1 The Australian bush fly (*Musca vetustissima*) as a potential vector in the transmission
2 of foodborne pathogens at outdoor eateries.
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4 Running title: Flies, BBQs, and Pathogens
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Abstract

Australian outdoor activities are often accompanied by a barbeque (BBQ) with family, friends and guests, which are often interrupted by uninvited guests in the form of the Australian bush fly, *Musca vetustissima*. We investigated the bacterial loading associated with the Australian bush in three different environments: on a cattle farm, in a typical urban area (shopping centre car park), and at a BBQ. The highest bacterial populations per fly were found to occur in a farm environment (~9.1×10^4 CFU per fly), while the bacterial population was lowest on flies caught in an urban environment (~ 1.9×10^4 CFU per fly). The median CFU per fly caught near a BBQ was ~5.0×10^4. *Escherichia coli* was the most commonly isolated potential pathogen, while *Shigella* was the least common bacterial isolate that was screened for. All isolated foodborne pathogens or indicator bacteria were screened for antibiotic resistance against commonly prescribed antibiotics. This revealed a very high prevalence of multi-drug resistance, especially among the *Salmonella* and *Shigella* isolates of 94 and 87 % resistance respectively against amoxilillin, roxythromycin and cefaclor.

Keywords: Flies, *Musca vetustissima*, Farm, Barbeque, Bacteria, Antibiotic resistance, Vector

Introduction

Australia is a country where in typical summer tradition most outdoor activities are accompanied by a barbeque (BBQ) with family, friends and guests - some of which come uninvited. The uninvited guests are those that crawl and swarm around areas where both raw and cooked foods are prepared and consumed. One of those annoyances is the Australian bush fly: *Musca vetustissima*. As part of the typical lifecycle of the Australian bush fly there is an increase in the fly's population in the months of December and January, the middle of the Australian summer (Greenberg, 1973). The Australian bush fly breeds and spends its initial stages of growth in cattle dung (Heath, 1989): given this start to life and their typical habits of populating rural areas near animals and dung, they are likely vectors in the spread of potential pathogens and foodborne diseases. Various studies have been conducted in relation to the common house fly, *Musca domestica*, and its potential to be a vector in the transfer of pathogens to humans (Echeverria *et al*., 1983; Olsen, 1998; De Jesus *et al*., 2004; Barro *et al*., 2006; Macovei and Zurek, 2006) – to date there have been only a limited number of studies in relation to the Australian bush fly to carry and transfer pathogens to humans. Weinstein (1991) reported that the Australian bush fly could act as a vector in the transmittance of *Neisseria gonorrhoeae* as the causal agent of conjunctivitis, while a strong correlation was identified between the incidence of trachoma and the seasonal presence of *M. vetustissima* in three aboriginal communities in North-Western Australia (da Cruz *et al*., 2002). Furthermore, the Australian bush fly was a prime suspect in the uncontrolled transmission of rabbit haemorrhagic disease virus from an Australian quarantine facility in 1995 (McColl *et al*., 2002). This study investigated the prevalence of a series of Gram negative foodborne pathogens on the Australian bush fly, as captured in three different outdoor

environments. The isolated foodborne pathogens were tested for antibiotic susceptibility using three commonly prescribed antibiotics for human use.

Materials and Methods:

Sampling.

Flies were captured in three different environments, namely: [1] on a cattle farm (three independent sampling days); [2] in a typical built-up urban area that did not have a significant presence of parkland (shopping centre carpark) (six independent sampling days); and [3] at an outdoor food preparation and consumption (BBQ) area during which time a number of people were preparing and consuming barbequed foods in a garden setting (three independent sampling days). All samplings were conducted during periods of the day when the temperature rose above 25 °C. In the farm environment, individual flies were easily caught with a sterile net. In the BBQ and urban environments, individual flies were caught with sterile nets by using human sweat as bait. This was achieved by the use of a volunteer who exercised to induce perspiration – flies were caught off the person's clothing. The same volunteer was used during all sampling sessions. All samples were processed the same day they were collected. A total of 86 flies were caught on a farm; 126 at a BBQ; and 92 in an urban area. Half of the flies caught from each individual environment were used to determine the microflora on the surface of the flies, while the other half was used for the total fly microflora. A sub-sample (two from each sampling event) of each batch of flies caught was preserved in 95 % ethanol and set aside for subsequent identification according to the CSIRO identification keys (Anon, 1991; Anon, 2006).

