Ectoparasites burden of House mouse (Mus musculus, 1758) from Hai’l region, Kingdom of Saudi Arabia

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ABSTRACT
The House mice (Mus musculus Linnaeus, 1758), play an important role in the transmission of diseases, both in humans and livestock, through ectoparasite carried on their feces, urine and hair remnants. The purpose of this study was to investigate the ectoparasites infestation, as well as their quantitative and qualitative abundance and, prevalence in the house mice captured from Hai’l region, Kingdom of Saudi Arabia (KSA). Parasitological investigations were performed on 70 house mice trapped during 2012–2013 from two localities (Hai’l City residential area and Al-Khitah agricultural farm habitats in Hai’l region). Captured mice were identified as males (34.3% and 48.6%) and females (65.7% and 51.4%) from the residential and agricultural farm habitats, respectively. The findings of the study showed that the sex ratio of the mice found in different habitats did not influence the level of ectoparasite infestation (P > 0.05). Therefore, we combined only sex-wise samples for each habitat and isolated habitats treated separately for our subsequent analyses. A total of 514 ectoparasites individuals belong to four species were recovered from the mice, which included 339 of flea (Xenopsylla cheopis Rothschild, 1903), 39 of sucking lice (Polyplax spinulosa Burmeister, 1835), 37 of sucking lice Polyplax serrata Burmeister, 1839), and 99 of mite species (Laelaps echidninus Berlese, 1887).

The presence of zoonotic parasites indicates that Mus musculus as a reservoir, might represent a danger to the public health particularly in the two sampled areas. Results also suggest an increasingly need for further studies to assess the role of the ectoparasites of house mice and their possible involvement in transmission of diseases among these areas.

1. Introduction

Arthropod ectoparasites are organisms that inhabit another organism’s skin or outgrowths (the host) for time periods (Wall and Shearer, 2008). Most species of ectoparasites are invertebrates belong to Arachnida and Insecta classes (Wall and Shearer, 2008). The former class essentially includes mites and ticks while the latter one contains lice and parasitic fleas. The life cycles of mites, ticks, and fleas which include four stages (eggs, larva, nymph and adults) whereas lice life cycle include only three stages (eggs, nymph and adults) that can live between 21 days and 28, over a year (Dryden, 1993; Norval and Horak, 2004). Many of these ectoparasites are host-specific species (mostly lice) while others (as numerous ticks) parasitize a wide range of mammalian hosts species (Hamid, 2016). Nevertheless, mammalian parasites are either ectoparasites (for example ticks, fleas, mites and lice) or endoparasites such as numerous intestinal parasites (e.g., nematodes, cestodes, and trematodes).

Rodents are known to carry a variety of parasites that can cause diseases for both humans and some animals species (Beck and Folster-Holst, 2009; Meerburg et al., 2009), through contamination of food with their feces, urine and hair remnants (Durden and Page, 1991). They also serve as reservoirs for different forms of viruses, bacteria and helminths which cause zoonotic diseases, such as rodent-borne hemorrhagic fever, swamp fever, salmonellosis, typhus, plague, Lyme disease, toxoplasmosis, schistosomiasis, nematodes and tapeworms (Davis et al., 2002; Brown, 2004; Villafane et al., 2008; Meerburg et al., 2009; Rafique et al., 2009; Worldbank, 2010).
Other zoonoses include rat bite fever, relapse fever, monkey pox and the mouse mammary tumor virus (MMTV) which may lead to breast cancer in humans (Indik et al., 2005). Perhaps the most well know zoonotic disease in history is plague or “Black Death”, associated with rodents and other mammalian species (Davis et al., 2002; Adjemian et al. 2007; Worldbank, 2010). The close association between rodents, humans and livestock therefore constitutes a risk factor for the transmission of these diseases (Worldbank, 2010).

The house mice (Mus musculus Linnaeus, 1758), are small species of mammal belong to the order Rodentia with a very wide distribution. They are getting benefits from living in humans settlement, farms, outbuildings, stores and other structures. Therefore, house mice were generally considered as sources for various pathogens responsible for significant human morbidity and mortality in urban centers around the world (Adjemian et al., 2007; Singleton et al., 2007; Kia et al., 2009; Allymehr et al., 2012; Roble et al., 2012; Hayashimoto et al., 2014).

