Effects of peri-mortem infection on the entomofauna of decomposing buried human remains – a metadata analysis.

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Abstract

The role of infectious disease as a cause of death is undeniable. The affect infectious disease may have on decomposition after death is less well established. Furthermore, virtually no information is available regarding the effects of burial conditions in such circumstances, despite that numerous clandestine burials occur each year. Although many aspects of post-mortem pathology are well understood and provide frequent insight in medicolegal investigation, where buried bodies are concerned, there is great variation in the decomposition processes, depending on extrinsic and intrinsic conditions.

Criminal burials and hurriedly dug clandestine graves are seldom deeper than 120cm allowing access to certain invertebrates, excluding others that only develop in unburied bodies. Numerous studies have reported on such clandestine graves with a purpose to facilitate forensic investigation, but our knowledge of decomposition in deeper graves lags behind, despite several often-cited papers of over a century ago.

The poor level of detail in deep-grave knowledge is in part due to resource deficiencies and ethical considerations, but in part due to lack of thorough investigation of

the data in papers of often cited prior work. To this end, a metadata analysis assessed a paper written by Dr Murray Galt Motter in 1898, providing detail of 150 disinterment events with linked medical records from City of Washington cemeteries. This paper, written more than a hundred years ago, was largely descriptive and the detailed data provided in a summary table were never fully analysed. The paper is often quoted despite these obvious oversights. The present study revisits this work, applying a frequency statistical analysis conducted using categorical data and chi-squared analysis. This new analysis reveals patterns and relationships so long 'locked-up' within the body of the table and provides greater understanding of the ef- fect of infectious disease on the abundance of species in the entomofauna associat- ed with deeply buried remains.

The data confirm that the presence of adipocere (saponification) is detrimental to development of soil entomofauna (($X^2 = 6{\cdot}64$, $df = 1$, $p<0{\cdot}01$)). Some species, in particular *Proisotoma sepulcralis* (Collembola), *Eleusis pallida* (Coleoptera) and *Conicera tibialis* (Diptera), were positively influenced by association with infectious disease cases ($p<0{\cdot}01$) while only *Piophila casei* (Diptera) demonstrated a negative association ($p<0{\cdot}05$).

Furthermore, the presence of peri-mortem infectious disease, while not necessarily a cause of death, influences post-mortem colonisation of the buried body by insects. The abundance of some species is enhanced, suggesting that bacterial burdens enhance decomposition in a manner favourable to insect feeding and hence abundance, by releasing compounds that the entomofauna feeds on.

Keywords: peri-mortem infection; deep-grave burial; post-mortem interval

Introduction

Infectious diseases, as distinguished from zoonotic diseases and non-communicable diseases (or NCDs) are caused by pathogenic microorganisms (bacteria, viruses, parasites or fungi) and are spread, directly or indirectly, from one person to another [1]. Peri-mortem infectious diseases are those present about the time of death, but are not necessarily the cause of death. The undeniable role of infectious disease as a cause of death is especially notable at times of trauma, such as post-operative crisis. Indeed, during a four-year study of 22,742 in-patient surgical procedures at Durham Regional Hospital and Duke University Medical Centre, 255 post-operative patients having surgical-site infections (SSIs) were examined and of those 7.8% died during initial postoperative hospitalisation [2]. Furthermore, the study concluded that patients who develop SSI have longer and costlier hospitalisations than patients who do not develop such infections, are twice as likely to die, are 60% more likely to spend time in ICU, and are more than five times more likely to be readmitted to the hospital [2]. Likewise, during examination of 3,754 combat-related deaths, 129 (3.3%) died of wounds after evacuation from a combat theatre and of those, in 55 cases (or 44% of evacuees), death was partially attributable to an infectious process (e.g., sepsis, pneumonia, wound infection) [3].

In low income countries, the predominant cause of death is from infectious diseases and the risk of acquiring these varies greatly depending on socioeconomic determinants such as poverty and environmental conditions [4]. Despite the direct link to poverty, and despite efforts to diminish the relative number of deaths caused by infectious diseases, nowhere in the world have these yet become a negligible cause of death [5].