Fly microflora extraction method

The surface microflora of the bush flies was extracted by placing each fly in a sterile 50 mL tube containing 10 mL of a saline extraction solution containing 10 g L^{-1} NaCl and 2 g L^{-1} K_2HPO_4. Each tube containing a single submerged fly was sonicated for 1 minute (Branson, 2200) and vortexed for 30 seconds, following which the tubes were placed in a head-over-head tumbler for one hour at ~ 40 rpm. The total fly microflora (surface microflora + internal microflora) was extracted by placing each individual fly in a sterile 50 mL tube and homogenising the insect in 10 mL of the extraction solution using a sterile glass rod. Each tube containing a single homogenised fly was sonicated for one minute (Branson 2200) and vortexed for 30 seconds, following which the tubes were placed on a shaking platform for 30 minutes at ~ 200 rpm.

In order to eliminate any confusion regarding the origin of the microflora from the flies caught using human sweat as bait; the clothes worn by the volunteer acting as "fly bait" were swabbed using a cotton swab (area = 5 cm^2), which was then processed as any other sample.

Microbial analyses

The total microbial population of the flies was determined by plating onto plate count agar (Oxoid). The presence of the Gram negative bacteria was determined in a qualitative manner. This involved a simple absence/presence testing in which a portion of the fly extract was enriched with an equal volume of double strength nutrient broth (Difco), following which the various bacteria were tentatively identified on specific media. *Escherichia coli* was determined on Eosin Methylene Blue agar plates (Oxoid); *Enterococcus faecalis* was determined on Slanetz and Bartley agar (Oxoid); while both *Salmonella* and *Shigella* spp. were determined on Salmonella-Shigella modified agar (Oxoid). General microscopic observations and standard

biochemical characterisation (Finegold and Baron, 1986) were employed to further corroborate the tentative identity of the bacteria.

Antibiotic resistance:

The three most commonly prescribed antibiotics for human use in Australia are Amoxicillin, Roxythromycin, and Cefaclor. Subcultures of all isolated potential pathogens were screened for their ability to grow on total plate count agar (Oxoid) within 48 hrs while incubated at 37 °C in the presence of 50 mg L^{-1} of the above-mentioned, pharmacy supplied antibiotics. Growth was scored as "+" or "–". Good to strong growth was scored as "+", while the absence of obvious growth (as compared to the control plate) was scored as "–". Individual bacterial cultures that scored "+" on more than one antibiotic were considered to be multi-drug resistant. Each antibiotic was screened individually.

Results

The total bacterial population associated with the Australian bush fly was found to differ with regards to the environment in which they were caught. The median total bacterial population was the highest for flies caught on a farm (~ 9.1×10^4 CFU per fly; range: 8.9×10^3 – 3.1×10^6 CFU per fly), while the lowest median populations were found on flies caught at a typical urban setting (~ 1.9×10^4 CFU per fly; range: 1.0×10^3 – 1.4×10^6 CFU per fly) (Fig. 1). Flies caught at a BBQ had a median total bacterial population of ~5.0×10^4 CFU per fly, ranging from: 1.0×10^3 – 2.9×10^6 CFU per fly. Regardless of the environment in which the flies were caught, the total bacterial population associated with the flies was always greater than the surface population (Fig. 1), with typical median bacterial population associated with the flies' surfaces at 1.0×10^4 CFU per fly. Similar to the total number of bacteria

associated with the flies, the spread of bacterial population values associated with the extremities (surface) of individual flies from a single environment varied greatly. For instance, the lowest bacterial population extracted from the surface of individual flies was about 100 CFU per fly, while the highest bacterial population found on the surface of a single fly was about 9.0×10^5 CFU per fly.

