Blowflies (Diptera: Calliphoridae) as potential mechanical vectors of the protozoan cyst and helminthic eggs in Kashmir Himalaya, India

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Abstract

Blowflies (Diptera: Calliphoridae) are an important group of non-biting flies that are potential mechanical vectors of protozoan and helminthic pathogens. The present study was carried out to isolate and identify protozoan cysts and helminthic eggs transmitted by the blowflies. Surveys were conducted at six different sites viz. butcher shops, fish markets, garbage piles, water bodies and open vegetation in Kashmir Himalaya. The flies were collected with the help of a sweeping net and using day old beef liver as bait from March 2021- March 2023. A total of 968 blowflies were collected, out of which 83 were found carrying at least one protozoan cyst and helminthic egg with six identified species of parasites. Garbage piles were recorded with the highest number of positive cases (10.81%) while human habitation had the highest transmission rate (3.3%). *Chrysomya megacephala* (Fabricius) were reported with the highest number of parasitic cyst and ova (one protozoan cyst and three helminthic eggs) while *Ascaris lumbricoides* and *Entamoeba coli* were found to be the abundant parasites reported from the surface of these flies. The number of parasites isolated from the surface of the blowflies was statistically significant (F = 9.073, df = 1 and p 0.014) indicating a positive association between the number of parasites isolated from blowflies and the collection sites.

Introduction

Blowflies (Diptera: Calliphoridae) often referred to as bluebottles, greenbottles and carrion flies are members of the non-biting flies which are essential in the fields of medical, veterinary, and forensic sciences. Due to their habit of foraging on or breeding in the garbage and other filth, these flies spread several veterinary and medically important infections, such as bacterial, viral, protozoan, and helminths among humans and other animal species (Reid, 1953; Greenberg, 1971; Aziz et al., 2016; Tomberlin et al., 2017; Dar and Mir, 2022). The larvae of several blowfly species parasitize earthworms, snails, toads, frogs, nesting birds, and other animals (Draber-Monko,1981). Blowflies have been reported as potential carriers of parasitic cysts and helminthic eggs (Sulaiman et al., 1988, Lawson and Gemmell, 1958). The global prevalence of parasites in non-biting flies as vectors was studied where blowflies were found with the highest prevalence as vectors (Liu et al., 2023). *Musca domestica* Linnaeus (Diptera: Musidae) has been extensively studied for its role as a mechanical vector in the transmission of protozoan cysts and helminthic eggs (Dipeolu, 1977; Echeverria et al., 1983; 1972; Khamesipour et al., 2018; Issa, 2019), but blowflies have been explored in dearth (Sulaiman et al., 1988, Lawson and Gemmell, 1958, Aziz et al., 2016; Tomberlin et al., 2017 and Liu et al., 2023). This study, the first of its kind in Kashmir Himalaya is a preliminary assessment to determine blowfly species having highest role in the mechanical transmission of protozoan cyst and helminthic eggs. The present study would help society in many ways including preventing disease transmission, improving the health care of humans and animals and to generate baseline data for futuristic studies of the flies associated with the transmission of the parasites.