From a post-mortem medicolegal perspective, the role of pathogens inherent in the deceased is important in firstly understanding possible cause of death, but secondly, because it is proposed here that it might affect estimating time of death. Estimations of time of death diminish in precision the longer the period post-mortem, and unfortunately few methods offer particularly good reliability or accuracy [6]. As decomposition progresses, estimation of death at autopsy gradually becomes replaced by other methods, including forensic entomology (the application of the study of insects to legal matters). Forensic entomological methods of post-mortem estimation rely on several theories, such as succession of necrophagous species or numerical evaluation of life cycle stages. A thorough knowledge of species involved and their respective behaviours is vital. Forensic entomologists frequently examine unburied human remains, but on occasion, buried remains are examined, although such cases largely are restricted to legal investigation or experimental studies at human taphonomic research facilities.

Examination of buried human remains and the associated entomofauna is infrequently encountered and opportunities to evaluate these are rare. Useful data embedded in an often-quoted paper by Dr Murray Galt Motter, published in 1898 [7], a period of early development of forensic entomology, have largely gone un-analysed for over a century, because the paper is largely descriptive. A metadata analysis of the detailed tables supplied in Motter's paper forms the foundation of the research results discussed in the current paper. Numerous papers since Motter's have dealt with insects associated with burial in various conditions and durations asserting that the larvae of some species burrow down to the body, but seldom more than 0.5 to 0.6m [8-17]. A particular concern with some of these studies is that they focus on surrogate carrion and may, in reality, tell us little about what happens in the human body. Nonetheless, carrion ecology aids and assists work on human remains by way of back-ground or benchmarking data. While most burial papers deal with hurriedly dug clandestine graves, seldom deeper than 120cm [18], there is clearly also importance in understanding terrestrial sub-soil invertebrates (the entomofauna) of graves deeper than this for forensic purposes, especially where exhumation is required during the re-opening of prior cases for insurance purposes and evaluation of mass graves [12,15]. It is noteworthy that decomposition in clandestine graves without a coffin, may differ from that in deeply dug graves with a coffin [19]. In addition, with a revival in recent years for 'natural burials' in which little or no chemical preservation is used and which frequently use biodegradable coffins and no burial vault, there may be implications for correct recording of infectious disease persistent at death. It is in the deep-grave (clandestine or natural) context that the current research results are embedded.

Specifically, this paper sets out to analyse the possible association between perimortem infectious disease and the entomofauna found in graves of more than one metre depth, using Motter's 1898 data [7]. These data report, *inter alia*, the conditions at burial and the entomofauna found during the exhumation of 150 graves in city of Washington cemeteries. As explained in the methods these data were benchmarked against Motter's presence/absence data for adipocere [7], on the understanding that adipocere generally excludes insects from a deceased body. The formation of adipocere (grave-wax or saponification) is a progression of neutral adipose fat and intrinsic lipases in a decomposition process (possibly driven largely by microbial activity) by which triglycerides degrade into fatty acids by hydrolysis and hydrogenation [19-22].

As a result of Motter's findings [7] and the elaboration there-upon via this metadata analysis, extrinsic conditions concerning whether or not the body was buried in a coffin (wooden or other), in a sealed crypt, or placed directly in the ground, what type of soil, pH and how much moisture there was, are clearly very relevant in the assessment of post-mortem interval in buried conditions using an entomofauna analysis.

Methods

A metadata analysis for the abundance of species within the entomofauna associated with grave-depth and the inherent conditions associated with each case, was carried out in a Microsoft Excel spreadsheet and SPSS v22.0.0 under licence to Bournemouth University, using Motter's previously un-analysed 1898 dataset [7] as a platform. Over the intervening century, taxonomic changes have demanded a detailed update of taxa encountered according to modern species catalogues [23]. For the sake of brevity, species analysed in this research are referred to by the short binomen in the text of this paper; the full currently valid binomen is included in Table 1.

