2,3b}$	$1.8 \pm 0.4^{1,2b}$

NB: newborn.
Values are expresses as mean ± standard deviation.
Different numbers indicate significant statistical differences between columns ($p < 0.05$).
Different letters indicate significant statistical differences between rows ($p < 0.05$).

3.2. *Lectinhistochemistry*

SBA lectin bound to small PC granules, whereas Con A only weakly labeled their cytoplasm. The remaining lectins showed no staining of PC at any small intestinal section or age. Representative images are shown in Fig. 2.

3.3. *Immunohistochemistry*

Positive cells to lysozyme were recognized from gestational day 293 at the bottom of the crypts in all the intestinal sections and animals. These cells were negative to PCNA antibody. Representative images are shown in Fig. 3.

3.4. *Morphometry*

3.4.1. *PC size*

During the fetal stage the highest size of PC was found in the duodenum. A gradual increase in their size was found in the ileum from birth up to 45-days-old. Then, no significant morphometric differences were observed at any intestinal region (Table 1).

3.4.2. *PC number*

Fetuses from gestational day 293 showed no significant difference in PC number among the three intestinal sections. In the neonates the number of PC was higher in the duodenum, but in 16–21-days-old animals the number increased in the ileum. Adult alpacas showed lower number of PC than offsprings, but similar to that of fetuses (Table 2).

4. Discussion

In the present work the morphology and histochemical characteristics of PC in alpacas at different developmental stages were analyzed. PC location was similar to that described in other mammals (Vásquez et al., 2014; Nolte et al., 2005) including members of the family Camelidae (Abdel-Magied and Taha, 1995), and they were found in all the small intestinal sections from gestational day 293. The morphology of these cells has been described in rats (Sundström and Helander, 1980), guinea pigs (Vásquez et al., 2014; Satoh et al., 1990), mice (Satoh et al., 1992), rabbits (Zanuzzi et al., 2008; Abdel-Magied and Taha, 1995), horses (Takehana et al., 1998), sheeps (Ergün et al., 2003) and humans (Ehrmann et al., 1990). In all these species PC are pyramidal with easily recognized

granules by traditional histochemical techniques. In alpacas they were pyramidal, but some PC showed a more columnar shape, with apical eosinophilic cytoplasm and barely defined granules when stained by HE. PC in fetuses, young and adult alpacas were immunohistochemically positive to lysozyme, a reliable marker of PC in horses (Takehana et al., 1998; Masty and Stradley, 1991), mice (Peeters and Vantrappen, 1975) and humans (Erlandesen et al., 1974). The lack of positive immunoreactivity to PCNA supports that they belong to the stable epithelial population at the studied times, and help to indirectly differentiate them from the rest of the proliferating cells of the crypt.

Martínez et al. (2009) and Zanuzzi et al. (2008) studied the lectinhistochemical binding pattern of PCís granules in rabbits and rats, respectively. These authors reported that DBA, SBA and WGA bound to PCís granules with variable intensity, thus indicating the presence of carbohydrate residues, like β-B-*N*-acetil-glucosamine, *N*-acetilneuraminic acid, α-D-*N* acetilgalactosamine and β-D-*N* acetilgalactosamine. In contrast, in humans and mice the granules can be labeled by UEA-1, which has high affinity to fucose. In the present study only SBA lectin bound to the granules, suggesting variability in their carbohydrate composition among species.

To determine and compare PC number we analyzed those cells positive to lyzozyme antibody, and compare differences at different stages of development. Although the use of this method may underestimate the final total number, it was the more appropriate technique to fairly distinguish them in the alpaca. Lyzozyme positive-cells were arranged in small groups (2–3) or dispersed between non-positive cells at the base of the crypts in all the intestinal sections from day 293 of pregnancy. The presence of PC from the fetal stage was also reported in human fetal small intestine by Mallow et al. (1996), supporting an early differentiation, as it has been well-documented by several other authors (Putsep et al., 2000; Ouellette et al., 1989). In addition, this early differentiation may indicate that PC are necessary to actively participate in the intestinal defense mechanisms during lactancy, to protect neonates against infectious enteric diseases, the main cause of perinatal mortality in alpacas (Rosadio et al., 2012).

Immediately after birth PC gradually increased in size and on the following days the size was similar in all the intestinal sections. In jejunum and ileum the number of PC increased in neonates from days 28–36, a different result from that reported by Lira et al. (2012), possibly because they studied animals from birth up to 21-days-old. We also found differences in the morphometric data with respect to other species studied, such as *Cavia porcellus* (Vásquez et al., 2014), and rabbit (Zanuzzi et al., 2008). The increase in PC number with age may be associated with the greater expression of antimicrobial peptides, such as defensins, reported in the intestinal epithelium of young alpacas (More et al., 2011).