Materials and methods

Kashmir is the northernmost region of India, spanning 15948 square kilometres and situated between 33.22^{o} and 34.40^{o} N and 73.34^{o} and 75.30^{o} E. Its southern and southwest borders are formed by the Pir Panjal range, while its northern and northeastern borders are formed by the vast Himalayan and north Kashmir range, which divide it from the icy plateau of Ladakh (Hussain, 1987). Surveys were carried out seasonally from March 2021- March 2023 at the selected study sites viz. near human habitations, butcher shops, fish markets, garbage piles, water bodies and open vegetation across the Kashmir Himalaya. These sites were selected keeping in view that blowflies are carriers of animal and human enteric parasites and can disseminate them through contaminated sites and food. The flies were collected with the help of a sweeping net and day-old rotten beef liver used as a bait. After collection specimens were transferred into collection vials having at the bottom, a layer of cotton soaked with ethyl acetate which narcotise the flies after some time. The collection method was adapted of Datta et al. (1997), Nandi (2002) and Akbarzadeh et al. (2015). The narcotised flies were immediately brought to the Entomology Research Laboratory, Department of Zoology, University of Kashmir, where identification was done by examining the fly under a Labomed binocular stereo zoom microscope and running them through the keys of Whitworth (2006, 2010), Falk (2015), Akbarzadeh et al. (2015) and Jones et al. (2019). After identification, each individual fly was transferred into 15 ml graduated centrifuge tube containing 0.85% normal saline and the fly was thoroughly shaken for 2 minutes to create a suspension containing different stages of parasites attached externally to the body of fly after that each individual fly was removed and preserved in 90% ethanol along with necessary details (Fotedar et al., 1992; Ogunniyi et al., 2015). For the examination of parasitic ova/cysts, 10 ml of washing was centrifuged at 3000 rpm for 5 minutes, after which the supernatant and pellets were decanted. The sediments were then placed on a glass slide to form a thin smear, which was then examined under a Magnius MLX microscope with and without 1% Lugol's iodine stain (Fotedar et al., 1992; Ogunniyi et al., 2015). The protozoan cyst and helminthic eggs were identified based on descriptions by Faust et al. (1970); Chatterjee (1975) and Soulsby (1982). The photomicrographs of the parasitic cysts and eggs were taken using Redmi Note 8 Pro and enhanced with Adobe Photoshop 7.0 while the data obtained were analysed using Microsoft Excel software (2019) and PAST 4.03.

Results

During the three-year survey from March 2021- March 2023, 968 flies were collected from six different collection sites viz. near human habitations, butcher shops, fish markets, garbage piles, and water bodies across the Kashmir Himalaya, India. The Garbage piles were found with the highest number of flies (222) and positive cases (24) followed by water bodies (182; 17), butcher shops (176; 12), fish markets (169; 14), open vegetation (129; 7) and human habitation (90; 9) (Fig. 1) respectively. A total of 83 calliphorid flies with six identified species as *chrysomya megacephala* (Fabricius), *Chrysomya rufifacies* Macquart, *Calliphora vicina* Robineau-Desvoidy, *Lucilia sericata (*Meigen), *L. cuprina (*Wiedemann) and *L. porphyrina (*Walker) were found positive for the helminthic eggs and protozoan cysts while as *Calliphora vomitoria (*Linnaeus) and *Lucilia papuensis (*Macquart) were found negative for the helminthic eggs and cysts (Table 2). Among the six examined parasites, one species belongs to Protozoa as *Entamoeba coli*, two

species to Cestoda as *Taenia saginita* and *Hymenolepis nana* and three species to Nematoda identified as *Ascaris lumbricoides, Trichuris trichiura* and *Enterobius vermicularis* (Fig. 2). Out of six selected sites garbage piles were found positive for all the parasitic species with a transmission rate of 2.7 followed by fish markets with four parasitic species and a transmission rate of 2.3, water bodies with four parasitic species and a transmission rate of 2.19, human habitation with three parasitic species and a transmission rate of 3.3, butcher shops with three parasitic species and a transmission rate of 1.7, and open vegetation with two parasitic species and a transmission rate of 1.5 (Table 1). Among the identified blowfly species, *Chrysomya megacephla* (Fabricius) were found to have the highest prevalence of parasites (n = 5) followed by *Calliphora vicina* Robineau-Desvoid (n = 4), *Chrysomya rufifacies* Macquart (n = 3), *Lucilia sericata (*Meigen) (n = 3), L. *cuprina (*Wiedemann) (n = 3) and *L. porphyrina (*Walker) (n = 1). The data obtained during the study was also subjected to one-way ANOVA and it was observed that the number of parasites isolated from the surface of the blowflies was statistically significant (F = 9.073, df = 1 and p 0.014) and a positive association between the number of parasites isolated from blowflies and the collection sites.