One of the shortcomings of Motter's (1898) publication is that his summary table presents sample codes instead of exact numbers of specimens, while in the text, the dialogue was generalised rather than being numerical [7]. Consequently the tables require interpretation by cross-reference with the listed content in Motter's paper, which may account for why this particular publication has received so little attention. Accordingly, initial assessment was carried out using a scale as follows:

1. single specimen mentioned, or taxon mentioned in the singular

2. multiple specimens designated as 2 or taxon mentioned in the plural (2 or -*ae*)

3. ‘a few’ specimens mentioned (3 - 5)

4. ‘numerous’ specimens mentioned (6 - multiples of ten)

5. ‘myriads’ of specimens mentioned (100+)

It is likely that items assigned to categories two, three and four overlap to some extent, because the term ‘pupae’ (for example) could refer to two or a few or numerous specimens. Therefore, unless otherwise specified, simple plural terms were conservatively allocated to category 2.

Values were summed for each species to provide relative frequency data, from which a shortened species list was compiled including all species having a relative abundance greater than one. Nonetheless, the evaluations used in this analysis were made by cross reference to Motter's 1898 text [7], which carries explanations of the collected material.

Another shortcoming of Motter’s data was that some species listed in the taxonomic list, didn’t appear numerically in the graves-list and were consequently considered unquantifiable and excluded from the analysis. Accordingly, only species that could be assessed categorically were included and most of the eliminations were in any respect adventitious species having little to do with decomposition. Oligochaetes and undetermined specimens were removed from the analysis.

In his paper, Motter annotated the medical conditions present in the deceased at the time of death [7]. Although these in no way suggest cause of death, it was considered possible that the presence of an infectious condition may have some bearing on composition of the microbial and entomological fauna during decomposition.

Many old fashioned medical terms were used in the original data, describing conditions now better understood and/or known by another name. So as to make the analysis meaningful in modern medical terms, each of Motter's listed ailments was redefined [24] as follows: 1. No condition listed; 2. Accidental death; 3. Cardiovascular disease (CVD); 4. Cerebrovascular conditions (CVA (=Apoplexy), Cerebral congestion, Cerebral embolism (CAGE)); 5. Hepatic colic and Obstruction of the bowls; 6. Hypothermia; 7. Infectious diseases (Bronchitis, Cholera, Diarrhoea, Diphtheria, Malaria (=Intermittent fever), Pertussis, Pleuritis, Pneumonia, Tetanus, Tuberculosis (=Consumption, Phthisis), Typhoid); 8. Kidney disease (Nephritis, Uraemia and Bright's disease); 9. Malnutrition and Starvation; 10. Meningitis and Hydrocephalus; 11. Neurological conditions (Epilepsy (=Convulsions)); 12. Non-communicable inflammatory infections (Dysentery, Enteritis, Epysipelas (Cellulitis), Gastritis, Hepatic abscess, Membranous croup, Peritonitis); 13. Respiratory (non-infectious): Oedema and Hemorrhage of the lungs; 14. Senility (meaning old age); 15. Still-born and Infant deaths.

Following this, the modern conditions were categorised into associated conditions and then numerically coded for further analysis. Generally undefined conditions that arise from multiple illnesses or which are symptomatic were henceforth eliminated, leaving those conditions arising from infection (commutable and non-commutable) and those conditions not associated with infection.

By analysing Motter's presence/absence data for adipocere using a simple chi-squared test, the dataset could be benchmarked, prior to analysing the infection data in greater depth and without further need for data manipulation. Given that very little is known about deep burial faunae, and given that the data we have access to is historic, we could only be confident of the results of the meta-analysis for the deep burial fauna, if we knew other details matched known findings for other parameters. The analysis of adipocere enabled that confidence.

Further analyses were carried out on a presence/absence basis, to eliminate gross errors possible by the above categorisation. Chi-squared analysis in SPSS was used to determine independence between species and attributes; replaced by Fisher's exact test with Monte Carlo estimation where low expected values demand a more rigorous level of testing. Results were expressed at 95% or in some instances, 99% significance.

