

Muscid Diptera as potential vectors of bacterial agents on dairy farms in the northern region of Paraná, Brazil

Dípteros muscóides como potenciais vetores de agentes bacterianos em fazendas de exploração leiteira na região Norte do Paraná, Brasil

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Abstract

The aim of this study was to determine the isolation frequency and microbial sensitivity profiles of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. isolated from synanthropic flies captured in entomological traps placed near the milking parlors of 30 dairy farms located in Northern Paraná, Brazil. In total, 192 flies were captured, and the isolation frequencies of muscids (21/30 = 70%) and caliphorids (27/30 = 90%) were significantly higher than that of sarcophagids (7/30 = 23.3%). Bacteriological tests on the internal contents and external surfaces of the flies showed that *E. coli* was present only in muscids (14.3%) and caliphorids (33.3%). *Salmonella* spp. was isolated from 9.5% of the muscids, 7.4% of the caliphorids, and 14.29% of the sarcophagids. *Staphylococcus* spp. was isolated from 28.5% of the muscids and 29.6% of the caliphorids. *E. coli* isolation was more frequent in flies captured on farms at which domestic chickens were housed near the milking parlors ($p = 0.031$) and which did not use sugarcane for animal feed ($p = 0.042$). Two out of 27 (7.4%) *Staphylococcus* spp. strains presented a phenotype of coagulase enzyme production. Ceftriaxone, ciprofloxacin, enrofloxacin, and gentamicin were the most effective antibiotics against *E. coli* and *Salmonella* spp. Tetracycline was the least effective antibiotic against the isolates. The *Staphylococcus* spp. strains isolated did not show the phenotype of oxacillin resistance. We conclude that regional flies are potential mechanical vectors of microbial agents able to cause enteritis in goats, mastitis in cows, and contamination of milk products for human consumption.

Key words: Synanthropic flies, dairy cattle, and infectious agents

Resumo

Moscas sinantrópicas são vetores mecânicos de enfermidades infecciosas para humanos e animais. O presente estudo pretendeu avaliar a presença de *Escherichia coli*, *Salmonella* e *Staphylococcus* spp na população de moscas das famílias Calliphoridae, Muscidae e Sarcophagidae capturadas em 30 fazendas de leite na região Norte do Paraná. As capturas foram realizadas com armadilhas entomológicas contendo iscas a base de fígado bovino, penduradas em árvores próximas as salas de ordenhas das fazendas. Lavados da superfície externa e fluidos extraídos do abdome esmagado das moscas foram semeados em agar sangue ovino a 5%, agar Mac Conkey e agar Baird-Parker e submetidos a enriquecimento seletivo para *Salmonella* em caldo Rappaport-Vassiliadis e tetrationato Muller-Kauffmann, seguido de

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plaqueamento em agar Xilose Lisina Desoxicolato (XLD) e agar *Salmonella-Shigella*. Os microrganismos isolados foram classificados bioquimicamente. Foram capturadas 192 moscas pertencentes às famílias Muscidae, Calliphoridae e Sarcophagidae. Nas fazendas pesquisadas, as capturas de *Muscidae* (21/30 = 70%) e *Calliphoridae* (27/30 = 90%) foram mais frequentes do que as capturas de *Sarcophagidae* (7/30 = 23,3%). *E. coli* foi isolada apenas de muscídeos (14,3%) e califorídeos (33,3%). *Salmonella* foi isolada de 9,5% dos muscídeos, 7,4% dos califorídeos e de 14,29% dos sarcófagídeos capturados. *Staphylococcus* spp foi isolado de 28,5% dos muscídeos e 29,6% dos califorídeos. Os isolamentos de *E. coli* foram mais comuns e moscas capturadas em fazendas que mantinham criações de aves próximas ao local ($p = 0,031$) de ordenha e que não utilizavam cana-de-açúcar para alimentação dos animais ($p=0,042$). Conclui-se que as moscas presentes em fazendas de leite da região apresentam grande importância sanitária, visto que os agentes isolados são capazes de causar enterites em bezerros, mastites nas vacas e contaminação de produtos lácteos produzidos no local.