Table 1. Transmission rate of parasitic cysts/eggs among blowflies from the collection sites

Sample source	Blowflies examined	Number of positive flies	Parasitic egg/cyst retrieved	% of positive flies	Transmission rate in %
Butcher shops	176	12	3	6.81	1.7
Fish markets	169	14	4	8.28	2.3
Garbage piles	222	24	6	10.81	2.7
Human habitation	90	9	3	10	**3.3**
open vegetation	129	7	2	5.42	**1.5**
water bodies	182	17	4	9.34	2.19
Total	968	83		50.66	13.69

Table 2. Blowfly species with the name of the parasite whose cyst/ egg retrieved from their body surface

Blowfly Species	Parasitic Eggs/ Cyst Retrieved
chrysomya megacephala (Fabricius)	*Ascaris lumbricoides**
	*Enterobius vermicularis**
	*Trichuris trichuria**
	*Taenia saginita***
	*Entamoeba coli****
Calliphora vicina **Robineau-Desvoidy**	*Ascaris lumbricoides*
	Enterobius vermicularis
	*Hymenolepis nana***
	Entamoeba coli
Chrysomya rufifacies **Macquart**	*Ascaris lumbricoides*
	Enterobius vermicularis
	Entamoeba coli
Lucilia sericata **(Meigen)**	*Ascaris lumbricoides*
	Trichuris trichuria
	Entamoeba coli
L. cuprina **(Wiedemann)**	*Ascaris lumbricoides*
	Enterobius vermicularis
	Entamoeba coli
L. porphyrina **(Walker)**	*Entamoeba coli*
Lucilia papuensis **(Macquart)**	None
Calliphora vomitoria **(Linnaeus)**	None

*Nematode; **Cestode; ***protozoan

Discussion

Several studies on the function of flies as mechanical carriers of harmful microorganisms and on the possibility spread of intestinal protozoan and helminths in humans have been conducted (Greenberg, 1971). Non-biting flies are frequently found in residential areas and on farms, where they coexist closely with both people and animals (Yu et al., 2018). Non-biting flies mostly pollute water sources, food, meat, and animal feed through body-surface contact (Ahmadu et al., 2016). When animals and humans consume food or drink contaminated with parasite cysts or eggs, they get indirectly infected. Non-biting flies have the ability to transfer parasite eggs and cysts via contact with human and animal skin, in addition through water and food. This enhances the risk of infection in both humans and animals (Adenusi *et al*., 2013, Getachew et al., 2007). It is likely that different kinds of non-biting flies carry different types of parasites (Graczyk et al., 2005). The house fly (*M. domestica*) has been the subject of