Results

Of the 150 graves disinterred during Motter's 1898 study, 123 included grave fauna amounting to approximately 74 invertebrate taxa (including some undetermined to species). The most informative data come from thirteen species (Table 1) representing taxa encountered in 5 or more disinterments (relative frequency >1), representing the top 70.2% relative abundance. The remaining species represent 30% of the overall abundance and consist of species encountered in very low numbers and of little interest in the decomposition sense, many being adventitious.

Overall, for most species the presence of adipocere is a deterrent ($F = 21{\cdot}75$, $p<0{\cdot}05$), with some species completely absent where adipocere is present (Figure 1). Motter made some interesting observations: beetles, and sometimes Collembola, were occasionally found working inside the bones, entering through the nutrient canals etc. and were sometimes found within layers of adipocere or between bone and adipocere [7]. Besides these notable exceptions, insects are significantly deterred by the presence of adipocere.

Specifically, for all but two species, adipocere acted as a barrier to colonisation (X^2 = 6·64, *df* = 1, *p*<0·01) resulting in significantly fewer numbers compared to graves lacking adipocere. There was no significant difference in the abundance of the spiders *Cicurina brevis* and *Eidmannella pallida*, although the low abundance in the sample nevertheless makes them marginal to the analysis, on top of which they are predatory and hence play no role in the actual process of decomposition and are as likely as not to crawl across the surface of adipocere, i.e. the substance is inconsequential to their activities, whereas it is not insubstantial as a barrier to decomposers.

Having established that the adipocere data provided by Motter, agrees with the notion that, for the most part, adipocere inhibits insect activity in the grave, there is greater confidence in the results obtained for the analysis of infectious disease.

Types of ailment were tested in several ways, in the first instance according to presence or absence of an ailment of any kind, but secondly and most importantly, according to whether or not an ailment was infectious or not. A significant difference (*F* =27·00, *p*<0·01) was obtained for the hypothesis that the presence of some ailment prior to death has an effect on community composition and further analysis found that eight of the 13 most abundant species were significantly (*p*<0·01) more abundant where some ailment was noted prior to death (Figure 2).

Specific ailments were then analysed to determine effects on the community structure of the soil dwelling entomofauna, with a significant result (*F* = 258·09, *p*<0·01) demonstrating that infectious diseases and infant deaths (which may include respiratory infection) were significantly more likely to enhance the abundance of soil dwelling entomofauna than other categories of illness (Figure 3). Of particular note, *Piophila casei* demonstrated a significantly negative association with non-infectious cases (p<0.05). In other words, where non-infectious disease occurred prior to death, *P. casei* was absent.

Association with infectious disease was strongly significant (p<0·001). In particular, *Proisotoma sepulcralis* , *Eleusis pallida* and *Conicera tibialis* were significantly (p<0·01) more abundant in burials where infectious disease was noted (Figure 4). Less obviously abundant, but nevertheless still significant (p<0·01) were *Julus* sp. and *P. casei*. The remaining species were either negatively associated or returned a non-significant result. In addition, those cases listed as having peri-mortem respiratory disease, also peaked significantly (p<0·01) for *P. sepulcralis* and *C. tibialis* (but not for *Eleusis pallida*).

Curiously, only *C. tibialis* demonstrated a significant (p<0·01) increase in abundance in cases associated with still-born and infant deaths. While we cannot turn back time to assess the underlying cause of death, it could be assumed that at least some of these were infectious cases.