We further investigated the presence of a range of Gram negative bacteria that are commonly associated as foodborne pathogens. *Escherichia coli*, *Enterococcus faecalis* and *Salmonella* spp. could be detected at significant frequencies on all outdoor sites examined (Fig. 2). In most cases the prevalence of these Gram negative bacteria was higher at the total-fly bacterial population, compared to their presence on the body surfaces of the flies only. *E. coli* was the most commonly encountered Gram negative bacterium regardless of the environment they were caught in. *E. faecalis* was the second most commonly encountered enteric bacterium on the flies sampled in this study, with approximately 70 % of all flies carrying the bacterium. *Salmonella* was found at the highest frequency in typical urban environments, while the prevalence of *Salmonella* was the lowest on flies caught at BBQs. The prevalence of *Salmonella* caught at farms was approximately 63 %. On the other hand, compared to the prevalence of *E. coli*, *E. faecalis* and *Salmonella*, the prevalence of *Shigella* was relatively low. The rate of suspected *Shigella* ranged from 20 % to about 40 %, with the highest prevalence on flies caught near a BBQ area (Fig. 2).

To evaluate the potential of the Australian bush fly to act as a vector of bacteria that carry antibiotic resistance, we screened the pathogenic isolates for their ability to grow in the presence of three commonly prescribed antibiotics in Australia. In most instances the observed antibiotic resistance against the prescription drugs used in this study was high (Table 1). Amoxicillin is a typical first-line of defence antibiotic to which none of the *E. faecalis* isolates showed any resistance at 50 mg L^{-1},

however forty percent of the *E. coli* isolates showed resistance against amoxicillin. The vast majority of the tentative *Salmonella* and *Shigella* isolates showed resistance against amoxicillin at 50 mg L^{-1}, while all of the *Salmonella* and *Shigella* isolates were resistant against roxythromycin and cefaclor (Table 1). There was only a moderate antibiotic resistance against roxythromycin among both *E. coli* and *E. faecalis*, while a relatively high resistance was observed with regards to cefaclor (57 and 83 % respectively).

Discussion:

In an attempt to assess the potential of the Australian bush fly, *Musca vetustissima*, to act as a carrier of potential foodborne pathogens, the bacterial populations associated with the surface microflora and the total fly microflora were ascertained. The number of bacteria associated with flies caught at a cattle farm were comparable to earlier findings which showed that stable flies also had a variable bacterial loading at approximately the same numbers (Mramba *et al*., 2006). The fact that bacterial populations were the highest among farm caught flies is not surprising since the Australian bush flies prefers to breed in cattle dung (Heath, 1989). Similarly, it is not surprising that flies caught in a typical urban area had a relatively low bacterial loading since their immediate environment mainly consisted of concrete with little to no vegetation let alone animals. The flies caught near an operational BBQ were in a garden setting with ample vegetation but no direct evidence of animal activity, as a result the bacterial loading on these flies was lower than those caught on a farm but higher than those caught in an urban setting. The spread of bacterial population values associated with the body surfaces of individual flies from a single environment varied greatly. Regardless of the environment in which the flies were caught, the total bacterial population associated with the flies was always greater than

the surface population (Fig. 1). The inclusion of fly intestinal (faecal) material suggests that a portion of the total bacterial population values can be attributed to faecal matter of the flies. These observations are in agreement with those from Barro et al. (2006) who found that houseflies had the potential to excrete up to 2.2×10^3 CFU per faecal sample.

