Analysis of the spread of arachnoentomosis pathogens as an environmental risk in the implementation of acclimatization programs for large phytophages in the steppe biome (a case study of steppe research station “Orenburg Tarpania”)

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Abstract. In this report, we compiled a species composition database relating to arachnoentomosis pathogens in animals living in the Orenburg region and potentially dangerous for the implementation of projects for the conservation movement of large phytophages. Horseflies (family Tabanidae) are represented by two subfamilies, seven genera and twenty-eight species of blood-sucking insects. Gadflies are represented by three families (Hypodermatidae, Gastrophilidae, and Oestridae), six genera, and twelve species. Parasitic mites of the order Acariformes are represented by three families (families Sarcoptidae, Psoroptidae and Demodecidae), four genera, and eight species. Based on our own studies, it was found that gadflies, in particular Gastrophilus intestinalis, represent the greatest danger among the pathogens of arachnoentomosis to animals kept in the Steppe Research Station “Orenburg Tarpania” managed by the Steppe Institute, Russian Academy of Sciences-Urals Branch (Przhevalsky horses, kiangs, two-humped camels, yaks, downy goats). Wool samples, skin scrapings and scarificat taken for microscopy from the premises for keeping animals kept both in the station and in private farmsteads revealed no sarcoproid mites.

1. Introduction

In the Orenburg region, work is underway to study the acclimatization of ungulates in the Steppe Research Station “Orenburg Tarpania” managed by Steppe Institute, Russian Academy of Sciences-Urals Branch, as part of projects on the reintroduction of large phytophages into steppe communities. The station’s collection of steppe animals includes Przewalski’s horses, kiangs, yaks, camels and downy goats. Animals are kept in semi-free ranging conditions in spacious enclosures with natural vegetation. The research station is located in the immediate vicinity of Preduralskaya Steppe, Orenburg State Nature Reserve that implements the project of breeding a semi-free population of Przhevalsky horses.

Reintroduction is defined as the return of a species that has disappeared from nature to its historical habitat. Activities for the return of endangered species of animals should be scientifically based, with calculated future prospects, and carefully planned. At the first stage, reintroduction involves acclimatization of animals to new environmental conditions. Acclimatized animals are at risk of being invaded by new parasites. Infestation can occur during interaction with local livestock, service personnel, or animal welfare items, which can lead to unforeseen negative consequences. In this study, we present a register of arachnoentomosis pathogens living in the Orenburg region, representatives of...
the families *Tabanidae*, *Hypodermaidae*, *Gastrophilidae* and *Oestridae* from the order *Diptera* and the families *Sarcoptidae*, *Psoroptidae* and *Demodecidae* from the order *Acariformes*. Primary studies were conducted to identify parasites in the station area and surrounding villages. The selection of the considered groups of parasites was justified by cases of diseases registered in the Orenburg region over the past decade. In addition, we also considered parasites that could cause significant disturbance of animals in natural landscapes.

Thus, the study of parasitic arthropods (distribution, abundance, species specialization, impact on the host organism) in the Orenburg region will allow us to assess possible risks among acclimatized animals and make forecasts for development of the reintroduction project.

2. Materials and Methods
Based on scientific literature review relating to the problem of arachnoentomoses in domestic animals of the Orenburg region (horses, cattle, camels), we compiled a register of parasitic arthropods living in the project implementation region.

Horseflies (family *Tabanidae*) are the most active among hematophages. The transmission of many pathogens of zooanthroponoses from sick individuals to healthy ones is quite possible during the blood-sucking of mammals by female horseflies [1].

Gadflies (families *Hypodermaidae*, *Gastrophilidae* and *Oestridae*), as with horseflies, also play an important epizootic and epidemiological significance. They are involved in the transmission of a number of infectious diseases. But the greatest harm is caused by gadflies in the larval phase, as the development of their preimaginal phases occurs in the body of animals. Long-term parasitism of these insects causes a chronic course of entomoses, inflammation of vital organs and general intoxication of the animal organism. Gadflies remain poorly studied, and the species composition, range, biology, population ecology, and phenology of these insects need further research [2].