Noticeably and not unexpectedly, the two spider species (*C. brevis* and *Eidmannella pallida*) and the calliphorid fly *Cochliomyia macellaria*) are not only present in low abundance (Table 1), but also demonstrate no significant change in abundance when either a peri-mortem ailment or an infectious disease is present. Conversely, *P. sepulcralis* (Collembola), *Eleusis pallida* (Coleoptera) and *C. tibialis* (Diptera) demonstrate a negative association with cases for which no ailment is listed and a positive association with infectious disease cases (p<0·01)

Discussion

Evaluating to what extent peri-mortem conditions affect rates of decay and hence estimation of time of death is well established [6,25]. Furthermore, peri-mortem *treatment* of a pre-existing condition affects the rate of decay and this has significant bearing on medico-legal estimation of time since death of decomposing human remains [26]. Taking this one step further, it is clearly equally important to understand how peri-mortem disease affects rates of decay. Given the predominant accelerative role played by insects associated with decay on the surface, it follows that understanding the entomofauna associated with deep-graves may provide post-mortem evidence, especially with respect to post-mortem interval and especially if peri-mortem disease enhances abundance of the soil associated within the grave context. Thus, the understanding that decomposition is slowed by burial in coffin-style graves at depth in comparison to clandestine graves in soil with no coffin [19], needs careful evaluation.

The research reported here provides clear evidence that the presence of peri-mortem infection enhances certain entomofaunal interactions. Consideration is therefore required in exhumation investigations where the presence of infectious disease is known, as the enhanced entomofaunal interactions will accelerate decomposition above that of a grave situation lacking infectious disease. That is, as with surface decomposition and shallow clandestine surface graves, not all burial decomposition occurs at the same rate and the rate of decomposition is dependent of multiple variables.

With the possible exception of members of the genus *Dissochaetus* Reitter 1885 (Coleoptera, Cholevidae), the presence of adipocere (or grave-wax) is known to result in the exclusion of insects on a deceased body [27]. The results of the entomofauna associated with the presence/absence of adipocere noted in Motter's data, confirmed the general concept of exclusion of insects by adipocere, with the obvious caveat that he also observed beetles (undetermined) burrowing between bone and layers of adipocere.

Of particular note, the significant associations of *Proisotoma sepulcralis* (Collembola), *Eleusis pallida* (Coleoptera) and *Conicera tibialis* (Diptera) with infectious disease cases ($p<0{\cdot}01$) links well with the forensic post-mortem necrophagous community and these species may be considered potential indicators for investigating post-mortem interval under these conditions. Of particular interest are the long periods of isolation recorded for post-mortem necrophagous communities in deep-dug graves. Massed ("thousands of specimens") Collembola (*Sinella (Coecobrya) tenebricosa* Folsom, 1902 (Entomobryidae)) and an unknown number of *C. tibialis* were recovered from an embalmed exhumation after 28 years from a 1.8m deep unsealed casket in an unsealed concrete vault, in Battle Creek Michigan [28]. In another example, *C. tibialis* was recovered in large numbers (unspecified) from an exhumation from a 2m burial at Guadalajara in Spain after 18 years [29].

It is already known that intrinsic bacterial flora in the alimentary canal provides a key source of bacteria in the initial stages of decomposition resulting in putrefaction of tissue and that ante-mortem injury or trauma (e.g. damage to the skin, laceration, etc.) result in increased decomposition by allowing agents of decay (bacteria, insects) better access [19]. While, decomposition is accelerated in deaths from infectious diseases [30], it is also decelerated in deep burials by reduced temperature profiles and anaerobic conditions, which in turn result from decreased gaseous diffusion and hence increased CO_2 concentration [19]. An increase in decomposition rate for bodies where peri-mortem disease was implicated (compared to those not associated with peri-mortem disease) is a result of the combination of increased ante-mortem bacterial load, pre-existence of bacteria in the blood and organs, and the likelihood of an elevated temperature at the time of death mitigated against by duration of exposure prior to burial. These same post-mortem attributes also enhance insect activity and the conclusion drawn from the current analysis is that peri-mortem infection not only affects decomposition processes, but it demonstrably enhances insect activity, hence accelerating the decomposition rate. This has potential complications for estimations of post-mortem analysis in exhumations and should clearly be accounted for during such investigations. This is especially relevant given our previous understanding that lower temperatures due to burial depth, subsequent decreased gaseous diffusion and hence increased CO_2 concentration with associated anaerobic conditions, slowed decomposition rates.