Locally, house mice are found throughout the Kingdom of Saudi Arabia in habitats ranging from semi-arid, arid, mountain and sand-dune habitats (Harrison, 1972; Buttiker and Harrison, 1982; Musser and Carleton, 2005). Harrison (1972) and Buttiker and Harrison (1982) reported about 15 rodent species inhabit the Kingdom of Saudi Arabia. Some of them are commensal species such as the roof rat (Rattus rattus), the house mouse (Mus musculus) and the Norway or brown rat (Rattus norvegicus). Al-Khalili (1984) captured five wild rodents species which include Acomys dimidiatu homericus, Gerbillus dasyurus, Praomys formats Yemeni, Eliomys melanurus and Meriones Rex buries, in the south-west of Saudi Arabia. Additionally, Al-Rajhi et al. (1993) and El-Bahrawy and Al-Dakhil (1993) conducted other rodent surveys in Riyadh City and Hanifah Valley, and reported Rattus rattus rattus, Acomys dimidiatu, Meriones libycus, R. rattus frugivorous, R. rattus alexandrines and Mus musculus.

House mice were not mentioned in most conducted studies on rodent populations (El-Bahrawy and Al-Dakhil, 1993; Morsy et al., 1994, Al-Ahmed and Al-Dawood, 2001). However, it is an urgent of any region to record and catalogue the ectoparasites of house mice to understand risks of infection due to the parasites intensity, prevalence and distribution as well as understanding the factors that modify zoonotic pathogen prevalence among wild and/or commensal populations to prevent human infections.

The purpose of this study was to investigate the ectoparasites infestation, as well as their quantitative and qualitative abundance and prevalence in the house mice from Hai’l region, Kingdom of Saudi Arabia. The research specific objectives were to survey and determine the infestation and prevalence of ectoparasites , particularly on house mice, to predict the type of ectoparasites, and identify gaps in our knowledge which may lead to a better understanding on the epidemiology and pathogenesis of related diseases.

2. Materials and methods

2.1. Study area

The study was conducted within the region of Hai’l which is located between Latitude 25° 16’ 34” and 28° 53’ 16” N and Longitude 39° 26’ 52” and 44° 22’ 42” E, Kingdom of Saudi Arabia (Fig. 1).

Two sites were select (Marshal, 1981; Zahedi et al., 1984) ed: Hai’l City Habitat, Residential Areas (27° 06’ N, 41° 31’ E) and
Al-Khitah Agricultural Habitat, sub-urban/cultivated Areas (27° 59’ N, 40° 58’ E). Rodents were captured from March 2012 to December 2013 using the wire-box live traps (Fig. 2).

Active burrows and drops were recorded at targeted habitats that represent most common signs of rodents activity (Jones et al., 1996; Zeller et al., 2001). Live traps were baited with cucumbers, potatoes, carrot, tomatoes, or onions, and then placed near the rodent burrows before dusk and collected after dawn.

During the study period the mice trappings were performed on a monthly basis for three consecutive days as one hundred and twenty live traps were set randomly at the farm habitats and urban sites, respectively.

In each farm, the sampling grid consisted of 20 traps placed at 10 m intervals along 5 transect lines and then moved to another location in the same site until the end of the observation. The captured rodents were examined for Mus musculus identification by morphological measurements and physical appearances, according to Musser and Carleton (2005). They were then transported to the Biology Department, University of Hai'l for further investigations.

Animal trapping and handling were carried out in compliance with the Ethical Committee of the University of Hai'l. For each specimen, the following parameters were reported: capturing position, sex, body mass (using a digital balance, Milton UK balances), and external morphometric measurements (using a digital vernier caliper).