most research, including some lab investigations and surveys of the frequency of transmitted parasites. Though other fly species are not as common in human habitations and animal settings as the house fly (*M. domestica*), in certain nations with unsanitary conditions, including Africa and Asia, the prevalence of parasites carried by these fly species has increased linearly (Monzon et al., 1991). A study on the human intestinal parasites associated with non-biting flies in Ile-Ife Nigeria was carried out in which nine human intestinal parasites reported with *Entamoeba coli* in high prevalence followed by *Ascaris lumbricoides*, garbage piles were found with the highest number of flies while public latrines with the highest transmission rate (Ogunniyi et al., 2015). *Chrysomya megacephala* (Fabricius) was found to be the predominant fly species that carried the eggs of the roundworm *Ascaris lumbricoides*, the pinworm *Trichuris trichuria*, and the hookworm *Necator americanus* on the adult body surface of the fly during research on cyclorrhaphan flies as mechanical vectors of human helminths in Malaysia (Sulaiman et al., 1988). The external surface of the non-biting flies contained the eggs of the human helminths *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm *N. americanus*. Since they feed and reproduce in filthy places like faeces, garbage piles, dead organic matter, etc., blowflies (Diptera: Calliphoridae) are also non-biting flies that act as potential mechanical vectors to the spread of various protozoan cysts and helminthic eggs. The role of blowflies as mechanical vectors was investigated in the current study, and it was found that they act as potential mechanical vectors of both protozoan cyst and helminthic eggs. *Chrysomya megacephala* (Fabricius) were reported as the dominant species carrying the eggs of *Ascaris lumbricoides*, *Enterobius vermicularis*, *Entamoeba coli*, *Trichuris trichuria* and *taenia saginita* as also illustrated in the studies of (Sulaiman et al., 1988, Ogunniyi et al., 2015, Chaiwong et al., 2014, Liu et al., 2023). The Garbage piles were found with the highest number of flies (222) and positive cases (24) as also reported in the study of Ogunniyi et al. (2015), because such contaminated sites attract more blowflies which in turn increases the risk for the transmission of more parasitic eggs and cysts among the animals and humans. The rate of transmission was highest in human habitations due to the fact that most insect vectors live and breed near human habitations and in areas with domestic animals. Our results also demonstrated the role of other medically important blowflies in the spread of various parasites (Table 2) which has not been reported in any other study because research on the function of other blowflies in the spread of protozoan cysts and helminthic eggs is still in its very early stages. Studies on the role of blowflies as potential mechanical vectors are scarce and hence deserve further investigations in the transmission of different protozoan and helminthic parasites.

Declarations

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Statements & Declarations

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Competing Interests

The authors declare that there is no conflict of interest

Author Contributions

T. A.D. and A.H.M. conceived and planned the manuscript. T.A.D. carried out the survey and collection of the fly specimens. A.H.M. contributed to the interpretation of the results and took the lead in writing the manuscript. Both the authors made final approval of the manuscript to be published

Data Availability

Not applicable

Ethics Approval

Not applicable

Consent to Participate

Not applicable

Consent to publish

Not applicable

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Figures

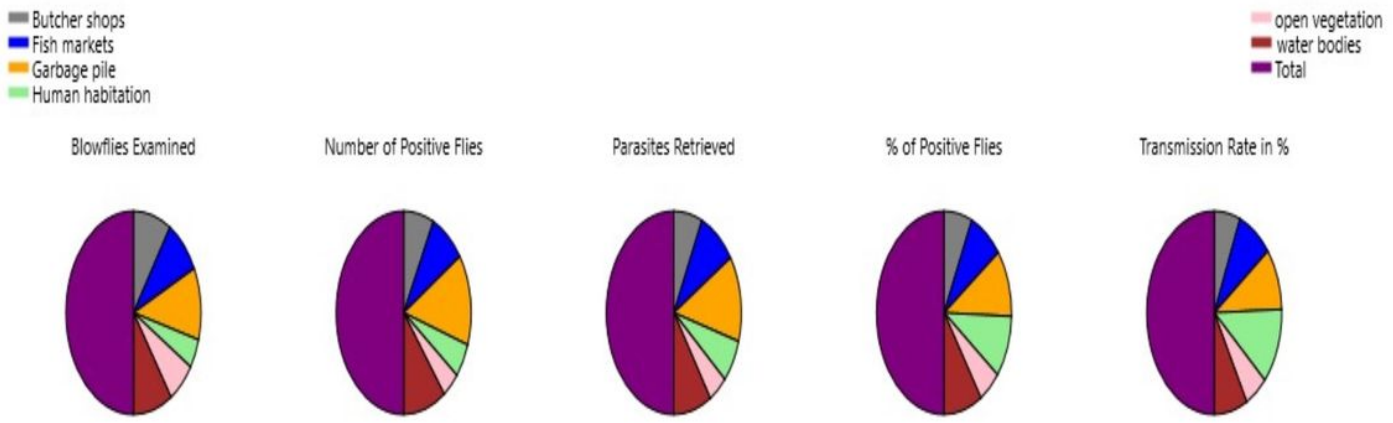

Figure 1

Isolation rate of parasitic cyst/eggs and their transmission rate among the selected collection sites across the Kashmir Himalaya, India.