Parasitic mites from the order *Acariformes* are the causative agents of chronic skin infestations. Their veterinary value is also significant [1].

Up-to-date information on the spread, frequency and ongoing prevention measures of arachnoentomoses for the period 2014-2020 is based on veterinary reporting data on the domestic animals incidence in municipalities of the region.

The obtained material is ranked according to the risk of disease spreading and is represented as a schematic map depicting the epizootic situation in the region. The graphic material is compiled using the ArcGIS 10.2 software.

In the research station area, visual examination of acclimatized animals was carried out for the presence of parasites, traces of their vital activity or clinical manifestations of parasite-caused diseases. A similar examination was done in relation to animals kept in private farmsteads of the Sazan village, located 3 km from the research station. The selected samples of wool, skin scrapings and samples of infrastructure containing animals were examined for the presence of mites according to the methods of Friedberg and Frener, N. N. Bogdanov, G. Z. Shik, A.V. Alfimova, M. G. Khatin [3]. Description of the morphology of gadfly eggs is made according to K. Y. Grunin [4].

3. Results and Discussion
Faunal studies of horseflies (family *Tabanidae*) in landscapes of the Southern Urals (Orenburg region) revealed 28 species of horseflies from 7 genera and 2 subfamilies of these insects [5, 6] (table 1).
Table 1. Fauna of the Tabanidae family identified in the Orenburg region.

| Subfamily   | Genus  | Species         | Abundance | Status   |
|-------------|--------|-----------------|-----------|----------|
| Chrysopsinae| Silvius| Silvius vituli  | Not identified |         |
|             | Chrysops| Chrysops caecutiens | Uncommon |         |
|             |         | Chrysops pictus | Uncommon |         |
|             |         | Chrysops relictus | Uncommon |         |
| Tabaninae   | Tabanus| Tabanus bromius | Common    |         |
|             |         | Tabanus miki    | Not identified |         |
|             |         | Tabanus maculicornis | Uncommon |         |
|             |         | Tabanus sudeticus | Uncommon |         |
|             |         | Tabanus bovinus  | Uncommon |         |
|             |         | Tabanus glaucopis | Uncommon |         |
|             | Atylotus| Atylotus rusticus | Uncommon |         |
|             |         | Atylotus quadrifarius | Not identified |         |
|             | Hybomitra| Hybomitra muehlfeldi | Rare |         |
|             |         | Hybomitra lurida | Uncommon |         |
|             |         | Hybomitra nitidifrons | Uncommon |         |
|             |         | Hybomitra confiformis | Uncommon |         |
|             |         | Hybomitra distinguenda | Uncommon |         |
|             |         | Hybomitra ciureai | Uncommon |         |
|             |         | Hybomitra nigricornis | Uncommon |         |
|             |         | Hybomitra lundbecki | Uncommon |         |
|             |         | Hybomitra montana | Uncommon |         |
|             | Heptatoma| Heptatoma pellucens | Not identified |         |
|             | Haematopota| Haematopota italica | Uncommon |         |
|             |         | Haematopota turkestanica | Rare |         |
|             |         | Haematopota crassicornis | Uncommon |         |
|             |         | Haematopota pallidula | Uncommon |         |
|             |         | Haematopota ptuufalfa | Common |         |
|             |         | Haematopota bimaculata | Common |         |

Gadflies in the Orenburg region are represented by 12 species belonging to 6 genera from 3 families (tables 2-4).

Table 2. Species composition of gadflies from the family Hypodermatidae, inhabiting the Orenburg region, and their characteristics.