2.2. Collection of ectoparasites from hosts

Mice were individually anesthetized in a jar containing a cotton pad moistened with chloroform. Then brushed in a deep white plate containing 70% ethyl alcohol to dislodge all ectoparasites as far as possible, using a relatively hard brush. Ectoparasites were then extracted with forceps while the pelage and skin were examined carefully. The preceding procedure was repeated several times until all ectoparasites were fully eliminated from the entire body of the host. Furthermore, the jar plates were examined for any residual ectoparasites that were subsequently examined by a compound binocular microscope (Leitz, Germany). The ectoparasites were then sorted using a finer scale brush, positioned for each group in a separate container and stored in labeled specimen tubes containing 70% alcohol waiting to processed further.

Fleas, and lice were mounted later in Hoyer’s medium (gum arabica 30 gm, chloralhydrate 200 gm, glycerine 20 cc, and water 500 cc, whereas mites were stored in Oudeman’s fluid (mixture of 85 attributes of 70% alcohol, 5% from each glycerin and glacial acetic acid). Subsequently, under the microscope, specimens were identified from the prepared slides, its numbers were calculated and photographs were taken by using a wild MPS 11 camera, mounted on a leitz 20 dialux microscope (Leitz Wetzlar, Germany). Morphological identifications of ectoparasite specimens were performed using the standard identification keys for each group (i.e, fleas, mites and lice) by Chandler and Read (1961). The ectoparasites and hosts were collected and stored as voucher specimens at Biology Department, Faculty of Science, Hai'l University, KSA.

2.3. Data analyses

Data was coded and computerized by using Statistical Sciences for the Social Sciences (SPSS) version 19.2 for windows. Descriptive statistics have been calculated for the host and ectoparasite to quantify the host and ectoparasites infestation patterns per the preferable mice’ sex, and sample/population by using hosts’ infection as covariate.

According to Bush et al. (1997), Rozsa et al. (2000), and Krasnov et al. (2002), the following terms and quantitative parameters have been adopted: number of infected animals (n), total number of parasites (N), total number of specific parasite (Ni), and total number of infected and non-infected animals (Z). The following indexes and parameters were cacuated for each parasite: Mean Intensity (MI) = the number of a specific parasite living on an infected host; Mean Abundance (MA) = the number of a particular parasite species living per any host (includes infected as well as uninfected host); Prevalence or percent of infestation (P) = the number of host infected with specific parasites divided by a number of the tested host, and usually represented by a percentage (Bush et al., 1997). As well as, Constituent Ratio (C) = the total number of specific parasite (Ni) divided by the total number of parasites (N) that multiply by 100; and Infection Index (I) = the total number of parasites (N) multiply by the ratio of the number of infected hosts (n) to square root of all the captured animals (Z2).

Therefore, the prevalence, indices, mean density and mean intensity of parasites distribution are suitable descriptors to quantify parasites in a sample host. Subsequently, general ectoparasites prevalence and intensity were compared across two isolated habitats (Hai’l City residential areas and Al-Khitah agricultural farms) by a t-test. Additionally, total ectoparasites, fleas, sucking lice and mesostigmatid/gamasid mites were assessed using Spearman correlation (Ludwig and Reynolds, 1988) to test for the abundance differences between the two habitats and any possible differential patterns. Also, ANOVA was used to recognize how the different ectoparasite groups respond to the different habitats.

Fig. 2. Rodents collection from Al-Khitah agricultural farms, image view for (a) Rodents Burrows, (b) Live Trap, and (c) Targeted area.
3. Results and discussions

3.1. Mus musculus ectoparasites spectrum

A total of 70 mice (35 from each habitat) were caught and examined for ectoparasites from the two isolated habitats in Hai’l region (Fig. 1; Table 1).

All the mice were separated into two sex groups; males (34.3% and 48.6%) and females (65.7% and 51.4%) from residential and agricultural farm habitats, respectively. No significant differences (P-value > 0.05) were found in mice occurrence per habitat (Table 1). The gender of the mice occurrence per habitat did not affect the ectoparasite infestation rate (T = 1.221, d.f. = 34, P-value = 0.230 for Hai’l; T-value = 1.205, d.f. = 34, P-vale = 0.056 for Al-Khitah). In addition, there were no significant differences in rodent sex at P < 0.05 considering one way-ANOVA for locations and dependent capture sex for the comparison of rodent group infestation (F (1, 68) = 0.056 and P = 0.813).

