Infection avoidance behaviour in adult fruit flies is sex-specific and depends on prior exposure to a viral pathogen

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Abstract

Infection avoidance behaviours are the first line of defence against pathogenic encounters. Behavioural plasticity in response to internal or external cues can therefore generate heterogeneity in infection. We tested whether *Drosophila melanogaster* exhibits infection avoidance behaviour during foraging, and whether this behaviour is modified by prior exposure to Drosophila C Virus (DCV) and by the risk of DCV encounter. We examined two measures of infection avoidance: (1) the motivation to feed in the presence of an infection risk and (2) the preference to feed on a clean food source over a potentially infectious source. We found no clear evidence for preference of clean food sources over potentially infectious ones. However, infection avoidance was present in female fruit, which were less motivated to feed when presented with a risk of encountering DCV, but this was only the case if they had been previously exposed to this viral pathogen. We discuss the relevance of plasticity in avoidance behaviours during ecologically relevant scenarios such as foraging for host fitness and pathogen spread.

Key-words: Infection, avoidance behaviour, Drosophila, DCV, foraging

Background

Hosts vary considerably in their ability to acquire and transmit infection (Barron et al., 2015; Fellous et al., 2012; Paull et al., 2011; Susi et al., 2015; Vale and Little, 2009). Given the ubiquitous presence of pathogens and parasites in natural environments, mounting a timely and efficient immune response to all possible pathogenic challenges would be physiologically costly and ultimately ineffective. Individuals capable of reducing the probability of contacting parasites, infected conspecifics or infectious environments can therefore not only prevent the deleterious effects of infection, but also circumvent the undesirable energetic costs of immune responses, including immunopathology (Barron et al., 2015; Curtis, 2014). Avoiding infection is therefore the first line of non-immunological defence against infection(Parker et al., 2011), and it is known to occur across a broad range of host taxa, including humans (Curtis, 2014; Moore, 2013).

Like most traits, infection avoidance behaviours are likely to vary according to the context of infection, and pathogens are major drivers this context (Barron et al., 2015; Curtis, 2014; Lazzaro and Little, 2009; Moore, 2013; Vale et al., 2008; Wolinska and King, 2009). Pathogens may alter host responses in two ways. First, by altering the immuno-physiology of the host during infection, pathogens can alter host behaviour (Adamo, 2006; Adelman and Martin, 2009). Second, pathogens also modify the host external environment by increasing the likelihood of exposure to novel infections, and these external cues of infection risk are also known to influence host behavioural responses (Barron et al., 2015; Curtis, 2014). Understanding variation in infection avoidance behaviours therefore provides an important functional link between the neurological, behavioural and immunological processes that together govern the spread of disease (Adamo, 2006).

Insects are ideal systems to investigate the interplay between infection and behaviour (Adamo, 2006; Parker et al., 2010). The fruit fly *Drosophila* is especially amenable to these studies, as it is one of the best model systems for host-pathogen interactions (Neyen et al., 2014) and behavioural ecology and genetics (Dubnau, 2014; Sokolowski, 2001). One of the best-studied pathogenic interactions in *Drosophila* is the host response to systemic and

enteric infection with Drosophila C Virus (DCV) (Dostert et al., 2005; Ferreira et al., 2014). DCV is a horizontally transmitted +ssRNA virus that naturally infects the fly gut (Huszar and Imler, 2008), causing intestinal obstruction, severe metabolic dysfunction and eventually death (Chtarbanova et al., 2014). As a consequence of its pathology, female flies infected with DCV are also known to exhibit behavioural modifications, such as reduced locomotion and increased sleep (Vale and Jardine, 2015). The Drosophila-DCV interaction therefore offers a powerful system to investigate the ecological consequences that may arise from the physiological and behavioural effects of enteric viral infections.

In the present study we used a combination of controlled experimental infections and foraging choice assays, to test whether adult *D. melanogaster* are able to avoid potentially infectious environments when foraging for food, and if avoidance behaviour is modified in response to virus exposure history and to different risks of acquiring DCV infection. We find evidence for avoidance behaviours in the form of reduced motivation to feed according to the risk of infection. However, these effects were only present in female flies, indicating potentially important sexual dimorphism in infection avoidance, and were only present when females were previously exposed to DCV.