E. coli was the most commonly isolated bacterium, followed by *E. faecalis*, *Salmonella* spp. and *Shigella* spp. (Fig. 2). *E. coli* has also frequently been connected with houseflies (Kobayashi *et al*., 1999; Alan and Zurek, 2004; De Jesus *et al*., 2004) including in association with outdoor food preparation areas (Barro *et al*., 2006). The high frequency of *E. faecalis* observed in this study is very similar to previous findings, which showed that the common housefly carried this bacterium at a frequency of about 88 % (Macovei and Zurek, 2006). Flies caught on farms are typically carriers of *Salmonella* (Barber *et al*., 2002; Ugbogu *et al*., 2006; Holt *et al*., 2007). Since the Australian bush fly usually relies on cattle dung for the early stages of its life cycle (Heath, 1989), the high prevalence of *Salmonella* in other outdoor areas is not unexpected. The prevalence of *Shigella* in association with flies is not uncommon, as Béjar *et al*. (2006) and Ugbogu *et al*. (2006) reported that flies were effective vectors for the transmission of this pathogen. The housefly and other insects have on many occasions been associated with the potential to spread pathogens (Grübel *et al*., 1997; Osato *et al*., 1998; Nayduch *et al*., 2002; De Jesus *et al*., 2004; Ekdahl *et al*., 2005; Pai *et al*., 2005; Sela *et al*., 2005; Tatfeng *et al*., 2005). The communicative nature of roving between contaminated environments and human outdoor eateries presents the Australian bush fly as a significant potential threat to human wellbeing.

The frequent use of antibiotics in general and animal medicine has seen a dramatic increase in the incidence of bacteria that are resistant to these often crucial antimicrobial agents. The decreased effectiveness of antibiotics can result in infections that are more difficult to combat and impose a significant drain on public health funding. The acquisition of antibiotic resistance is often associated to very direct exposures to the subscription of antibiotics: e.g. hospital-acquired, or through the therapeutic or prophylactic use of antibiotics in food-producing animals (Graham *et al*., 2009). The latter has arguably been linked to an increase in antibiotic resistance in foodborne pathogens (Teuber, 1999; Threlfall *et al*., 2000). However, a more indirect route of antibiotic resistance acquisition via flies has recently been shown (Macovei and Zeruk, 2006; Petridis *et al*., 2006).

The prevalence of antibiotic resistance among the potential pathogens isolated from the Australian bush fly in this study is a worrying occurrence. This could mean that *M. vetustissima* is not just a probable vector for the spread of foodborne pathogens, it could also mean that an infection caused by these bacteria would be difficult to combat with typical first-line of defence anti-bacterial drugs. We further investigated whether the various bacterial isolates had resistance against more than one antibiotic tested. We found that *E. faecalis* was sensitive to all combinations of the antibiotics tested, while such combination would probably only be moderately effective against *E. coli* (Table 1). On the other hand, the very high prevalence of antibiotic resistance among the *Salmonella* and *Shigella* isolates suggests that infections caused by these potential pathogens would be difficult to combat. While the prevalence of antibiotic resistance among the potential foodborne pathogens isolated from the bush flies in this study is a worrying fact, the high level of antibiotic resistance overall could also have widespread implications regarding the efficacy of therapeutic antibacterial drugs used to address infections in farm animals.

Our research shows that the Australian bush fly has the capacity to be a vector in the spread of potential pathogens. Furthermore, the high incidence of antibiotic resistance against the commonly prescribed antibiotics is a public health concern that could have wide ranging implications. Hence, it is important that food vendors, people at outdoor eateries, and people operating barbeques protect all foods (cooked and raw) from contact with the Australian bush fly.

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Disclosure statement:

No competing financial interests exist.