Palavras-chave: Moscas sinantrópicas, bovinocultura de leite, agentes infecciosos, Norte Pioneiro do Paraná

Introduction

Non-hematophagous synanthropic flies belong to the order Diptera, and are known to be mechanical vectors that carry infectious and parasitic diseases to human and animals. These insects carry pathogenic microbial agents from contaminated organic substrates that they use for reproduction and feeding, especially feces from livestock and decaying organic matter (LEVINE; LEVINE, 1991). The flies most likely to cause sanitation issues belong to the families Sarcophagidae (sarcophagus flies), Muscidae (house flies), and Calliphoridae (blow flies) (GRACZYK et al., 2001), which are distinct in the substrates they use for reproduction and in their synanthropic habits. Several pathogenic microorganisms are frequently isolated from these Diptera, especially *Salmonella* spp., *Yersinia enterocolitica*, *Edwardsiella tarda*, *Shigella sonnei*, *Escherichia coli*, *Klebsiella* spp., methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (RAHUMA et al., 2005).

On dairy farms, synanthropic flies may transmit microbial agents related to several conditions, such as enteritis, mastitis, and dermatitis, to animals (BRAVERMAN et al., 1999; FÖRSTER et al., 2009). Some species are known to be vectors of bacterial pathogens associated with bovine mastitis, an infection of the mammary glands that results in reduced milk production and increased somatic

cell counts, with important effects on public health (OLIVER et al., 2005). In locations with high rates of infestation, the flies can also transfer infectious agents to milk products, such as fresh cheese (CARDOZO et al., 2009).

The aim of this study was to evaluate the microbial sensitivity and the isolation frequency of *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. isolated from washed surfaces and the internal contents of synanthropic flies captured on 30 farms in the northern region of Paraná and to evaluate the risk factors associated with the isolation of these microorganisms.

Material and Methods

The study was performed between February and March 2011 on dairy farms located in the municipalities of Andirá (latitude 23° 03', longitude 50° 13', altitude of 470 m), Bandeirantes (latitude 23° 06', longitude 50° 22', altitude of 420 m), and Abatiá (latitude 23° 18', longitude 50° 18', altitude of 620 m), in the northern region of the state of Paraná, Brazil. Thirty farms were randomly selected from a total of 42 farms participating in projects for the technological development of the dairies that produce commercial milk products in the region.

For fly capture, entomological traps built from 2-L plastic bottles were used, adapting a methodology described by Dias et al. (2009). Each trap was built

using two bottles, with the bottoms removed to allow one to fit inside the other. The bottle on the inside was painted black and the one on the outside was left transparent. Before being used, the traps were disinfected with 70% ethanol. Each trap was then hung from a tree at a minimum distance of 20 m from the milking parlors and at a height of 1.0-1.70 m above the ground. Inside each trap, 10 g of bovine liver was attached to a metallic hook as bait. Flies entered the trap from the below landed on the bait, and then proceeded in the direction of the light, where they were caught in the transparent bottle. After 24 h at the collection points, the traps were removed, sealed with tape, placed in refrigerated isothermal boxes and transported to laboratories for performance of microbiological tests in less than 4 hours.

During fly capture, a structured questionnaire composed of closed questions that had been previously tested was given to the employees or the owners responsible for handling the milking, with the purpose of collecting epidemiological information associated with the presence of insects on the property. The questionnaire included questions concerning poultry and swine husbandry on the farm, the use of lay stalls for disposal of animal waste, the manner of milking, the alimentary supplements offered in troughs (ground sugarcane and concentrates), and the control of rodents.

The captured flies were inactivated in a freezer at -12°C for 30 min and identified with the aid of a stereoscopic microscope using taxonomic keys specific for each family (CARVALHO; MELLO-PATIU, 2008) For each farm, the flies belonging to the families Muscidae, Calliphoridae, and Sarcophagidae were divided into separate sterile containers. Between one and five samples from each family were randomly selected from each container to undergo bacteriological tests.