Alteration of decomposition rate and subsequent post-mortem assessments are common-place and careful assessment of conditions are required during post-mortem estimation. In the case of burial, and perhaps this also applies to surface cadavers, the status of peri-mortem infection has great relevance in time of death assessments.

Conclusion

The paper demonstrates quite clearly that the presence of peri-mortem infection does significantly affect the decompositional entomofauna in deep grave burial.

While deep-grave conditions are seldom of forensic interest, there are occasions (such as exhumations of war graves or civil enquiries) where thorough knowledge of the interactions below ground need to be clearly understood and not assumed (as is presently the case). Furthermore, there is increased popularity for natural burials, in which the absence of preserving chemicals in the presence of peri-mortem infection may be of critical concern - this may well result in medicolegal concerns. That certain species are positively affected by peri-mortem infection means that we can harness knowledge about these indicator species and use that to determine presence or absence of infection or make necessary adjustments to estimated post-mortem intervals.

Table 1. Relative frequency of occurrence of grave faunas

Binomen	Family	Higher taxonomy	Insta
Proisotoma sepulcralis (Folsom, JW, 1902)	Isotomidae	Collembola	7
Eleusis pallida LeConte 1863	Staphylinidae, Osoriinae	Coleoptera	5
Conicera tibialis Schmitz, 1925 puparia	Phoridae	Diptera	4
Uropoda depressa Banks in Motter 1898	Parasitidae	Acarina	2
Actobius umbripennis LeConte 1863	Staphylinidae, Staphylininae	Coleoptera	1
Julus sp.	Julidae	Myriapoda	1
Virgoiulus minutus (Brandt, 1841)	Blaniulidae	Myriapoda	1
Helicodiscus parallelus (Say, 1821)	Gastropoda	Mollusca	1
Piophila casei (Linnaeus, 1758) puparia	Piophilidae	Diptera	1
Rhizophagus sculpturatus Mannerheim, 1842	Monotomidae	Coleoptera	
Eidmannella pallida (Emerton, 1875)	Araneae	Arachnida	
Cicurina brevis (Emerton, 1890)	Araneae	Arachnida	
Cochliomyia macellaria puparia	Calliphoridae	Diptera	

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Highlights

- Peri-mortem infection significantly affects decompositional of buried cadavers
- Entomofauna of buried cadavers is significantly affected by Peri-mortem infection
- Abundance of some Collembola, Coleoptera and Diptera increases with infectious burial
- These indicator species show potential for investigating post-mortem interval
- Presence or absence of peri-mortem infection can be determined post-mortem

Declaration of interests: There are no competing interests to declare.