Alpacas are precocial species in which a well-development of intestinal crypts is identified at birth (Lira et al., 2012). PC are also present from birth in the small intestine of *Cavia porcellus*, another precocial species, and their number increase during the first postnatal weeks (Vásquez et al., 2014). In contrast, in altricial species, such as the mice, these cells appear from day 7 after birth (Bry et al., 1994).

There are specie-specific differences in the distribution and number of PC in the small intestine in adults. In human a higher number of PC was reported in duodenum and ileum (Singh, 1971), whereas Behnke and Moe (1964) found a progressive increase in the number from duodenum to ileum in rats of 2–4 weeks-old, and Cheng et al. (1969) reported the highest density in the duodenum and jejunum of adult mice. In adult sheep, Ergün et al. (2003) described a heterogeneous distribution throughout the small intestine, although the higher density of cells was found in the jejunum. In the present work we found that PC number in the ileum of adult alpacas is higher than in neonates and offsprings. Palacios et al. (2004) and Rosadio et al. (2010) have reported catarrhal and necrotic enteritis in the jejunum and ileum of neonate alpacas and offsprings caused by coccidios. In addition, Palacios et al. (2005) described the occurrence of catarrhal, hemorrhagic and fibrous enteritis in the jejunum and ileum of young alpacas with colibacilosis and enterotoxemia. Therefore, the lower number of PC found in the intestine of neonates alpacas and offsprings may explain the more frequent occurrence of enteric diseases when compared to adult animals.

Thus, it is evident that the differences in PC number depend on several factors, including the species, the age of the animals, the health status, and also on the methodological criterion used for the quantification; thus, comparisons between different species or within the same species at different ages are difficult.

In conclusion, in the present study we showed the presence of PC in the small intestine of fetuses, young and adult alpacas. The early differentiation of PC in the small intestine of alpacas, as well as the increase in their number during the first two weeks of life, suggest their role in the intestinal defensive functions against enteric diseases that occur mainly during lactancy.

Conflict of interest

The authors declared that they have no conflicts of interest with respect to their authorship or the publication of this article.

Acknowledgments

This study was supported by grants from project N° 173 FINCyT-IB-2013 (INNOVATE-PERU). We thank Dr. Raúl Rosadio for his contributions to improve the experimental design of the study, and María Elena Salazar for her technical assistance in image processing.

References

Instituto de Patología de las Fuerzas Armadas de los Estados Unidos de América (A.F.I.P), Métodos Histotecnológicos, Washington, DC: Publicado por el Registro de Patología de los Estados Unidos de América, (1995) 276p.

Abdel-Magied, E.M., Taha, A.A.M., 1995. A comparison of the structure of granular cell T type in the intestinal epithelium of the camel with Paneth cells of the rabbit. J. King Saud. Univ Sci. 7 (1), 51–62.

Ayabe, T., Satchell, D.P., Pesendorfer, P., Tanabe, H., Wilson, C.L., Hagen, S.J., Ouellette, A.J., 2002. Activation of paneth cell α-defensins in mouse small intestine. J. Biol. Chem. 277 (7), 5219–5228.

Behnke, O., Moe, H., 1964. An electron microscopic study of mature and differentiating paneth cells in the rat, especially of their endoplasmic reticulum and lysosomes. J. Cell. Biol. 22, 633–652.

Bevins, C.L., Salzman, N.H., 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat. Rev. Microbiol. 9 (5), 356–368.

Bevins, C.L., 2004. Paneth cell and the innate immune response. Curr. Opin. Gastroenterol. 20, 572–580.

Bevins, C.L., 2005. Events at the host-microbial interface of the gastrointestinal tract. V. Paneth cell alpha-defensins in intestinal host defense. Am. J. Physiol. Gastrointest. Liv. Physiol. 2, 173–176.

Bohe, M., Borgstrum, A., Lindstrom, C., Ohlsson, K., 1984. Pepsin-like immunoreactivity in human paneth cells. Digest 30, 271–275.

Bradbury, P., Gordon, K.C., 1990. Connective tissues and stains. In: Theory and Practice of Histological Techniques. Churchill Livingstone, New York, pp. 119–142.

Bry, L., Falk, P., Huttner, K., Ouellette, A., Midtvedt, T., Gordon, J.I., 1994. Paneth cell differentiation in the developing intestine of normal and transgenic mice. Proc. Natl. Acad. Sci. USA 91, 10335–10339.