| Genus       | Species            | Characteristics                                                                                                                                 |
|-------------|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Hypoderma   | Hypoderma bovis    | The fly is parasitic on cattle, horses, sheep, goats, buffalos. Larvae of the 1st stage parasitize in the adipose tissue of the spinal canal. Larvae of the 2nd and 3rd stages migrate to the back and coupling area. Gadflies produce one generation per year. |
|             | Hypoderma lineatum | Parasitizes cattle, horses, goats and yaks. Larvae of the 1st stage parasitize in the throat, submucosal layer and muscular coat of the esophagus. Larvae of the 2nd and 3rd stages are localized in the back and coupling. Gadflies produce one generation per year. |
| Pallasiomyia| Pallasiomyia antilopum | Parasitizes under the skin of the back in the saiga antelope. In the Orenburg region, it was recorded on the left bank of the Ural River, on the right bank of the Ilek River, and also in the city of Buzuluk [1].  |
Since *N. lineatum* and *H. Bovis* live together in the Orenburg region, all phases of the development of the first one are significantly shifted to earlier periods. *H. lineatum* advanced its phenology by six weeks compared to that of *H. bovis*. Thus, the emergence time for *N. lineatum* is May-June, and for *N. bovis* it ranges between June and September [2, 7, 8, 9].

**Table 3.** Species composition of gadflies from the family *Gastrophilidae*, inhabiting the Orenburg region, and their characteristics.

| Genus        | Species                  | Characteristics                                                                                                                                 |
|--------------|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| *Gastrophilus* | *Gastrophilus pecorum*   | The parasite of the horse, Przewalski's horse, donkey, kulan and zebra. Larvae of the 2nd and 3rd stages parasitize on the surface of the mucous coat of the stomach, oral cavity and esophagus, at the root of the tongue, on the soft and hard palate, in the rectum. One generation per year. The gadfly emergence time ranges between July and September [8]. |
|              | *Gastrophilus veterinus* | Parasitizes horses and donkeys. Larvae of the 1st stage parasitize on the inner surface of the host gums (gingival tissue), larvae of the 2nd and 3rd stages parasitize in the duodenum, stomach. The gadfly produces one generation per year. The emergence occurs in June-August [8]. |
|              | *Gastrophilus haemorrhoidalis* | Parasitizes horses, donkeys, mules. The 1st stage larvae parasitize in the oral cavity, larvae of the 2nd and 3rd stages do so in the duodenum, stomach, pharynx and larynx, in the rectum of the horse. One generation of gadflies per year. The emergence of this gadfly in the Orenburg region was recorded in May-October [8]. |
|              | *Gastrophilus inermis*    | Parasitizes horses. The 1st stage larvae parasitize in the mucous membrane of the cheeks, larvae of the 2nd and 3rd stages parasitize in the stomach and rectum. One generation. The emergence occurs in June – August [8]. |
|              | *Gastrophilus intestinalis* | Horse and donkey parasite. The 1st stage larvae parasitize in the mucous membrane of the tongue, larvae of the 2nd and 3rd stages parasitize in the stomach, duodenum and esophagus. One generation per season. In Orenburg region, the emergence is recorded in July – September [8, 9]. |

The gadfly larvae from genus *Gastrophilus* of the 2nd and 3rd stages were found in Przhevalsky horses in Preduralskaya Steppe, Orenburg State Nature Reserve, during diagnostic deworming (dehelminization). Larvae in small numbers were obtained from all sex and age groups of animals [10-12]. In addition, the *Gastrophilus* gadfly larvae of the 2nd and 3rd stages were obtained in 2020 from a two-year-old Przhevalsky mare kept in the Steppe Research Station “Orenburg Tarpania”.

Visual examination of the animals kept in the camp showed that gadflies lay eggs on the coverts in the withers and forelegs of Przewalsky horses and camels. A similar pattern was observed in the horses kept in subsidiary farms in the village of Sazan. Single eggs are white-yellowish in color. The structure of the egg surface has the form of frequent transverse stripes. The attachment appendage is about half the length of the egg. The shape of eggs was wedge-shaped, extending to the free end, on which, apparently, the cap was located. According to the timing of gadfly egg collection (August-September), their laying location on the body of animals and their morphology, we can conclude that these eggs belong to *Gastrophilus intestinalis*. 
Table 4. Species composition of gadflies from the family *Oestridae*, inhabiting the Orenburg region, and their characteristics.