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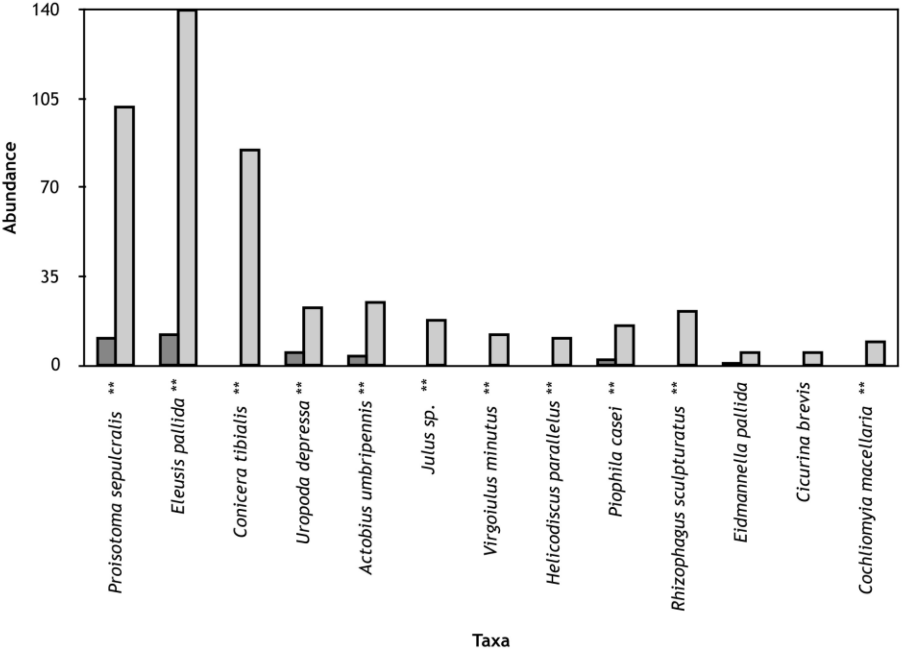
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Abundance
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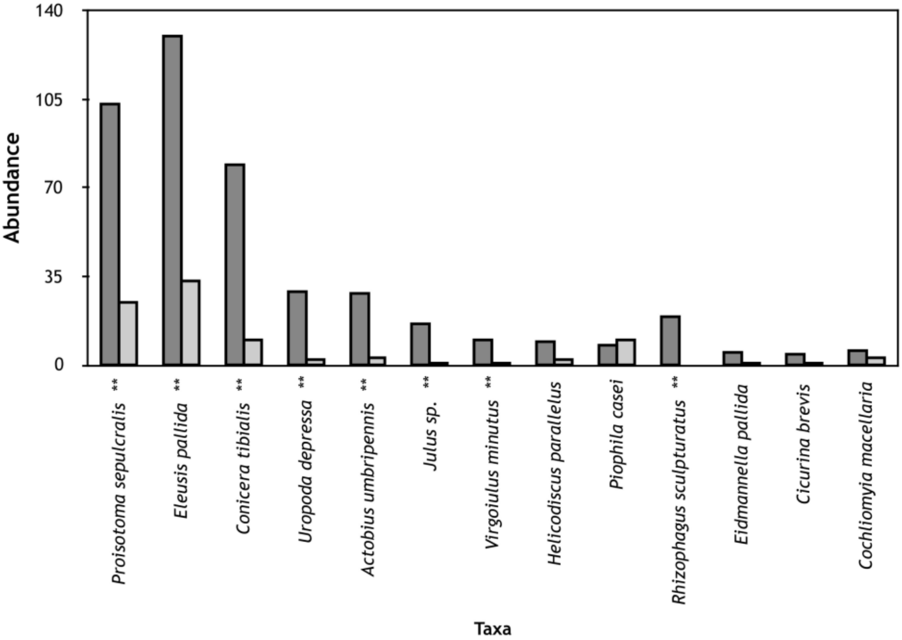
Eleusis pallida **
Conicera tibialis **
Uropoda depressa **
Actobius umbripennis **
Julus sp. **
Virgoiulus minutus **
Helicodiscus parallelus **
Piophila casei **
Rhizophagus sculpturatus **
Eidmannella pallida
Cicurina brevis
Cochliomyia macellaria **
Taxa