Methods

Fly and virus stocks

All flies used were from a long-term laboratory stock of Wolbachia-free *Drosophila melanogaster* Oregon R line, maintained on Lewis medium in standard conditions: 25^{o}C, with a 16:8h light:dark cycle. Fly stocks were routinely kept on a 14-day cycle with non-overlapping generations under low larval densities. The DCV culture used in this experiment was grown in Schneider Drosophila Line 2 (DL2) as described in (Vale and Jardine, 2015). Ten-fold serial dilutions of this culture (diluted in Ringers buffer solution) were aliquoted and frozen at -80ºC for long-term storage before use.

Prior virus exposure

Flies used in the foraging choice assays were obtained by preparing 10 vials of Lewis medium and yeast containing ten mated females. Flies were allowed to lay eggs for 48 hours resulting in age-matched progeny reared in similar larval densities. Two to three days after eclosion, these progeny were exposed to DCV via the oral route of infection (Vale and Jardine, 2015), in order to test the effect of previous exposure to virus on avoidance behaviour during foraging. Briefly, single-sex groups of 20 flies were placed in vials containing agar previously sprayed with DCV ("exposed" to 50 µl of 10^8 viral copies/ml) or the equivalent volume of Ringers buffer solution as a control ("not exposed"). This procedure produced 10 replicate vials of either healthy or virus-exposed male or female flies. DCV exposure using this protocol typically results in ~20% mortality (Fig. S1 and (Vale and Jardine, 2015)).

Foraging choice assays

Following 5 days of virus exposure, we set up independent foraging choice assays in cages - cylindrical transparent plastic containers (12 cm in diameter) containing two equally spaced plastic vials of standard Lewis fly medium and yeast. For each combination of "exposed" and "not exposed" male or female flies, we set up two sets of cages to simulate different risks of infection: a "no risk" environment, with two clean vials (sprayed with sterile Ringers solution), and a "high-risk" environment where one of the vials was sprayed with DCV, as described above. Six replicate 20-fly groups were allocated to the "high-risk" chambers and four replicates to the "no risk" chambers, resulting in a total of 40 independent foraging choice cages. Flies were added to the chamber from a neutrally placed hole in the lid, and the number of flies that settled on each vial was recorded every 30 minutes for six hours. Care was taken to randomise the position of the cages so that the orientation of the light did not influence the choice of the flies in any systematic way.

Statistical Analysis

To measure infection avoidance, we took two approaches. First, we hypothesised that the motivation to feed would be lower in environments where the risk of infection is higher (Curtis, 2014). We therefore compared the motivation to feed between the "no risk" and "high-risk" cages, measured

as the proportion of flies inside a cage that chose to feed on any of the provided food sources. We also asked whether flies that chose to feed showed any evidence of avoiding potentially infectious food sources. For this analysis we focussed on the proportion of flies choosing the clean food source over the infectious food source in the "high risk" cages. In both analyses of 'motivation to feed' and 'infection avoidance', data on the proportion of flies choosing each food source within each replicate cage were analysed with a generalised linear model assuming binomial error and logit link function, and included fly 'sex', 'previous exposure' and 'infection risk' as fixed effects. 'Replicate cage' was included as a random effect, nested within treatments. We also analysed the average motivation to feed and infection avoidance across all time points, in a model including "time" as a random effect. Treatment specific contrasts were used to test the significance of pairwise comparisons. Analyses were carried out using JMP 12 (JMP).

Results

Once inside the cages, only a fraction of flies chose either of the food sources provided, and this motivation to feed increased over time for flies in all treatment groups (χ^2_1= 11.00, p=0.001; Fig. S2). The rate at which motivation increased differed between sexes ('Time × Sex' interaction, χ^2_1= 12.47, p=0.0004), and on average female flies showed greater motivation to feed than males (χ^2_1= 5.01, p=0.025), with 67% of female and 36% of male flies making a choice to feed on any of the provided substrates during the 6 hours of observation (Fig. 1).

Across the entire six-hour observation period, the motivation to feed differed between sexes, and depended both on their previous exposure and on their current risk of infection ('Sex' × 'risk of infection' × 'Previous exposure' interaction, χ^2_1= 21.82, p<0.0001). The proportion of males choosing any food substrate did not vary with previous exposure to DCV in either high-risk (χ^2_1= 2.21, p=0.137) or no-risk environments (χ^2_1= 0.09, p=0.764; Fig. 1).