| Genus       | Species                  | Characteristics                                                                                                                                 |
|-------------|--------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| *Oestrus*   | *Oestrus ovis*           | Parasite of sheep and goats. The 1st stage larvae are localized on the inner surface of the lower turbinated bones, nasal septum, the wall of the nasal cavity. Larvae of the 2nd and 3rd stages are localized in the maxillary sinuses, frontal sinuses, and horn cavities [8]. |
| *Cephalopina* | *Cephalopina titillator* | Parasitizes camels. The *Cephalopina titillator* larvae parasitize in the head cavities (whorls of turbinated bones, ethmoidal bone, upper and middle nasal passages) and in the nasal pharynx of camels. The gadfly produces two generations per year. Gadfly flying occurs in May-June, and in September-October [8]. |
| *Rhinoestrus* | *Rhinoestrus purpureus*  | Parasite of horses and donkeys. Larvae of the 1st stage are developed in turbinated bones, ethmoidal bone, in nasal passages. Larvae of the 2nd and 3rd stages are localized in ethmoidal labyrinth, in pharynx, in sinuses of the sphenoid bone, in larynx, in shells of olfactory bulbs. In the Orenburg region, one generation of *Rhinoestrus purpureus* per year was recorded [8]. |
|             | *Rhinoestrus latifrons*  | The larvae parasitize in cavities of the horse’s head [8].                                                                                     |

In natural and climatic conditions of the Orenburg region, *Oestrus ovis* produces one generation per year [2, 8]. The gadfly emergence occurs in late August to September [2, 9], there are also data on earlier dates of the beginning of *Oestrus ovis* emergence: from mid-June to mid-September [8]. In the Orenburg region, in the forest-steppe and steppe biomes, the prevalence of gadfly infestation in sheep was 81.8%, with the intensity of 24.6 larvae [13]. In addition, information is available on prevalence and intensity of *Oestrus ovis* infection in sheep in the Orenburg region, respectively, 69-97% and 11-49 larvae [8].

According to veterinary reports (2014-2020) on infestation of domestic animals with itch mites and scientific works on the spread of such mites in the Orenburg region, 8 species of acariform mites were identified. The selected species belong to 4 genera from the order *Acariformes* (table 5).

Table 5. Species composition of parasitic mites from the order *Acariformes* in the Orenburg region.

| Subfamily  | Genus   | Species                  |
|------------|---------|--------------------------|
| *Sarcoptidae* | *Sarcoptes* | *Sarcoptes equi*          |
|            |         | *Sarcoptes caprae*       |
|            |         | *Sarcoptes bovis*        |
| *Psoroptidae* | *Psorotes* | *Psoroptes equi*          |
|            |         | *Psoroptes caprae*       |
|            |         | *Psoroptes bovis*        |
| *Chorioptidae* | *Chorioptes* | *Chorioptes caprae*      |
| *Demodecidae* | *Demodex* | *Demodex bovis*          |

In Belyaevsky district, where the Steppe Research Station “Orenburg Tarpania” is located, *Sarcoptes bovis* and *Psoroptes bovis* were confirmed in the laboratory [9]. The species *Sarcoptes*
Caprae, Psoroptes caprae, and Chorioptes caprae were found in the adjacent Akbulak district, and also Sarcoptes bovis, Sarcoptes caprae, Psoroptes bovis, Psoroptes caprae, Chorioptes caprae were identified in the adjacent Sol-Iletsk city district [9, 14]. Demodex bovis was detected in Sorochinsky city district in 2019. Figure 1 shows schematic map of diseases caused by acarimorphic mites in the Orenburg region.

Figure 1. Schematic map depicting the occurrence of diseases caused by acarimorphic mites within Orenburg region: A – sites of diseases, B – sites of diseases occurred in a number of successive years, 1 – sites of sarcoptic mange in cattle, 2 – sites of sarcoptic mange in sheep and goats, 3 – sites of sarcoptic mange in horses, 4 – sites of psoroptic mange in cattle, 5 – sites of psoroptic mange in sheep and goats, 6 – sites of psoroptic mange in horses, 7 – sites of chorioptic mange in sheep and goats, 8 – sites of follicular cattle mange.