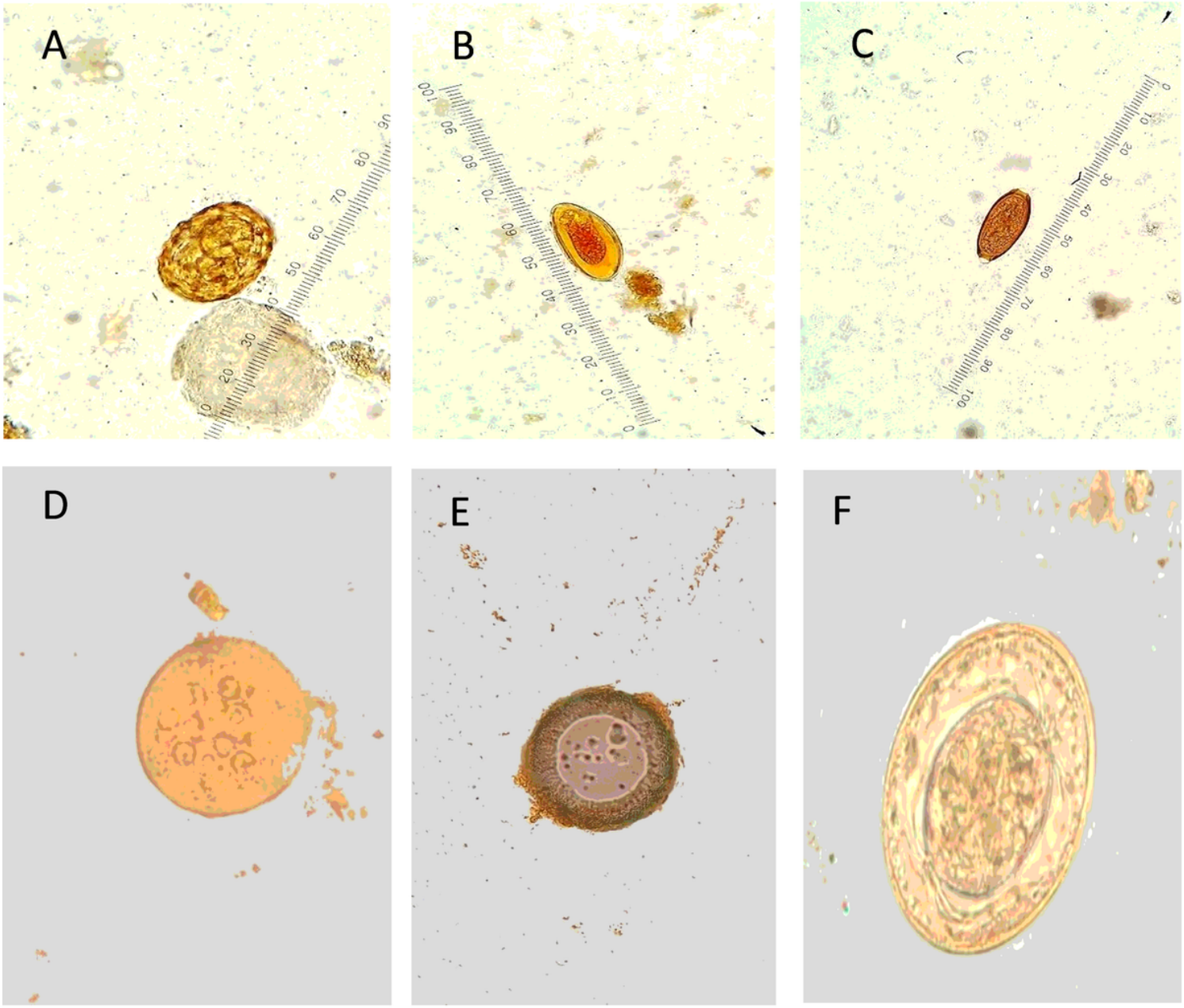

Figure 2

Cyst and eggs of certain parasites isolated from the surface of the blowflies: A. *Fasciola hepatica*; B. *Enterobius vermicularis*; C. *Trichuris