Separate bacteriological tests were performed on the internal and external portions of the fly body. External samples were obtained by washing the

captured exemplars in 3 mL of sterile physiological solution in 5-mL glass tubes, using a vortexer. To collect the contents of midgut and hindgut, the captured insects were first washed in 70% ethanol for 5 min for superficial disinfection. The insects were then washed with sterile physiological solution to remove any remaining traces of ethanol. The washed flies were placed in sterile tubes with 3 mL sterile physiological solution, and the abdomen and cephalothorax were crushed using sterile forceps, followed by vortexing of the tubes.

The fluids obtained from the internal and the external parts of the flies were separately cultured on 5% bovine blood agar and Baird-Parker agar supplemented with egg yolk and potassium telluride for the isolation of *Staphylococcus aureus* and MacConkey agar for the isolation of enterobacteria. After 24-48 hours of incubation at 35°C , the microorganisms isolated on the plates were identified using Gram staining and morphological and biochemical characteristics (MURRAY et al., 2003). For the isolation of *Salmonella*, 0.1 mL fluid was removed from each inoculum for culture in tubes containing Rappaport-Vassiliadis broth, and 1 mL was removed for culture in tubes containing Muller-Kauffmann tetrathionate broth. The cultures were incubated at 35°C ($\pm 1^{\circ}\text{C}$) for 18 h and then plated on xylose lysine desoxycholate (XLD) agar and *Salmonella-Shigella* agar. The plates were incubated for 24-48 hours at 37°C . The isolated microorganisms were identified using Gram staining and morphological and biochemical characteristics, as well as their reactivity in a serodiagnostic test using anti-*Salmonella* polyvalent serum (Probac®).

The microbial sensitivity of the isolates was determined using the classic method of disk diffusion. Bacterial inocula grown on brain-heart infusion broth in a final volume of 0.1 mL, corresponding to 0.5 on the MacFarland turbidity scale, were spread over the surface of plates containing Mueller-Hinton agar. Filter paper discs impregnated with the antibiotics ampicillin (10 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), enrofloxacin (5

mcg), gentamicin (10 mcg), sulfamethoxazol + trimethoprim (25 mcg), and tetracycline (30 mcg) were then added to the surface of the agar. Paper discs containing cefalexin (30 mcg), oxacillin (1 mcg), and penicillin G (10 UI) were used for *Staphylococcus* spp. strains. After incubation of the plates at 37°C for 24 h, the microorganisms' growth inhibition halos were compared with the accepted standards for classification and classified as sensitive, partially sensitive, or resistant. (NCCLS, 2005).

To determinate whether the capture frequencies differed between flies in the families Muscidae, Calliphoridae, and Sarcophagidae, Fisher's exact test was used. The same test was used to analyze whether the isolation frequency of *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. differed between the fly families. To evaluate the correlations between the isolation rates of the various fly families and the information collected using the epidemiological questionnaires, Fisher's exact test was used. For all analyses, a *P*-value <0.05 was considered statistically significant (PAGANO; GAUVREAU, 2004).

Results

At the time of fly capture, each farm housed an average of 34.7 bovines (range: 9-93), including both calves and adults, with an average production of 90.7 L of milk per farm and 9.36 L per lactating animal. In total, 192 flies were captured, of which 114 belonged to the family Calliphoridae, 64 to Muscidae, and 14 to Sarcophagidae. The capture of flies belonging to Calliphoridae was observed on 27 farms (90%), Muscidae on 21 farms (70%), and Sarcophagidae on 7 farms (23.2%). Muscids and caliphorids were captured significantly more frequently than sarcophagids (*P* <0.05).

A total of 12 *E. coli* strains were isolated. At 3 of the 21 (14.3%) farms, flies of the family Muscidae were captured, and *E. coli* was isolated from external surface samples or internal abdomen contents from these flies. In contrast, *E. coli* was isolated from flies of the family Calliphoridae at only 7 of the 27 (33.3%) farms evaluated, and was never isolated from flies of the Sarcophagidae family (Table 1). The frequencies of *E. coli* isolation did not differ between the families Calliphoridae and Muscidae (*P* = 0.2396). *E. coli* was isolated more frequently from flies captured on farms with domestic chickens (*P* = 0.031) and those not providing sugarcane in the animal troughs (*P* = 0.042).