References:

Anonymous The insects of Australia. 2nd ed. CSIRO, division Entomology, Melbourne University Press, 1991

Anonymous Feedlot flies: Identifying the problems and some solutions. Meat and Livestock Australia. Queensland Government Department of Primary Industries and Fisheries, 2006

Alan MJ and Zurek L. Association of *Escherichia coli* O157:H7 with houseflies on a cattle farm. Appl Environ Microbiol 2004;70:7578-7580

Barber DA, Bahnson PB, Isaacson R, et al. Distribution of *Salmonella* in swine production ecosystems. J Food Prot 2002;65:1861-1868

Barro N, Savadogo A, Ouattara CAT, et al. Carriage of bacteria by proboscises, legs, and faeces of two species of flies in street food vending sites in Ouagadougou, Burkina Faso. J Food Prot 2006;69:2007-2010

Béjar VC, Chumpitaz JC, Pareja EC, et al. *Musca domestica* Como vector mecanico de bacterias enteropatógenas en mercados y Basurales de Lima y Callao. Rev Peru Med Exp Salud Publica 2006;23:39-43

Da Cruz L, Dadour IR, McAllister IL, et al. Seasonal variation in trachoma and bush flies in north-western Australian aboriginal communities. Clin Experiment Ophthalmol 2002;30:80-83

De Jesus AJ, Olsen AR, Bryce JR, et al. Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). Int J Food Microbiol 2004;93:259-262

Echeverria P, Harrison BA, Tirapat C, et al. Flies as a source of enteric pathogens in a rural village in Thailand. Appl Environ Microbiol 1983;46:32-36

Ekdahl K, Normann B, and Andersson Y. Could flies explain the elusive epidemiology of campylobacteriosis? BMC Infect Dis 2005;5:11 doi:10.1186/1471-2334-5-11

Finegold SM, and Baron EJ. Chapter 27: Enterobacteriaceae. Baily and Scotts' Diagnostic Microbiology. 7th ed. The CV Mosby Company, St Louis MO. 1986

Graham JP, Price LB, Evans SL, et al. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. Sci Total Environ 2009; 407:2701-2710

Greenberg B. Flies and Disease. Vol.1: Ecology, classification and biotic associations. Princeton University Press, NJ. 1973

Grübel P, Hoffman JS, Ching FK, et al. Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*. J Clin Microbiol 1997;35:1300-1303

Heath J. The fly in your eye. Viacorp Pty. Ltd. The James family trust. Available online at: www.viacorp.com/flybook/fulltext.html 1989

Holt PS, Geden CJ, Moore RW, et al. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella* serovar Entertidis-challenged hens. Appl Environ Microbiol 2007;73:6030-6035

Kobayashi M, Sasaki T, Saito N, et al. Houseflies: Not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. Am J Trop Med Hyg 1999;61:625-629

Macovei L and Zurek L. Ecology of antibiotic resistance genes: Characterization of Enterococci from houseflies collected in food settings. Appl Environ Microbiol 2006;72:4028-4035

McColl KA, Merchant JC, Hardy J, et al. Evidence for insect transmission of rabbit haemorrhagic disease virus. Epidemiol Infect. 2002;129:655-663