Therefore, we combined only sex-wise samples for each habitat as isolated habitats were considered independent to avoid geographic variations (Abdel-Rahman et al., 2009). Even though, some studies (Marsh, 1981, Lareschi, 2010) have shown relationships between the host sex and ectoparasite infestation due to the morphological, biological and ecological differences between males and females.

However, more Mus musculus (30; 85.71%), from the inhabited area were found to be infested with ectoparasites compared to a lesser fewer host individuals (27; 77.18%) from agricultural farms (Table 1).

This distribution might be ascribed to warm microclimates, relatively small home range and social behavior in house mice (Allymehr et al., 2012; Szenczi et al., 2012). Furthermore, the close contact between mice in the occupied building can cause a shift from territorial behavior to a hierarchy of individuals, and the presence of different pollutants and the high number of human occupants that may increase the survival, breeding success and activity of arthropods (Frynta et al., 2005; Szenczi et al., 2012).

Hence, both areas showed a high prevalence of infected host (0.8286, and 0.8000) in the urban and the rural habitats, respectively (Table 1).

Table 2 present an overview of parasitism on the Mus musculus form Hai’l region.

Overall, the distribution of parasites affecting either one group, two groups or more than two. Therefore, groups of parasites pattern was observed by 45.71% and 22.86%; 17.14% respectively from Hai’l residential areas and 17.14%; 20.00% and 40.00% respectively from Al-Khitah Agricultural habitat.

In Hai’l region, with respect to the patterns of infection in both habitats, the rate of singal infection was higher in the residential habitat (i.e., 45.71%) as that of the agricultural farms have only 22.9%. while mixed-infections (40.00%) have been reported for the agricultural farms which may be attributed to their contact with livestocks in that habitat. Our results are in line with other parasitological studies and infection status (Lello et al., 2005; Bordes and Morand, 2011).

3.2. Mus musculus ectoparasites diversity

A total of 514 individual parasites (187 from inhabited building and 327 from agricultural farm habitats, respectively) belong to four species were identified and recovered from Mus musculus, which inluced 339 of flea (Xenopsylla cheopis Rothschild 1903), 39 sucking lice (Polyplax spinulosa Burmeister 1835), 37 sucking lice (Polyplax serrata Burmeister 1839) and 99 of mite (Laelaps echidninus Berlese 1887) are shown in (Figure 3; Table 3).

Among all captured mice, fleas were the most abundant ectoparasite (65.95%), which were found primarily in each infected mouse. Lower numbers of other ectoparasites including mesostigmatid mites (19.26%) and sucking lice (14.79%) were recorded within all habitats.

Interestingly, no ticks were found in the sampled mice from urban and urban sampled that can be explained by several factors, including: low host capture in the arid environment, and/or the parasite existence status as many parasites are present on their
host throughout the year and others only during a specific period (Meerburg et al., 2009; Solanki et al., 2013). In any case, lice are obligate parasites that cannot survive off the host and have no developmental stages free-living in the environment as fleas and ticks (Durden and Musser, 1994).

Finally, correlation tests of total ectoparasites infections, fleas, mesostigmatid mites and sucking lice abundance were negatively correlated between the host habitats (r = −0.525; d.f. = 69; P = 0.001 for all ectoparasites abundance; r = −0.393; d.f. = 69; P = 0.017 for fleas abundance; and r = −0.420; d.f. = 69; P = 0.012 for the mesostigmatid mites abundance). But there were no significant differences in sucking lice abundance (r = −0.083, d.f. = 69, P = 0.635) between sub-urban area of the agricultural farm and residential area habitats. However, ANOVA test for ectoparasites (4 groups, Table 3) with respect to total ectoparasites indicate significant differences (P-value < 0.05).