Table 1. Pathogenic microorganisms isolated from the external surface and internal contents of synanthropic flies captured near the milking parlors of dairy farms in the northern region of Paraná, 2012 (n = 30).

Family		Microorganism		
		<i>Escherichia coli</i>	<i>Salmonella</i> spp	<i>Staphylococcus</i> spp
Muscidae	External	2/21 (9,5%)	1/21 (4,7%)	6/21 (28,5%)
	Internal	1/21 (4,7%)	2/21 (9,5%)	0/21 (0%)
	Total	3/21 (14,3%)	2/21 (9,5%)	6/21 (28,5%)
Calliphoridae	External	6/27 (22,2%)	1/27 (3,7%)	8/27 (29,6%)
	Internal	6/27 (22,2%)	1/27 (3,7%)	0/27 (0%)
	Total	9/27 (33,3%)	2/27 (7,4%)	8/27 (29,6%)
Sarcophagidae	External	0/7 (0%)	0/7 (0%)	0/7 (0%)
	Internal	0/7 (0%)	1/7 (14,29%)	0/7 (0%)
	Total	0/7 (0%)	1/7 (14,29%)	0/7 (0%)

Source: Elaboration of the authors.

In total, 8 *Salmonella* spp. strains were isolated. The isolates were obtained from flies from all three families evaluated, from both external and internal samples, from between 0 and 14.1% of the farms on which the flies were captured (Table 1). The isolation frequency did not differ among the three families ($P = 0.8496$). *Salmonella* was isolated less frequently from flies captured on farms that kept domestic poultry ($P = 0.041$).

Twenty-seven *Staphylococcus* spp. strains were isolated from flies captured at 17 of the 30 evaluated farms (56.66%), and production of coagulase enzyme was observed in 2 isolates (7.4%). The strains were isolated from external samples of muscids and caliphorids in 28.5% (6/21) and 29.6% (8/27) of the farms, respectively (Table 1). Isolation of *Staphylococcus* was not significantly correlated with any of the risk factors examined (Table 2).

Table 2. Risk factors associated with the capture of synanthropic flies with positive isolations of *Escherichia coli*, *Salmonella*, and *Staphylococcus* spp. on dairy farms in the northern region of Paraná, 2012 (n = 30).

Variable	Microorganism								
	<i>E.coli</i>			<i>Salmonella</i>			<i>Staphylococcus</i>		
	Pos	Neg	P	Pos	neg	p	pos	neg	p
Poultry husbandry on the farm									
Yes	12 (40%)	12 (40%)	0,031*	2 (6,7%)	22 (73,3%)	0,041*	13 (43,3%)	11 (36,7%)	0,470
No	0 (0%)	6 (20%)		3 (10%)	3 (10%)		4 (13,3%)	2 (6,7%)	
Swine husbandry on the farm									
Yes	6 (20%)	5 (16,7%)	0,197	1 (3,3%)	10 (33,3%)	0,381	7 (23,3%)	4 (13,3%)	0,421
No	6 (20%)	13 (43,3%)		4 (13,3%)	15 (50%)		10 (33,3%)	9 (30%)	
Use of lay stalls for disposal of animal waste									
Yes	12 (40%)	14 (46,7%)	0,112	5 (16,7%)	21 (70%)	0,462	13 (43,3%)	13 (43,3%)	0,087
No	0 (0%)	4 (13,3%)		0 (0%)	4 (13,3%)		4 (13,3%)	0 (0%)	
Manner of milking									
Manual	9 (30%)	16 (53,3%)	0,304	4 (13,3%)	21 (70%)	0,627	14 (46,7%)	11 (36,7%)	0,633
Mechanical	3 (10%)	2 (6,7%)		1 (3,3%)	4 (13,3%)		3 (10%)	2 (6,7%)	
Ground sugarcane offered in troughs									
Yes	4 (13,3%)	13 (43,3%)	0,042*	2 (6,7%)	15 (50%)	0,367	12 (40%)	5 (16,7%)	0,082
No	8 (26,7%)	5 (16,7%)		3 (10%)	10 (33,3%)		5 (16,7%)	8 (26,7%)	