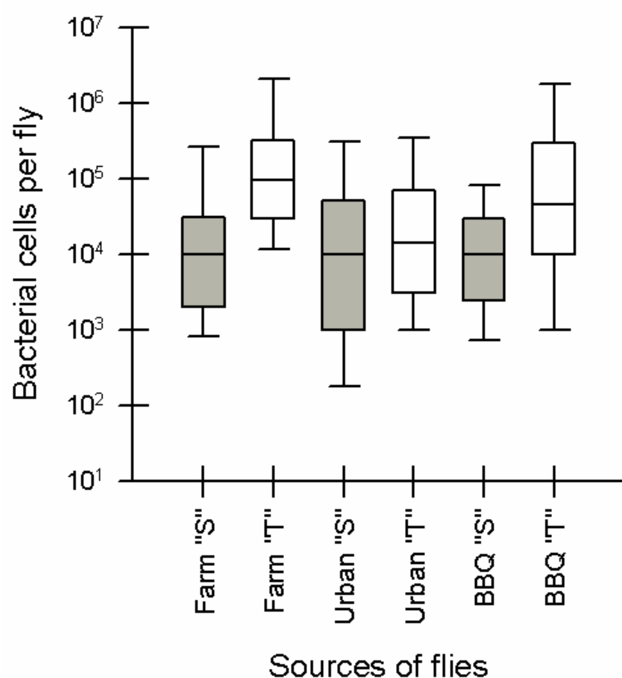
1 Mramba F, Broce A and Zurek L. Isolation of *Enterobacter sakazakii* from stable flies,
2 *Stomoxys calcitrans* L. (Diptera: Muscidae). J Food Prot 2006;69:671-673
3 Nayduch D, Pitman-Noblet G and Stutzenberger FJ. Vector potential of houseflies for
4 the bacterium *Aeromonas caviae*. Med Vet Entemol 2002;16:193-198
5 Olsen AR. Regulatory action criteria for filth and other extraneous materials: Part 3,
6 Review of flies and foodborne enteric disease. Reg Tox Pharmacol 1998;28:199-211
7 Osato M, Ayub K, Le H-H, et al. Houseflies are an unlikely reservoir or vector for
8 *Helicobacter pylori*. J Clin Microbiol 1998;36:2786-2788
9 Pai H-H, Chen W-C and Peng C-F. Isolation of bacteria with antibiotic resistance from
10 household cockroaches (*Periplaneta Americana* and *Blattella germanica*). Acta Trop
11 2005;93:259-265
12 Petridis M, Bagdasarian M, Waldor MK, et al. Horizontal transfer of shiga toxin and
13 antibiotic resistance genes among *Escherichia coli* strains in housefly (Diptera:
14 Muscidae) gut. J Med Entomo 2006;43**:**288-295
15 Sela S, Nestel D, Pinto R, et al. Mediterranean fruit fly as a potential vector of bacterial
16 pathogens. Appl Environ Microbiol 2005;71:4052-4056
17 Tatfeng YM, Usuanlele MU, Orukpe A, et al. Mechanical transmission of pathogenic
18 organisms: the role of cockroaches. J Vect Borne Dis 2005;42:129-134
19 Teuber M. Spread of antibiotic resistance with food-borne pathogens. Cell Mol Life
20 Sci 1999;56:755-763
21 Threlfall EJ, Ward LR, Frost JA, et al. The emergence and spread of antibiotic
22 resistance in food-borne pathogens. Int J Food Microbiol 2000;62:1-5
23 Ugbogu OC, Nwachukwu NC and Ogbuago UN. Isolation of *Salmonella* and *Shigella*
24 species from house flies (*Musca domestica*) in Uturu, Nigeria. Afri J Biotechnol
25 2006;5:1090-1091

Weinstein P. The Australian bush fly (*Musca vetustissima* Walker) as a vector of *Neisseria gonorrhoeae* conjunctivitis. Med J Aust 1991;155**:**717