3.3. Host parasites associations

The study found that agricultural fields and residential areas in the Hai’l region share similar ectoparasites when taking samples from the same location or near each other (Table 3). Some studies have demonstrated that the house mouse arthropods infection patterns differ among locations. For example, Clark (1970) reported only mites species (Ornithonyssus bacoti, Radfordia lemmina, and Myobia musculi) on Mus musculus, in Virgo County, Indiana, United States. Reeves and Cobb (2005) recorded the house mouse mite (Liponyssoides sanguineus); common rodent fur mites (Myocoptes musculinus and Myobia musculi), spiny rat mite (Laelaps echidninus); tropical rat mite (Ornithonyssus bacoti), and fur mites (Radfordia affinis) besides a sucking louse (Polyplax serrata) in structures with house mice infestations from South Carolina, U.S.A. Allymehr et al., 2012 documented mites (Dermanyssus gallinae, the red poultry mite; Myocoptes musculinus, and Ornithonyssus bacoti), a louse (Polyplax serrat), on poultry house mice in Northwest Iran. In addition, Iranian mites and ticks had the highest frequency (97.4%) and the lowest frequency (0.1%), respectively (Pakdad et al. 2012).

The quantity of ectoparasites (fleas, mite, and lice) was higher in semi-urban areas of the agricultural farms than from Hai’l inhabiting sites (i.e., 63.62% and 36.38%, respectively; Table 3). The higher ectoparasites density in the wild habitat was reported from India by Solanki et al. (2013). But a lower incident was reported in Kuala Lumpur city of Malaysia by Zahedi et al. (1984). Solanki et al. (2013) found that rodents captured (Rattus rattus, Rattus norvegicus and Mus musculus) from granary were infested with more ectoparasites (58%) compared to those from the residential areas (42%). While Zahedi et al. (1984) documented that ectoparasites, especially fleas and lice, were higher in urban areas than suburban sites. Nevertheless, different ectoparasites patterns exist throughout the globe as pointed out by several authors (Clark, 1970; Lello et al., 2005; Reeves and Cobb, 2005; Bordes and Morand, 2011; Allymehr et al., 2012; Pakdad et al., 2012).

3.4. Ectoparasites quantitative and qualitative indices

The quantitative and qualitative ectoparasites on the house mouse (Mus musculus Linnaeus, 1758) from Hai’l region were shown in Table 4. Two hundred and three individuals of fleas (Xenopsylla cheopis) were identified as the most common ectoparasite, associated with higher mean infection intensity and mean abundance of ectoparasites, followed by mesostigmatid mites, then sucking lice for the farm habitat and even higher than that of Hai’l City Residential habitat (Table 4).

The results are in line with others as the parasitic profile in the wild habitats has a higher probability of infection with ectoparasites due to the direct contact with other animals (Cai et al., 2014). Furthermore, feral mice have broad distribution, activity patterns and social interactions.
From an epidemiological point of view, prevalence is the proportion of a population found to have a condition of disease or a risk factor that was calculated for the different sites (Tables 1 and 5), as tacking specific aggregate parasites is urgently needed (Rozsa et al., 2000).

The prevalence of all ectoparasites together differed significantly between host habitats (T = 0.2962, P = 0.001). As well as significant differences were also evident for the fleas (T = 8.96, P = 0.002) and mesostigmatid mites (T = 9.61, P = 0.001) infestations. However, there were no significant differences (i.e., T = 2.6, P = 0.2301; and T = 2.2, P = 0.1521) in the sucking louse (Polyplax serrata, and P. spinulosa) abundance between host habitats.

Nevertheless, previous studies from the kingdom of Saudi Arabia (El-Bahrawy and Al-Dakhil, 1993; Morsy et al., 1994, Al-Ahmed and Al-Dawood, 2001) mentioned that rodents ectoparasites of rodents were small in the study area and linked to the extremely hot and very dry weather, mainly during the summer.

