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Foods introduced into Brazil through the border with Argentina and Uruguay: Pathogen detection and evaluation of hygienic-sanitary quality

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ABSTRACT

This study aimed to evaluate the presence of pathogens in, and the hygienic-sanitary quality of, commercialized foods of animal origin at the international border region of Rio Grande do Sul, Brazil. In total, 270 samples of raw and processed foods of animal origin were collected in Paso de los Libres, Argentina (n = 65 raw meat, n = 47 dairy products, n = 28 processed meat) and Rivera, Uruguay (n = 60 raw meat, n = 31 dairy products, n = 29 processed meat), or were seized by the Brazilian International Agricultural Surveillance System (Brazil-Argentina border) (n = 9 raw meat, n = 1 bush meat). The samples were subjected to the enumeration of aerobic mesophilic bacteria, enterobacteria, and coagulase-positive staphylococci, and were tested for *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. The virulence genes for *Salmonella* spp. (*hilA*, *invA*, *spvC*, *pefA*, and *sefA*), *L. monocytogenes* (*prs*, *inlA*, *inlC*, and *inlJ*) and *E. coli* O157:H7 (*uspA*, *eae*, rfb_{O157}, $fliC_{H7}$, *stx1*, *stx2*, and *hlyA*) were investigated using PCR assays. Raw products showed higher counts of aerobic mesophiles and enterobacteria compared to processed products ($P < 0.05$). There were no significant differences in aerobic mesophile or in enterobacterial counts between identical products according to origin (Argentina vs. Uruguay, $P > 0.05$). *Escherichia coli* O157:H7 was not detected in any of the samples tested. *Salmonella* spp. was detected in six (8%) raw products from Argentina. *Listeria monocytogenes* was isolated from five (6.66%) raw products originating in Argentina and 20 (16.66%) raw products from Uruguay. All 52 *E. coli* isolates carried the *uspA* gene, but only one carried the *eae* gene. The rfb_{O157}, $fliC_{H7}$, *stx1*, *stx2*, and *hlyA* genes were not detected. All *Salmonella* spp. isolates carried *hilA* and *invA* genes, but *spvC*, *pefA*, and *sefA* were not found. All *L. monocytogenes* isolates carried the *prs* gene; however, *inlA*, *inlC*, and *inlJ* genes were found in 20% of the isolates from Argentina and 95% of those from Uruguay. To our knowledge, this is the first microbiological study into the hygienic-sanitary quality of animal products in Brazil's land border region. *Salmonella* spp. and *L. monocytogenes* were detected in products of animal origin, constituting a public health concern and emphasizing the need for an active surveillance system to reduce the risk of foodborne pathogen introduction into Brazil.

1. Introduction

The international food trade, along with increased international travel, presents many benefits and opportunities but may also pose a risk to public and animal health. The transit of food, either by passengers traveling internationally or through illegal marketing in border regions, may constitute a source of foodborne pathogens, or serve as a vehicle for their introduction into countries considered being free from such microorganisms (Hueston et al., 2011; Noordhuizen et al., 2013; Swallow, 2012).

Due to its extensive borders (totaling 15,735 km), Brazil has established a large international sanitary inspection apparatus. Rio Grande do Sul (RS), a southern Brazilian state, shares approximately 724 km and 1000 km of its international land border with Argentina and Uruguay, respectively. Sanitary control of the entry of foods and animals into Brazil is the responsibility of the Ministry of Agriculture, Livestock, and Food Supply (MAPA), and is enforced through the International Agricultural Surveillance Service (VIGIAGRO). This service has checkpoints located in marine ports, airports, and at border crossings, and inspects live animals, products of animal origin, and baggage entering the country (Brasil, 2006a). At these checkpoints, VIGIAGRO inspects the luggage by sampling and seizes products of

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animal origin when they do not comply with the legal specifications. After seizure, these products are destroyed.

Currently, MAPA has authorized the import of foreign animal products, provided the following requirements are met: the amount per person must be eligible for personal consumption (10 kg of processed meat products and 5 kg of dairy products, egg, or processed fish products); and products must be sealed within their original packaging, with no signs of leakage or tampering. That products meet these requirements must be checked upon traveler entry into Brazil (Brasil, 2016).

However, significant challenges to the enforcement of these requirements exist. Surveillance, which may be deficient in detecting irregularities, is sometimes completely absent. In many places in Brazil, the requirement that baggage be checked at time of entry (Brasil, 2006a) is often not met because of factors such as lack of human resources, lack of funding, and administrative problems in some Federal Superintendencies of MAPA, as indicated by audits of VIGIAGRO carried out by members of the Federal Court of Accounts – TCU (Brasil, 2006b).

For these reasons, the illegal import of animal products is a widespread practice in many regions of Brazil, both at land borders (Pereira et al., 2017) and airports (Melo et al., 2014, 2015). This is particularly the case in the border region of RS, and it is estimated that > 60% of passengers traveling to Argentina or Uruguay return to Brazil carrying such products (raw or processed), but that < 10% have their baggage checked (Pereira et al., 2017).