In female flies however, previous exposure and current infection risk affected the motivation to feed on the provided food sources. When there was no risk of infection (Fig. 1, light grey bars) the motivation to feed was greater

in females that were previously exposed to DCV than in otherwise healthy, non-exposed females (χ^2_1= 104.11, p<0.001). Among females that were previously exposed to infection, we found that the presence of a risk of acquiring infection resulted in lower foraging effort - with just over 50% of flies making the choice to feed - compared to females in cages where there was no risk of acquiring infection, where over 80% of flies made the choice to feed (Fig. 1; χ^2_1= 168.48, p<0.001).

Once flies had made the choice to feed on one of the provided food sources, the choice between a clean and a potentially infectious food source was not affected by previous exposure to DCV ('previous exposure', χ^2_1= 0.513, p=0.47) in either male or females ('sex', χ^2_1= 0.595, p=0.44; Fig. 2).

Discussion

The ability to detect and discriminate between clean and potentially infectious environments is vital to avoid the adverse consequences of infection. In this study we tested if infection avoidance behaviour in *Drosophila melanogaster* is modified by its previous exposure to a viral pathogen and by the risk of infection with that same pathogen when encountered during foraging.

The higher motivation to feed of some female flies when the risk of infection was absent (Fig. 1) suggests flies were able to identify external cues of infection risk. Identifying infection cues is a general prerequisite of avoidance behaviours and occurs across a wide range of different taxa. For example, lobsters are known to detect and avoid virus-infected conspecifics (Behringer et al., 2006); fruit flies and nematodes are capable of avoiding pathogenic bacteria (Babin et al., 2014; Meisel and Kim, 2014); gypsy moth larvae are able to detect and avoid virus-contaminated foliage (Parker et al., 2010); sheep have been found to prefer to graze in parasite-poor patches (Hutchings et al., 2007); and it is has been argued that the disgust response in humans has evolved because it decreases contact with potential infection (Curtis et al., 2011). It is unclear how flies are able to detect food sources contaminated with a viral pathogen. In *Drosophila* and *C. elegans* avoidance of pathogenic bacteria is enabled by evolutionary conserved olfactory and chemosensory

pathways (Babin et al., 2014; Meisel and Kim, 2014), while avoidance of parasitic wasps appears to be mainly enabled by the visual sensory system (Kacsoh et al., 2013). While avoiding virus infected conspecifics is probably driven by visual cues of infection (Behringer et al., 2006), it remains unclear how virus-contaminated environments may be detected by *Drosophila*.

Infection avoidance, measured as a reduced motivation to feed, was clearest when flies had been previously exposed to infection (Fig. 1). In addition to responding to external cues of infection, internal physiological cues therefore also modify avoidance behaviour. Behavioural modifications due to infection are widely reported among animals (Barber and Dingemanse, 2010; Moore, 2013), and can be classified into (i) parasitic manipulation that enhances parasite transmission (Moore, 2013) (ii) sickness behaviours that benefit the host by conserving energetic resources during infection (Adelman and Martin, 2009), or (iii) side-effects of pathogenicity that do not benefit the host or the parasite (Barber and Dingemanse, 2010). Female flies infected with DCV are known to experience increased lethargy and sleep (Vale and Jardine, 2015), so these effects could also explain the reduced feeding activity we detected in female flies that had been previously exposed to DCV. Another potential explanation for reduced motivation to feed in previously exposed flies is infection-induced anorexia (Ayres and Schneider, 2009), a commonly described sickness behaviour (Adelman and Martin, 2009). However, it is unlikely that a lower motivation feed is simply a symptom of a "sick" fly, because it varied according risk of infection, and even reached 80% in exposed flies when foraging in a 'no risk' environment (Fig. 1). This suggests that flies are actively avoiding contact with the potentially contagious food source by lowering their foraging effort.