These ectoparasites can serve as a source of economic loss and impacts on health. For example, flea (X. cheopis) was the most abundant ectoparasite found primarily in every infected mouse and in addition to other flea-borne diseases, both the classic plague vector and endemic typhus fever. As far as the adult fleas' stage can suck blood from the host and mate with other fleas by producing up to 50 eggs per a day and function as a vector for plague, Yersinia pestis and Rickettisia typhi (Davis et al., 2002; Adjemian et al. 2007; Worldbank, 2010). House mice were susceptible to infection by mites and lice in both the urban and suburban habitats. As well as, rat louse (Polyplax spinulosa) can transmit marine typhus and plague from rat to rat as enzootic plague foci are known from KSA, although human cases are rare (Zahedi et al. 1984). Also, Laelaps echidninus, which was recorded in this survey, can transmit hemorrhagic fever, and although there are no registered events in the area.

Hence, Mus musculus from Hai’l region can represent a potential reservoir and a host of risk vectors for medical and veterinary arthropods within the KSA. We therefore suggest further research to include veterinary programs that interrupt the life cycle of mice parasitic organisms, and predict parasites transmission to susceptible livestock, domestic and wild animals.

### Table 4
Ectoparasites number (N), Mean intensity (MI), and Average abundance of Ectoparasites (MA) in the house mouse (Mus musculus Linnaeus, 1758) of Hai’l region (Hai’l City Residential Habitat, and Al Khitah Agricultural Farm Habitat).

| Ectoparasites          | Number (N) | Mean intensity (MI) | Average Ectoparasite abundance (MA) |
|------------------------|------------|--------------------|----------------------------------------|
| X. cheopis             | 136        | 5.44               | 3.89                                   |
| L. echidninus          | 22         | 1.83               | 0.63                                   |
| P. spinulosa           | 17         | 1.42               | 0.49                                   |
| P. serrata             | 12         | 1.33               | 0.34                                   |
| All ectoparasites      | 187        | 3.22               | 2.34                                   |
|                        |            |                    |                                        |

### Table 5
Quantitative indices of ectoparasites individual of the house mouse (Mus musculus) recovered from Hai’l region (Hai’l City Residential Structures, and Al-Khitah Agricultural Farm Habitats).

| Ectoparasite prevalence (P) | Constituent Ratio (C) | Infection Index (I) |
|-----------------------------|-----------------------|---------------------|
| X. cheopis                  | 71.43                 | 72.73               | 2.78                  |
| L. echidninus               | 34.29                 | 11.76               | 0.22                  |
| P. serrata                  | 25.71                 | 9.09                | 0.17                  |
| P. spinulosa                | 34.29                 | 6.42                | 0.09                  |

Thus, the present study represents the first documented distributional range for the house mice ectoparasites from Hai’l region, which in addition to other rare studies from the other regions (El-Bahrawy and Al-Dakhil, 1993; Morsy et al., 1994, Al-Ahmed and Al-Dawood, 2001) contributes to the distributional range of the previous mentioned ectoparasites that infest mice and other local rodents. All the ectoparasites recorded in this study were previously found on rodents worldwide and cause animals and human diseases (Worldbank, 2010). Nevertheless, the house mice are opportunistic and have sturdy adaptability to variable environments as infection communities, types and rates vary in different habitats.

4. Conclusions and recommendations

House mice in general have traditionally been a model and driving force for ecological and evolutionary understanding from early Darwinian days (Berry and Scriven, 2005). Mice are very adaptable to live with people, as they require very little space and only small amounts of food as they depend upon human for shelter and food, particularly in arid and semi-arid climates (Musser and Carleton, 2005; Soykan et al., 2009).

The present study reported different ectoparasites species (Xenopsylla cheopis; Polyplax spinulosa; Polyplax serrata and Laelaps echidnina) that parasitize commensal house mouse (Mus musculus) in Hai’l region. All the ectoparasites recovered in this study infested mice near or within the residential and cultivated areas that may warrant possible rodents-borne diseases in the region.

CRediT authorship contribution statement

Eltimad H. Abdel-Rahman: Conceptualization, Data curation, Writing - original draft, Writing - review & editing. Mohanad
Abdelgadir: Conceptualization, Data curation, Formal analysis, Writing - review & editing. Monif AlRashidi: Conceptualization, Data curation, Methodology, Writing - review & editing.

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Declarations

Author(s) declare that all works are original and not published in any other journal.

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