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Concentrates offered in troughs									
Yes	8 (26,7%)	17 (56,7%)	0,068	4 (13,3%)	21 (70%)	0,627	15 (50%)	10 (33,3%)	0,367
No	4 (13,3%)	1 (3,3%)		1 (3,3%)	4 (13,3%)		2 (6,7%)	3 (10%)	
Control of rodents									
Yes	9 (30%)	14 (46,7%)	0,597	3 (10%)	20 (66,7%)	0,329	10 (33,3%)	13 (43,3%)	0,660
No	3 (10%)	4 (13,3%)		2 (6,7%)	5 (16,7%)		3 (10%)	4 (13,3%)	

* $p < 0,05$; pos = positive isolation; neg = negative isolation; p = statistical significance.

Source: Elaboration of the authors.

The results of the sensitivity profiles of the isolates are summarized in Table 3. The most effective antibiotics against gram-negative microbes were ceftriaxone, ciprofloxacin,

enrofloxacin, gentamicin, and sulfazotrim, whereas ampicillin and tetracycline were the least effective. All the antibiotics tested were effective against *Staphylococcus* spp. (Table 3).

Table 3. Percentage of sensitive (Sens), partially sensitive (Parc), or resistant (Res) microorganisms isolated from synanthropic flies captured in dairy farms in the northern region of Paraná, 2012 (n = 30).

Antimicrobial agente	Microorganism					
	<i>E. coli</i> (N=12)		<i>Salmonella</i> spp (N= 8)		<i>Staphylococcus</i> spp (N= 27)	
	Sens	Parc/Res	Sens	Parc/Res	Sens	Parc/Res
Ampicillin	4/12 (33,3%)	8/12 (66,7%)	5/8 (62,5%)	3/8 (37,5%)	23/27 (85,19%)	4/27 (14,81%)
Cephalexin*	-	-	-	-	25/27 (92,59%)	2/27 (7,41%)
Ceftriaxone	12/12 (100%)	0/12 (0%)	8/8 (100%)	0/8 (0%)	24/27 (88,89%)	3/27 (11,11%)
Ciprofloxacin	12/12 (100%)	0/12 (0%)	6/8 (75,0%)	2/8 (25,0%)	26/27 (96,27%)	1/27 (3,70%)
Enrofloxacin	12/12 (100%)	0/12 (0%)	6/8 (75,0%)	2/8 (25,0%)	22/27 (81,48%)	5/27 (18,52%)
Gentamicin	12/12 (100%)	0/12 (0%)	6/8 (75,0%)	2/8 (25,0%)	27/27 (100,00%)	0/27 (0,00%)
Oxacillin*	-	-	-	-	27/27 (100,00%)	0/27 (0,00%)
Penicillin G*	-	-	-	-	19/27 (70,37%)	8/27 (29,63%)
Sulphazotrin	10/12 (83,3%)	2/12 (16,7%)	6/8 (75,0%)	2/8 (25,0%)	27/27 (100,00%)	0/27 (0,00%)
Tetracycline	6/12 (50,0%)	6/12 (50,0%)	0/8 (0%)	8/8 (100%)	19/27 (70,37%)	8/27 (29,63%)

* Evaluated only for *Staphylococcus* spp.

Source: Elaboration of the authors.

Discussion

Capturing flies in baited traps is a method frequently used to estimate the density of these insects in several types of environments (FERREIRA; LACERDA, 1993; DIAS et al., 2009). The capture proportions observed in the traps

set at the 30 farms are expected to reflect the fly populations associated with the conditions present in and around the milking parlors. The insects take shelter in the parlors during the hottest hours of the day, interacting with attractive substrates, such as animal excretions, feed remnants, and milk

residues (GERRY et al., 2011). The milking parlors present unavoidably precarious sanitary conditions, since a considerable amount of fresh feces are deposited there each day, hampering the control of fly populations and favoring the dispersion of insects to houses in urban and proximal rural zones (HOGSETTE et al., 2012).