The fact that only female flies demonstrated avoidance is an indication that any potentially adaptive effects of avoiding infection may be related to oviposition, which coincides with feeding. For flies previously exposed to DCV, avoiding infection would not confer substantial direct benefits given the physiological and behavioural costs of this infection (Arnold et al., 2013; Chtarbanova et al., 2014; Vale and Jardine, 2015), but would however reduce the exposure of future offspring to infection. While flies previously exposed to

DCV do not appear to immune primed following an initial viral exposure (Longdon et al., 2013), our results point to a sort of behavioural priming, where females previously exposed to infection avoid foraging in potentially infectious environments.

Conclusions

Using a combination of experimental infections and behavioural assays, we find evidence for avoidance behaviours in *Drosophila* in the form of reduced motivation to feed, which was most pronounced when flies were faced with an increased risk of encountering an infectious food source. However, these effects were only present in female flies, indicating potentially important sexual dimorphism in infection avoidance, and were only present when females were previously exposed to DCV. Understanding how avoidance behaviours may vary is therefore important for our understanding of how disease will spread in natural populations (Barron et al., 2015), and more broadly how pathogens might evolve in response to variation in host responses to infection (Boots and Bowers, 1999; McLeod and Day, 2015).

Competing interests

The authors declare that they have no competing interests.

Author contributions

PFV conceived the study. PFV and MDJ designed the experiment. MDJ carried out the experimental work. PFV analysed the data, wrote the manuscript and provided all research consumables.

Acknowledgements

We thank H. Cowan and H. Borthwick for helpful technical assistance and the members of the Vale, Obbard and Ross labs for helpful suggestions. This work was supported by a strategic award from the Wellcome Trust for the Centre for Immunity, Infection and Evolution (grant reference no. 095831), and by a Society in Science – Branco Weiss fellowship, both awarded to P. Vale. P.Vale is also supported by a Chancellor's Fellowship from the School of Biological Sciences, University of Edinburgh.

Figure legends

Fig. 1. The motivation to feed, measured as the proportion of flies in the cage that fed on any of the provided food sources. Single sex-groups of flies that had either been previously exposed to DCV or to a sterile inoculum were tested in a no-risk environment (choice between two clean vials; light grey) or a high-risk environment (choice between a clean vial and a DCV-contaminated vial; dark grey). Data are means ± SE.

Fig. 2. Infection avoidance, measured as the proportion of flies in the cage that preferred to settle on the clean food source relative to the DCV-contaminated food source. Single sex-groups of flies that had either been previously exposed to DCV or to a sterile inoculum were tested in a high-risk environment (choice between a clean vial and a DCV-contaminated vial). Data are means ± SE.