trichuria*; D. *Entamoeba coli*; E. *Taenia saginita*; F. *Hymenolepis nana*

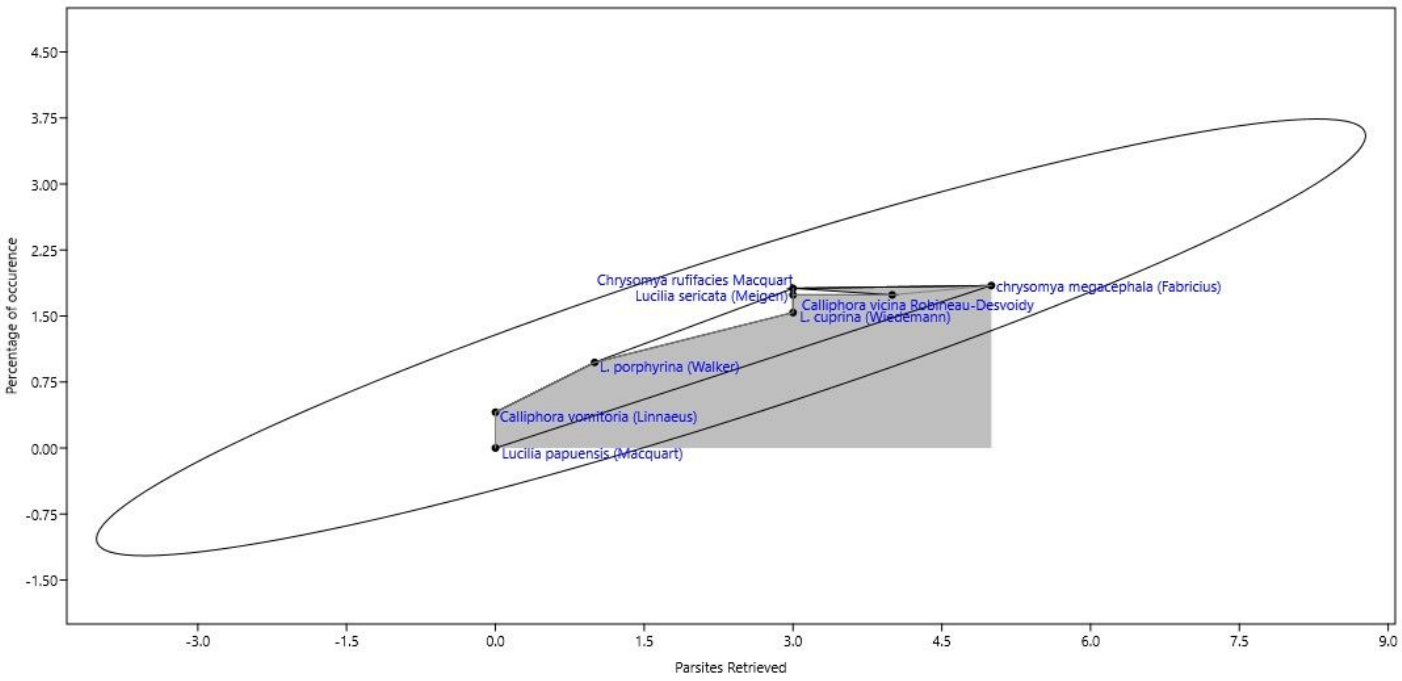

Figure 3

Percentage of occurrence of parasites among different species of calliphorids