We observed more flies from the families Muscidae and Calliphoridae than the family Sarcophagidae. These results differ from those of other studies describing the predominance of sarcophagid flies compared to muscid flies in rural areas of the state of Rio de Janeiro (MACEDO et al., 2011). The prevalence of Calliphoridae and Muscidae in the regions analyzed may be attributed to the greater adaptability of species, such as *C. megacephala* and *M. domestica* to the environmental conditions of tropical and subtropical regions and to their ability to proliferate in diverse trophic niches, compared to sarcophagids (PARALUPPI; CASTELLÓN, 1994). Moreover, the caliphorids, the group most frequently captured in the traps used, present a high invasive potential in anthropic and natural environments, where they prevail over indigenous flies (REIS et al., 1996).

E. coli, a microorganism typically found in feces, was isolated from muscids and caliphorids, possibility due to the coprophilic habits of these animals. *E. coli* was found in isolates from the exoskeleton and the internal contents of the flies, concordant with the results of other studies (FÖRSTER et al., 2009; BOUAMAMAA et al., 2010). These observations demonstrate the ability of flies to carry fecal bacteria with pathogenic potential to human beings and animals, especially in regards to highly endophilic flies frequently observed inside houses containing substrates and organic residues produced by human activity (EESA; EL-SIBAE, 1993).

Three relevant aspects concerning the isolation of *E. coli* should be emphasized. The first is related to the potential of the flies to transmit

enterotoxigenic strains of *E. coli* to newborn calves. Transmission can occur when the flies come in contact with feces from young and adult animals that asymptotically excrete enteropathogenic *E. coli* strains (DE RYCKE et al., 1986) and carry them to anatomic regions such as the udders of lactating cows, infecting nursing calves. A second important aspect is related to coliform mastitis, an acute condition with systemic effects caused by absorption of bacterial endotoxins (BURVENICH et al., 2003). Flies exposed to an environment containing *E. coli* can become colonized with the bacteria and might serve as a source for transmission of environmental mastitis to milk cows. The third aspect that we would like to highlight is associated with the possibility that flies carry fecal coliform bacteria to the milk produced on the farm. In 83.3% of the farms evaluated, milking was performed manually, a procedure that allows contact between the milk and flies present in the milking parlor. Milk sterilization methods are sufficient to kill the microorganism, but the bacteria might survive in raw milk and contaminate homemade fresh cheese (QUINTO; CEPEDA, 1997).

E. coli was isolated more frequently from flies captured from farms that kept poultry or broilers, often free, near the milking parlors. These results suggest that flies are attracted by the excrement deposited on the ground by the domestic chickens. These observations are consistent with other studies, suggesting that fresh chicken is a resource frequently used by flies for reproduction (MOON et al., 2001). *E. coli* was isolated less frequently from flies of the family Muscidae captured on farms that used sugarcane in animal troughs, compared to those that did not offer this food. These results suggest that sugarcane may influence the habits of the flies in regards to the search for substrates, and the resulting potential fecal contamination of the insects, since the profile of the pathogenic microorganisms carried by the flies depends, in part, on the type of substrate they utilize for nourishment or egg deposition. In the absence of a preferential

substrate, the flies search for alternative substrates (D'ALMEIDA; MELLO, 1996). Moreover, adult flies can retain pathogenic bacteria acquired in the larval phase (SCHUSTER et al., 2013).

Salmonella was isolated from flies belonging to all three families analyzed, most commonly from sarcophagids. The isolation proportions were similar to those described by Choo et al. (2011) in Malaysia. However, on the farms evaluated, no animals with enteric symptoms typical of salmonellosis were observed. Therefore, the isolation of *Salmonella* may have resulted from contact of the flies with feces from carrier animals that asymptotically excrete the bacteria, a very common situation in bovines infected by *Salmonella* (MOHLER; IZZO; HOUSE, 2009), or from other extra-fecal sources (PRADHAN et al., 2009). *Salmonella* cannot survive in the flies for prolonged periods, and therefore it can only remain present in the insect populations if they re-contaminate themselves every few hours (HOLT et al., 2007).