Figure 1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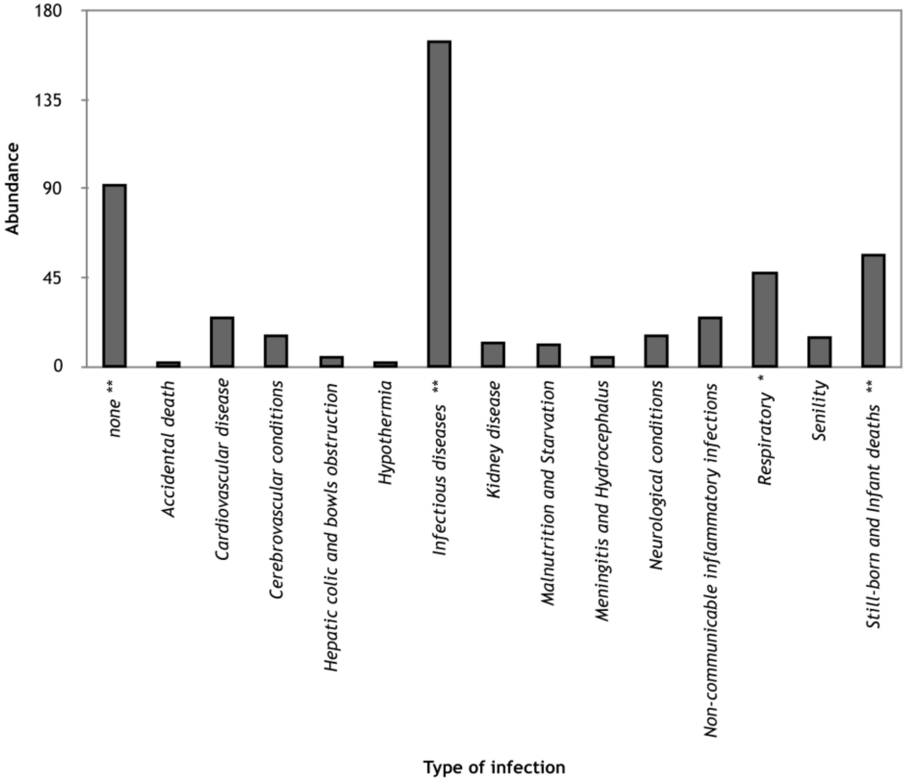
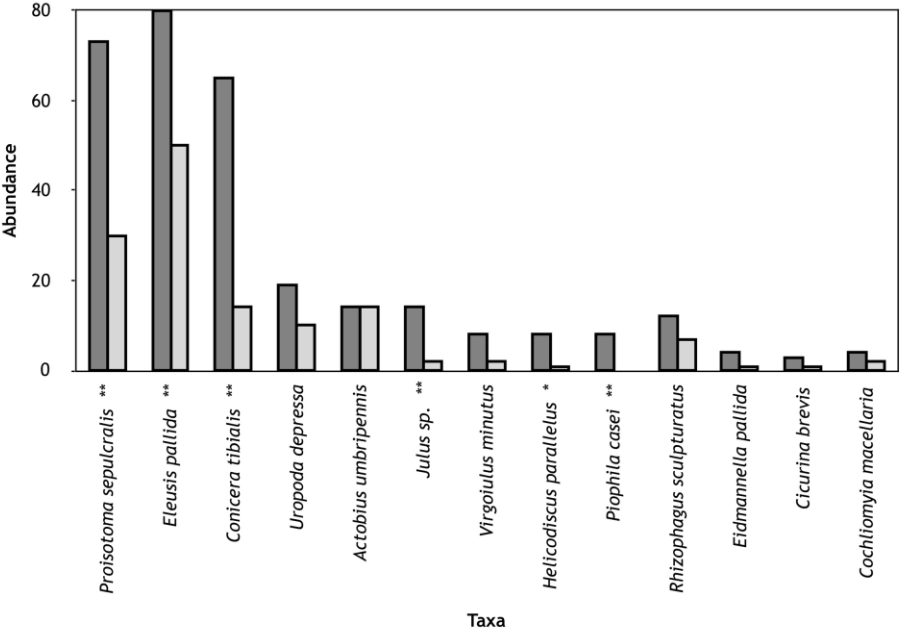
Rhizophagus sculpturatus **
Eidmannella pallida
Cicurina brevis
Cochliomyia macellaria
Taxa

Figure 2



180
135
90
45
0
Abundance
none **
Accidental death
Cardiovascular disease
Cerebrovascular conditions
Hepatic colic and bowls obstruction
Hypothermia
Infectious diseases **
Kidney disease
Malnutrition and Starvation
Meningitis and Hydrocephalus
Neurological conditions
Non-communicable inflammatory infections
Respiratory *
Senility
Still-born and Infant deaths **
Type of infection

Figure 3



80
60
40
20
0
Abundance
Proisotoma sepulcralis **
Eleusis pallida **
Conicera tibialis **
Uropoda depressa
Actobius umbripennis
Julus sp. **
Virgoiulus minutus
Helicodiscus parallelus *
Piophila casei **
Rhizophagus sculpturatus
Eidmannella pallida
Cicurina brevis
Cochliomyia macellaria
Taxa

Figure 4