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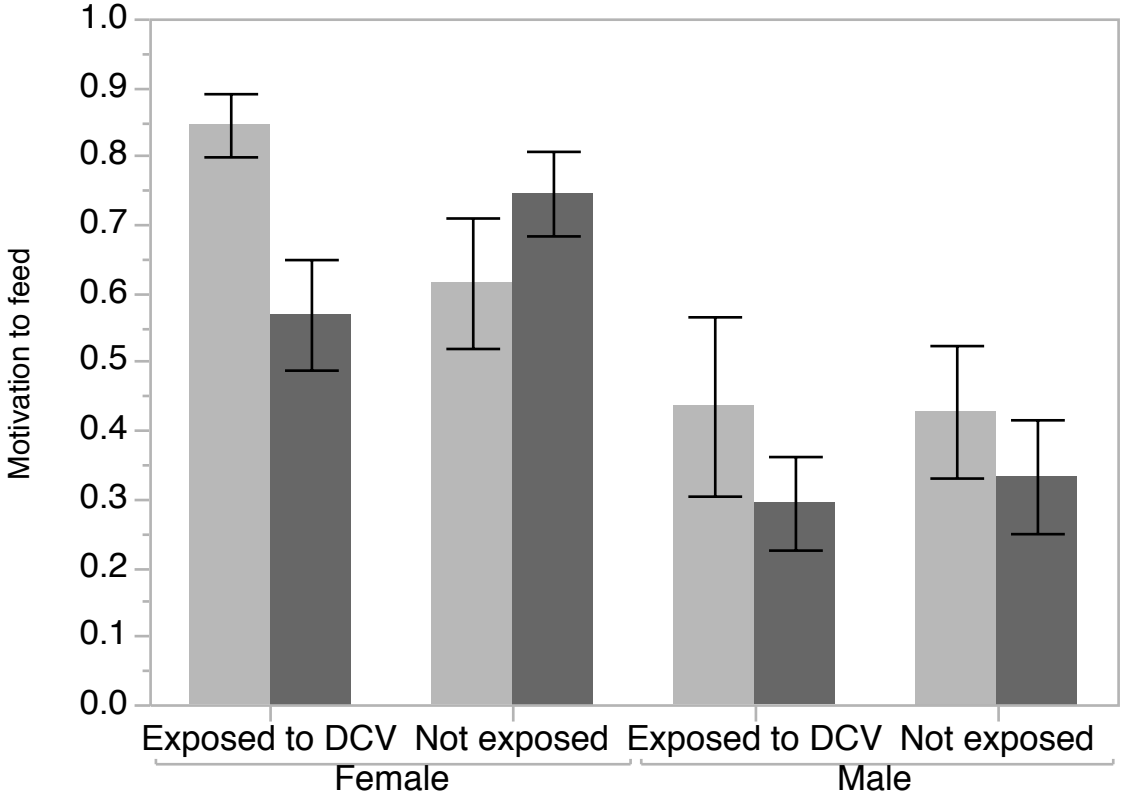
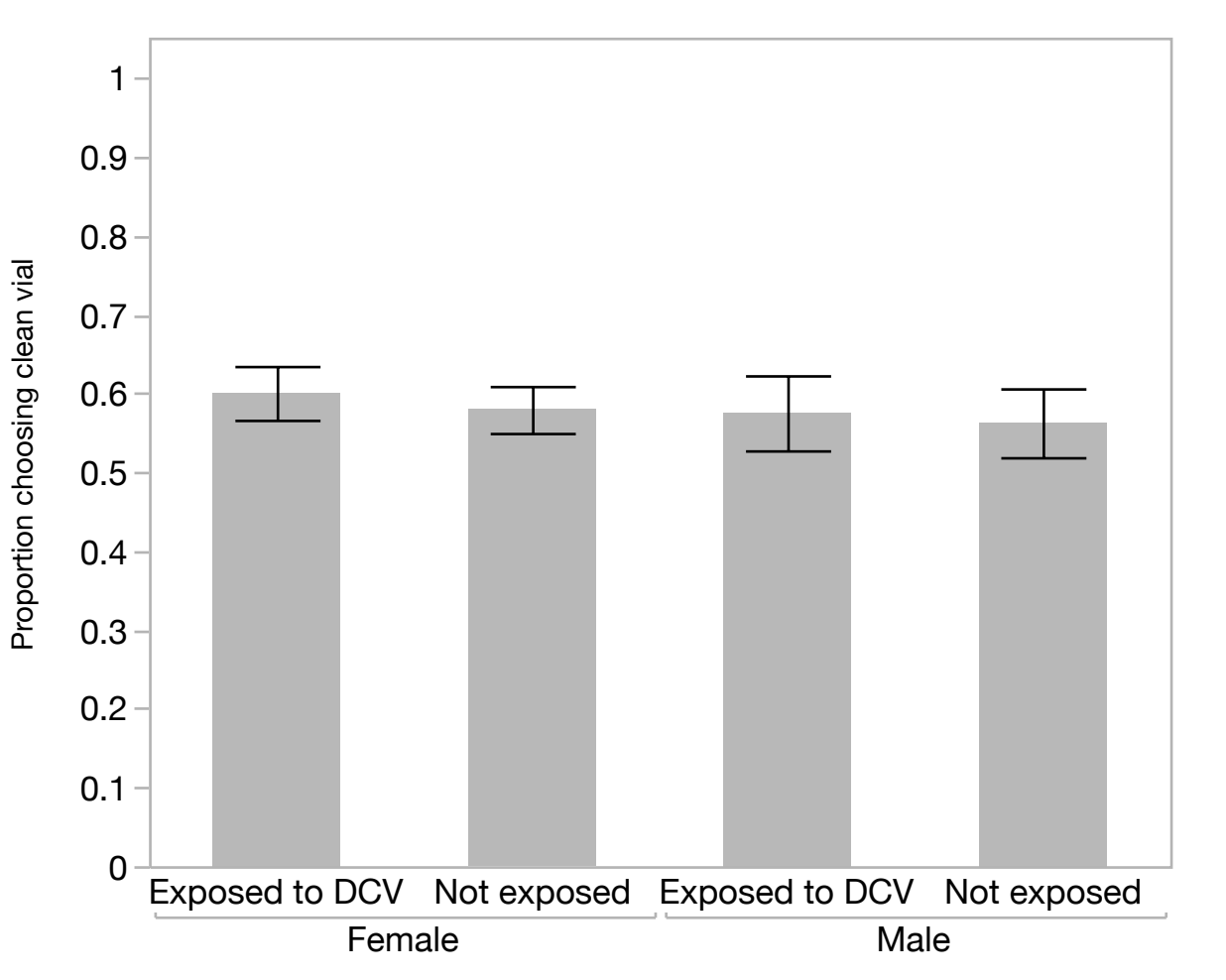
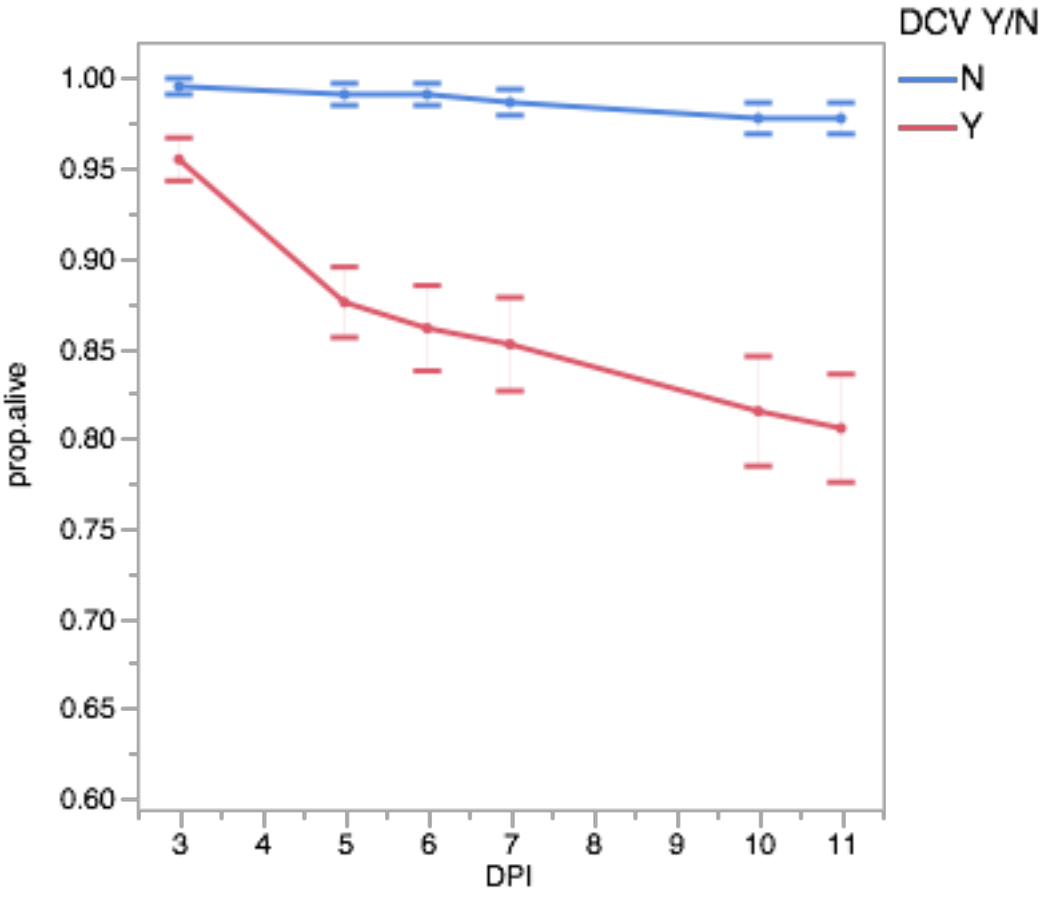

Motivation to feed
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Exposed to DCV
Not exposed
Exposed to DCV
Not exposed
Female
Male


Proportion choosing clean vial
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Exposed to DCV
Not exposed
Exposed to DCV
Not exposed
Female
Male


DCV Y/N
N
Y
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
prop.alive
3
4
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6
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DPI