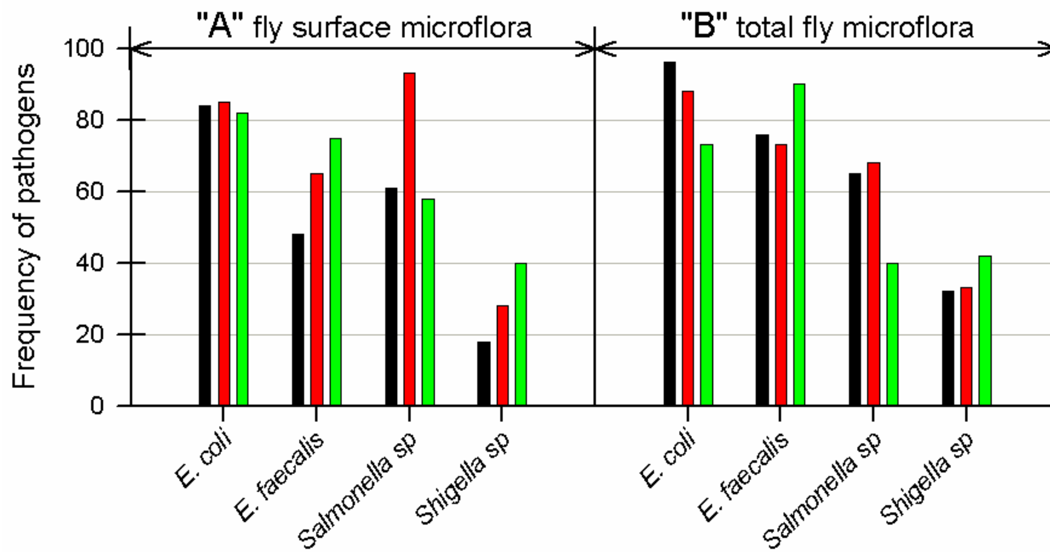
Table 1. Frequency of antibiotic resistance among the isolated Gram negative bacteria isolated from Australian bush flies.

	Antibiotics						
Bacteria	**A***	**R****	**C*****	**AR¶**	**AC¶¶**	**RC†**	**ARC††**
E. coli (236)	40 %	15 %	57 %	9 %	32 %	7 %	6 %
E. faecalis (198)	0 %	11 %	83 %	0 %	0 %	11 %	0 %
Salmonella sp. (173)	94 %	100 %	100 %	94 %	94 %	100 %	94 %
Shigella sp. (93)	87 %	100 %	100 %	87 %	87 %	100 %	87 %

Numbers in brackets indicate number of isolates tested. A* = amoxicillin at 50 mg L^{-1}; R** = roxythromycin at 50 mg L^{-1}; C*** = cefaclor at 50 mg L^{-1}; AR¶ = individual bacterial cultures with multi-drug resistance against both amoxicillin and roxythromycin; AC¶¶ = individual bacterial cultures with multi-drug resistance against both amoxicillin and cefaclor; RC† = individual bacterial cultures with multi-drug resistance against both roxythromycin and cefaclor; ARC†† = individual bacterial cultures with multi-drug resistance against all three antibiotics studied.

1 

2 **Figure 1**. The comparison of the total bacterial populations on the Australian bush fly
3 captured in three different environments, namely: cattle farm; typical urban area; and
4 at an outdoor BBQ. [Farm “S”] spread of bacterial loading on the surface of flies caught
5 on a cattle farm, n = 40; [Farm “T”] spread of bacterial loading in and on the entire flies
6 caught on a cattle farm, n = 40; [Urban “S”] spread of bacterial loading on the surface
7 of flies caught in a typical urban setting, n = 40; [Urban “T”] spread of bacterial loading
8 in and on the entire flies caught in a typical urban setting, n = 40; [BBQ “S”] spread of
9 bacterial loading on the surface of flies caught at an outdoor barbeque, n =60; [BBQ
10 “T”] spread of bacterial loading in and on the entire flies caught at an outdoor barbeque,
11 n = 60. Data as total bacterial population per fly; as found on either the surface of
12 individual flies or as found in the entire bush fly. Grey boxes represent the spread of
13 the bacterial population associated with the surface of the flies; while the white boxes
14 represent the bacterial populations found in and on the entire flies. The boxes represent
15 the median 50 % of the numerical data, while the whiskers represent the upper and
16 lower 25 %.



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2 **Figure 2**. Frequency of Gram negative bacteria associated to the Australian bush fly.
3 Shown are the bacterial populations associated with the external surface of individual
4 Australian bush flies [“A” fly surface microflora], and the bacterial populations
5 associated with the entire flies [“B” total fly microflora] caught at various outdoor
6 settings. Black bars: flies caught at a cattle farm; Red bars: flies caught at a typical
7 urban area; Green bars: flies caught at an outdoor BBQ.

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9