Because animal products seized by VIGIAGRO are destroyed, the health risks presented by illegally imported foods are not known with any certainty. The entry of these products without sanitary inspection presents a public health concern; as such food may not be fit for consumption and may contain pathogens. Thus, the objective of this study was to evaluate hygienic-sanitary quality through the enumeration of indicator microorganisms and detection of foodborne pathogens (*Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7) in imported raw and processed animal products commercialized in the border region of RS, Brazil.

2. Materials and methods

2.1. Sampling procedure

Samples were obtained in two ways: 1) through the acquisition of products of animal origin seized by VIGIAGRO-MAPA on the Brazil–Argentina border, 2) through direct sample collection from commercial establishments located in Argentina and Uruguay (Table 1).

1) Animal products were confiscated after baggage inspection carried out by the VIGIAGRO-MAPA, at Getúlio Vargas-Agustín Pedro Justo International Bridge (29°45′18″ S, 57°05′16″ W), between Uruguaiana (RS, Brazil) and Paso de los Libres (Province of Corrientes, Argentina). Of these samples, nine consisted of raw beef and one of bush meat (capybara - *Hydrochoerus hydrochaeris*), transported by travelers who had entered Brazil.
2) Additionally, sampling was performed at retail level in Paso de los Libres, Province of Corrientes, Argentina (29°43′00″ S, 57°05′00″ W) and in Rivera, Rivera Department, Uruguay (30°54′09″ S, 55°33′02″ O). Samples were imported into Brazil under the authorization and supervision of VIGIAGRO. In Paso de los Libres, 140 samples (n = 65 raw meat, n = 47 dairy products, n = 28 processed meat) were collected. In Rivera, 120 samples (n = 60 raw meat, n = 31 dairy products, n = 29 processed meat) were collected. The products were purchased from 16 commercial establishments (markets, grocery stores, and butchers); eight from each city, at three separate times.

Samples were collected between September 2015 and November 2016, cataloged, stored in plastic bags, transported in isothermal boxes containing recyclable ice, and stored at 4 °C until microbiological analysis.

Table 1
Number of products of animal origin evaluated at the Brazil-Argentina and Brazil-Uruguay border.

Products	Origin		Total
	Argentina	Uruguay	
Raw meat			
Beef	40[a]	28	68
Pork	15	20	35
Chicken	19	12	31
Bush meat	1[b]	0	1
Total	75	60	135
Dairy			
Yogurt	20	12	32
Cheese	17	10	27
Cream	7	1	8
Dulce de leche	3	8	11
Total	47	31	78
Processed meat			
Mortadella	11	8	19
Sausage	8	4	12
Salami	7	8	15
Ham	2	3	5
Pate	0	6	6
Total	28	29	57
Total	150	120	270

[a] Of the 40 beef samples of Argentine origin, 9 were obtained from VIGIAGRO (Brazil-Argentina border).
[b] Sample seized next to VIGIAGRO (Brazil-Argentina border).

2.2. Microbiological and molecular analysis

2.2.1. Enumeration of aerobic mesophilic microorganisms, enterobacteria, and coagulase-positive staphylococci

The preparation, weighing, and dilution procedures followed the protocol recommended by Brazilian legislation (Brasil, 2003). After tenfold dilutions in saline solution (0.9% NaCl), aliquots of 1 mL were inoculated onto Plate Count Agar (PCA, Difco™ 247940) for aerobic mesophilic microorganisms, and onto Violet Red Bile Glucose Agar (VRBGA, Difco™ 218661) for enterobacteria. The enumeration of coagulase-positive staphylococci was performed by inoculation of 0.1 mL onto Baird Parker Agar (BP, Difco™ 276819) with egg yolk tellurite emulsion (EY, Laborclin® 520089). VRBGA plates were incubated at 36 °C for 24 h and PCA and BP plates at 36 °C for 48 h, after which colony forming units (CFU) were enumerated. Presumptive colonies of staphylococci were tested for coagulase enzyme production by means of rabbit plasma coagulation. Results were expressed as log CFU/g or log CFU/mL.

2.2.2. Detection of Salmonella spp.

Detection of *Salmonella* spp. was performed as described by Andrews et al. (2016), with minor adaptations. Briefly, 25 g or 25 mL of sample was stomached with 225 mL buffered peptone water (BPW, Difco™ 218105) and incubated at 35 °C for 24 h. For selective enrichment, 0.1 mL and 1 mL of culture were transferred to 10 mL of Rappaport-Vassiliadis broth (RV, Difco™ 218581) and 10 mL of Tetrathionate broth (TT, Difco™ 249120), respectively. The RV and TT tubes were incubated at 42 °C and 37 °C, respectively, for 24 h. Thereafter, two selective media were used: Bismuth Sulfite Agar (BS, Difco™ 273300) and Xylose Lysine Deoxycholate Agar (XLD, Difco™ 278850). Cultures were incubated at 35 °C for 24 h. Presumptive colonies on XLD and BS plates were subjected to biochemical and serological tests with somatic polyvalent anti-*Salmonella* serum (Probac®, São Paulo, Brazil).

We further confirmed the identity of these isolates by PCR assay of