Contrary to the results observed for *E. coli*, *Salmonella* was isolated less frequently from flies captured on farms that kept poultry or broilers near the milking parlors. These results contradict those of other studies, which have shown that flies that cohabit with poultry are more prone to infection by *Salmonella* (HOLT et al., 2007).

Similar to *E. coli*, *Salmonella* can be transmitted by synanthropic flies to calves, cause mastitis in cows, and contaminate milk products made from raw milk. In addition, the flies may disperse this agent to food produced in nearby areas, such as rural communities and neighboring properties, and cause salmonellosis outbreaks in humans. Adequate control of fly populations can reduce the number of cases of diarrhea in humans in exposed areas by 23% (CHAVASSE et al., 1999).

Staphylococcus was isolated from the external surfaces of muscid and calliphorid flies. Of the 27 isolates of *Staphylococcus*, 2 (7.1%) were found to be producers of coagulase, the main virulence

marker for the genus, which is associated with cases of infectious mastitis in milk cows (SILVA; SILVA, 2005). The results observed are consistent with those of other studies demonstrating that the family Muscidae is able to carry both coagulase-positive and -negative *Staphylococcus* species (BOUAMAMAA et al., 2010).

The absence of *Staphylococcus* from the internal contents of the flies may be due to the secretion of peptides with antimicrobial effects in the digestive tract of the insects, which limit bacterial survival (NAYDUCH; CHO; JOYNER, 2013). Therefore, this microorganism presents a higher probability of transmission following superficial contact with the insects than with their feces or digestive juices. Considering that coagulase-negative *Staphylococcus* species are considered to be emergent mammalian pathogens (DUFOR et al., 2012), these results emphasize the importance of muscoid Diptera as potential mechanical vectors of staphylococcus mastitis among milk cows, possibly involving transmission mechanisms similar to those observed in cases of summer mastitis caused by *Arcanobacterium pyogenes* (BRAMLEY et al., 1985), in which the pus-containing milk is attractive to flies, which then becomes externally contaminated.

Ampicillin and tetracycline were the least effective antibiotics against *E. coli* and *Salmonella* spp. Resistance to these antibiotics has been observed in other studies of gram-negative microbial agents isolated from synanthropic flies (BOUAMAMAA et al., 2010), and is considered relatively common in enterobacteria isolated from bovine fecal samples (SAWANT et al., 2007), possibly due to the selection pressure arising from the massive, abusive, and unsupported use of sensitivity tests in livestock (TURNIDGE, 2004). Quinolones (enrofloxacin and ciprofloxacin), gentamicin, and ceftriaxone were the most effective antibiotics against the gram-negative agents isolated, consistent with the results of similar studies (BOUAMAMAA et al., 2010). Therefore, it is recommended that the dairy farms

utilize these antibiotics and derivatives of the same pharmacological line in a sensitive and controlled manner, with the aim of reducing selection pressure and the transmission of resistant pathogenic agents to synanthropic Diptera, since exchange of mobile genetic elements encoding antimicrobial resistance may occur in the digestive tract of these insects (AKHTAR; HIRT; ZUREK, 2009).

More than 70% of the *Staphylococcus* spp. strains showed resistance to the tested antibiotics. The least effective drugs against these agents were penicillin G and tetracycline, whereas the most effective was sulfamethoxazol in association with trimethoprim, oxacillin, and gentamicin. The high effectiveness of the tested antibiotics against the agent may be related to the elevated percentage of isolates classified as coagulase-negative, considering that some researchers have reported that strains of this agent isolated from bovines showed resistance less frequently than coagulase-positive species. On the other hand, these agents are known to be emergent mammalian pathogens, and the frequent use of antibiotics against mastitis may cause the emergence of multidrug-resistant strains of coagulase-negative *Staphylococcus* (DELLA LIBERA et al., 2010).

Conclusions

Synanthropic flies are potential vectors of *Salmonella* spp., *E. coli*, and *Staphylococcus* spp., bacterial agents associated with mastitis and enteritis in milk cows and with food contamination. Attractants associated with farming near the milking parlors may have influenced the population dynamics of muscid Diptera on the farms studied here.

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