Bustinza, V., La alpaca, 2001. Conocimiento De Gran Potencial Andino. Libro 1. Puno: Oficina De Recursos De Aprendizaje. Univ. Nacional del Altiplano, pp. 496.

Cheng, H., Merzel, J., Leblond, C.P., 1969. Renewal of Paneth cells in the small intestine of the mouse. Am. J. Anat. 126, 507–526.

Clevers, H.C., Bevins, C.L., 2013. Paneth cells: maestros of the small intestinal crypts. Annu. Rev. Physiol. 75, 289–311.

Deckx, R.J., Vantrappen, G.R., Parein, M.M., 1967. Localization of lysozyme in a Paneth Cell granule fraction. Biochem. Biophys. Acta 139, 204–207.

Eckmann, L., 2005. Defense molecules in intestinal innate immunity against bacterial infections. Curr. Opin. Gastroenterol. 21, 147–151.

Ehrmann, J., Malinsky, J., Gregar, I., 1990. Paneth cells and coeliac disease-quantitative, morphometric analysis. Acta Univ. Palacki. Olomuc. Fac. Med. 126, 187–201.

Elphick, D.A., Mahida, R., 2005. Paneth cells: their role in innate immunity and inflammatory disease. Gut 54, 1802–1809.

Ergün, E., Ergün, L., Asti, R.N., Kürüm, A., 2003. Light and electron microscopic morphology of Paneth cells in the sheep small intestine. Rev. Med. Vet. 154, 351–355.

Erlandesen, S.L., Parson, J.A., Taylor, T.D., 1974. Ultraestructural inmmunocytochemical localization of lysozyme in Paneth cells of man. J. Histochem. Cytochem. 22, 401–413.

Ghosh, D., Porter, E., Shen, B., Lee, S.K., Wilk, D., Drazba, J., Yadav, S.P., Crabb, J.W., Ganz, T., Bevins, C.L., 2002. Paneth cell trypsin is the processing enzyme for human defensin-5. Nat. Immunol. 3 (6), 583–590.

Goldstein, I.J., Hayes, C.E., 1978. The lectins: carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. 35, 127–340.

Karlsson, J., Pütsep, K., Chu, H., Kays, R.J., Bevins, C.L., Andersson, M., 2008. Regional variations in Paneth cell antimicrobial peptide expression along the mouse intestinal tract. BMC Immunol. 9, 37, http://dx.doi.org/10.1186/1471-2172-9-37.

Kelly, P.R., Feakins, R., Domizio, P., Murphy, J., Bevins, C., Wilson, J., McPhail, G., Poulsom, R., Dhaliwal, W., 2004. Paneth cell granule depletion in the human small intestine under infective and nutritional stress. Clin. Exp. Immunol. 135 (2), 303–309.

Keshav, S., 2006. Paneth cells: leukocyte-like mediators of innate immunity in the intestine. J. Leukoc. Biol. 80, 500–508.

Klaus, L., 1969a. The Paneth cell in disease. Gut 10, 804–811.

Klaus, L., 1969b. The paneth cell in health and disease. Ann. R. Coll. Surg. Eng. 44 (1), 23–37.

Lewin, K., 1969. Histochemical observations of Paneth cells. J. Anat. 105 (1), 171–176.

Lira, B., Cueva, S., Rodríguez, J., Ayón, M., Zanuzzi, C., Barbeito, C., Falcón, N., Vásquez, M., 2012. Identificación de las Células de Paneth en el intestino delgado de crías de alpacas en los primeros 21 días de edad. Rev. Inv. Vet. Perú. 23 (2), 132–146.

Liu, T.C., Gao, F., McGovern, D.P., Stappenbeck, T.S., 2014. Spatial and temporal stability of paneth cell phenotypes in Crohn's disease: implications for prognostic cellular biomarker development. Inflammation Bowel. Dis. 20 (4), 646–651.

Mallow, E.B., Harris, A., Salzman, N., Russell, J.P., De Berardinis, R.J., Ruchelli, E., Bevins, C.L., 1996. Human enteric defensins: gene structure and developmental expression. J. Biol. Chem. 271, 4038–4045.

Martínez, D.I., Camacho, J., Villanueva, I., Becerril, A., 2009. Cambios morfológicos producidos por el estrés sobre la población de células de Paneth. Rev. Med. Hosp. Gen. Mex. 72 (3), 129–135.

Masty, J., Stradley, R.P., 1991. Paneth cell degranulation and lysozyme secretion during acute equine alimentary laminitis. Histochemica 95 (5), 529–533.

Montalvo, C.E., 1966. Contribución al estudio de la morfología de la alpaca (Lama pacos). Estudio histológico e histoquímico del intestino. In: Tesis para optar el título profesional de Médico Veterinario. Facultad de Medicina Veterinaria. Universidad Nacional Mayor de San Marcos, Lima, Perú, pp. 34.

More, J., Manchego, A., Sandoval, N., Ramírez, M., Pezo, D., Lam-Chiok, K., Rivera, H., 2011. Detección genómica y expresión de péptidos antimicrobianos (α-y β-defensinas) en mucosa intestinal de crías de alpaca (*Vicugna pacos*). Rev. Invest. Vet. Perú. 22 (4), 324–335.

Nolte, M., Kotze, A., Van der Bank, F.H., Grobler, J.P., 2005. Microsatellite markers reveal low genetic differentiation among southern African *Camelus dromedarius* populations. J. Anim. Sci. 35 (3), 152–161.

Olivera, L.V.M., Zago, D.A., Jones, C.J.P., Bevilacqua, E., 2003. Developmental changes at the materno-embryonic interface in early pregnancy of the alpaca, *Lama pacos*. Anat. Embryol. 207, 317–331.

Otto, H.F., 1973. The intestinal Paneth cell Cytomorphology, ultrastructural pathology and function. A contribution to the lysozyme theory. Veroff. Morphol. Pathol. 94, 1–75.

Ouellette, A., Greco, R., James, M., Frederick, D., Naftilan, J., Fallon, J.T., 1989. Developmental regulation of crytdin, a corticostatin/defensin precursor mRNA in small intestinal crypt epithelium. J. Cell. Biol. 18, 1687–1695.

Ouellette, A.J., Satchell, D.P., Hsieh, M.M., Hagen, S.J., Selsted, M.E., 2000. Characterization of luminal Paneth cell alpha-defensins in mouse small intestine: attenuated antimicrobial activities of peptides with truncated amino termini. J. Biol. Chem. 275 (43), 33969–33973.

Ouellette, A.J., 2005. Paneth cell α defensins: peptide mediators of innate immunity in the small intestine. Springer. Semin. Immunopathol. 27, 33–146.

Palacios, C., Tabacchi, L., Chavera, A., López, T., Santillán, G., Sandoval, N., Pezo, D., Perales, R., 2004. Eimeriosis en crías de alpacas: estudio anátomo histopatológico. Rev. Inv. Vet Perú 15 (2), 174–178.

Palacios, C., Perales, R., Chavera, A., López, T., 2005. Caracterización anátomo-histopatológicas de enteropatía causantes de mortalidad en crías de alpacas. Rev Inv. Vet. Perú. 16 (1), 34–40.

Peeters, T., Vantrappen, G., 1975. The Paneth cell: a source of intestinal lysozyme. Gut 16, 553–558.

Pinard, C.L., Weiss, M.L., Brightman, A.H., Fenwick, B.W., Davidson, H.J., 2003. Evaluation of lysozyme and lactoferrin in lacrimal and other ocular glands of bison and cattle and in tears of bison. Am J. Vet. Res. 64 (1), 104–108.

Poindexter, B.J., Klein, G.L., Milner, S.M., Bick, R.J., 2009. Upregulation of defensins in burn sheep small intestine. Eplast 10, e6.

Porter, E.M., Liu, L., Oren, A., Anton, P.A., Ganz, T., 1997. Localization of human intestinal defensin 5 in Paneth cell granules. Infect. Immun. 65 (6), 2389–2395.

Porter, E.M., Bevins, C.L., Ghosh, D., Ganz, T., 2002. The multifaceted paneth cell. Cell. Mol. Life. Sci. 59 (1), 156–170.

Putsep, K., Axelsson, L.G., Boman, A., Midtvedt, T., Normark, S., Boman, H.G., Andersson, M., 2000. Germ-free and colonized mice generate the same products from enteric prodefensins. J. Biol. Chem. 275, 40478–40482.

Rennie, I.G., Parsons, M.A., 1981. Lysozyme distribution in human lacrimal glands and other ocular adnexa. Arch. Ophthalmol. 99 (10), 1850–1853.

Rodning, C.B., Erlandsen, S.L., Wilson, I.D., Carpenter, A.M., 1982. Light microscopic morphometric analysis of rat ileal mucosa: II. Component quantification of Paneth cells. Anat. Rec. 204, 33–38.

Rosadio, R., Londoñe, P., Pérez, D., Castillo, H., Veliz, A., Llanco, L., Yaya, K., Maturrano, L., 2010. Eimeria macusaniensis associated lesions in neonate alpacas dying from enterotoxemia. Vet. Parasitol. 168, 116–120.

Rosadio, R., Maturrano, L., Pérez, D., Luna, L., 2012. El complejo entérico neonatal en alpacas andinas. Rev. Invest. Vet. Perú. 23 (3), 261–271.

Satoh, Y., Ishikawa, K., Oomori, Y., Ono, K., 1989. Effects of cholecystokinin and carbamylcholine on Paneth cell secretion in mice: a comparison. Anat. Rec. 255 (2), 124–132.

Satoh, Y., Yamano, M., Matsuda, M., Ono, K., 1990. Ultrastructure of Paneth cells in the intestine of various mammals. J. Electron. Microsc. Tech. 16, 69–80.

Satoh, Y., Ishikawa, K., Oosmori, T., Takeda, S., Ono, K., 1992. Bethanechol and a G-protein activator, Na F/$AlCl_3$, induce secretory response in Paneth Cells of the mouse intestine. Cell. Tiss. Res. 269, 213–220.

Satoh, Y., Habara, Y., Ono, K., Kanno, T., 1995. Carbamylcholine- and catecholamine-induced intracellular calcium dynamics of epithelial cells in mouse ileal crypts. Gastroenterology 108, 1345–1356.

Singh, I., 1971. The distribution of Paneth cells in the human small intestine. Anat. Anz. Bd. 128, 60–65.

Stevens, A., 1990. The haematoxylins. In: Bancroft, John D., Stevens, Alan (Eds.), Theory and Practice of Histological Techniques. Churchill, Livingstone, New York, pp. 107–118.

Sundström, G., Helander, H.F., 1980. Quantitative electron microscopic studies on Rat Ileal Paneth Cells under various physiological and experimental conditions. Hepatogastroenterology 27 (4), 286–293.

Takehana, K., Masty, J., Yamaguchi, M., Kobayashi, A., Yamada, O., Kuroda, M., Park, Y.S., Iwasa, K., Abe, M., 1998. Fine structural and histochemical study of equine Paneth cells. Anat. Histol. Embryol. 27, 125–129.

Tang, Q.J., Wang, L.M., Tao, K.Z., Ge, C.R., Li, J., Peng, Y.L., Jiang, C.L., Geng, M.Y., 2006. Expression of polymeric immunoglobulin receptor mRNA and protein in human paneth cells: paneth cells participate in acquired immunity. Am. J. Gastroenterol. 101 (7), 1625–1632.

Toth, D.M., 1980. Ultrastructural changes in Paneth cells during hibernation in the ground squirrel *Spermophilus lateralis*. Cell. Tissue. Res. 211 (2), 293–301.

Vásquez, M., Pebe, E., Rodríguez, J.L., Lira, B., Falcón, N., Zanuzzi, C.N., Barbeito, C., 2014. Paneth cell identification in the small intestine of guinea pig offsprings (*Cavia porcellus*). Anat. Rec. 297 (5), 856–863.

Wehkamp, J., Chu, H., Shen, B., Feathers, R.W., Kays, R.J., Lee, S.K., Bevins, C.L., 2006. Paneth cell antimicrobial peptides: topographical distribution and quantification in human gastrointestinal tissues. FEBS Lett. 580 (22), 5344–5350.

Yasui, T., Tsukise, A., Miura, T., Fukui, K., Meyer, W., 2006. Cytochemical characterization of glycoconjugates in the apocrine glands of the equine scrotal skin. Arch. Histol. Cytol. 69 (2), 109–117.

Zanuzzi, C.N., Fontana, P.A., Barbeito, C.G., Portiansky, E.L., Gimeno, E.J., 2008. Paneth cells: histochemical and morphometric study in control and *Solanum glaucophyllum* intoxicated rabbits. Eur. J. Histochem. 52 (2), 93–100.

Zanuzzi, C.N., 2010. Efectos de la intoxicación con *Solanum glaucophyllum* (duraznillo blanco) sobre la proliferación, diferenciación y muerte celular en el intestino de conejos. In: Tesis para optar el Grado de Doctor en Ciencias Veterinarias, La Plata, Argentina : Fac. Cien. Vet. Univ. Nac. de la Plata, p. 286.

Zhang, Z., Liu, Z., 2016. Paneth cells: the hub for sensing and regulating intestinal microbiota. Sci. Chin. Lif. Sci., http://dx.doi.org/10.1007/s11427-016-5018-5.