Diagnosing sarcoptoidosis in animals kept in the Steppe Research Station “Orenburg Tarpania”, and in private farmsteads of the village of Sazan included examination of animals, microscopy of wool samples (Przhevalsky horses, camels, goats, yaks), skin scrapings (camels, goats, yaks), scarificat from the premises for keeping animals. Microscopy of the obtained samples revealed no mites.

4. Conclusions

The following groups of parasitic arthropods have been recorded in the Orenburg region:

1) Horseflies are represented by two subfamilies, seven genera and twenty-eight species of blood-sucking insects;

2) Gadflies are represented by three families, six genera and twelve species;

3) Parasitic mites from the order Acariformes are represented by three families, four genera, and eight species. Laboratory diagnostic tests on arachnoentomosis diseases in domestic animals are regularly performed in the region. The services systematically carry out preventive treatments against hypodermatosis, gasterophilosis and scabies. No veterinary statistics are kept concerning the similar diseases in wild animals inhabiting natural landscapes, which bears certain risks of infestation for...
newly imported animals after release into their natural habitat. The study of horseflies, gadflies and mites remains an urgent issue in environmental programs.

The prepared register of the species composition and, in part, distribution of arachnoentomosis pathogens will allow identifying the most favorable steppe areas for the reintroduction of large phytophages in the Orenburg region during the location selection process. The species composition of parasitic arthropods, their abundance and phenological features for each specific study area will reduce the environmental risks of conservation movement programs.

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References
[1] Polyakov V A, Uzakov U Ya and Veselkin G A 1990 Veterinary entomology and arachnology: spavochnik (Moscow: Agropromizdat) p 293
[2] Trukhachev V I, Tolokonnikov V P, Lysenko I O and Balega A A 2011 Gadfly diseases of animals (Stavropol: AGRUS) p 168
[3] Shabdarbayeva G S, Akhmetova G D, Turganbayeva G and Balgimbayeva A I 2013 Practical training in parasitology Textbook on arachnoentomology 2nd ed (Almaty: "S-Print") p 56
[4] Grunin K Ya 1953 Larvae of gadflies of domestic animals of the USSR (Moscow; Leningrad: Publishing House of the Academy of Sciences of the USSR) p 124
[5] Kucherov E V 1997 On the fauna of horseflies on the southern tip of the Urals Uspekhi entomologii na Ural Collection of scientific papers p 180
[6] Peterson A M and Chirov P A 2008 Faunistic review of the collection of horseflies (DIPTERA, TABANIDAE) of the Zoological Museum of the Saratov State University Entomological and parasitological studies in the Volga region 7 pp 33-35
[7] Grunin K Ya 1962 Fauna of the USSR Insects are diptera Subcutaneous gadflies (Hypodermatidae) (Moscow: Publishing House of the Academy of Sciences of the USSR) p 240
[8] Nepoklonov A A, Hipe T, Shpistzezer H and Dorzh Ts 1980 Animal diseases caused by gadflies (Moscow: Kolos) p 256
[9] Terentyeva Z H 2013 Parasitological studies of animals in the conditions of the Southern Urals Proceedings of the Orenburg State Agrarian University 3(41) pp 257-260
[10] Kristianovsky P I, Zharkikh T L and Platonov S A 2019 Helminthiasis of Przhevalsky horses in the Orenburg State Reserve Promising agricultural and food innovations Materials of the International Scientific and Practical Conference ed IF Gorlov pp 166-171
[11] Zharkikh T L, Kristianovsky P I, Bakirova R T, Petrov V Yu, Bulgakov E A, Khuzhakhmetova D E, Belimenko V V and Platonov S A 2019 Dynamics of intestinal parasite infection in Przewalski,s horses reintroduced to pre-urals steppe, Orenburg state nature reserve (Russia) Nature Conservation Research vol 4 S2 pp 23-30 doi: 10.24189/ncr. 2019.027
[12] Zvegintsova N S, Zharkikh T L, Kuzmina T A 2019Parasites of Przewalski, s horses (Equus Ferus Przewalskii) in Askania Nova biosphere reserve (Ukraine) and Orenburg state nature reserve (Russia) Nature Conservation Research vol 4 S2 pp 83-88 doi: 10.24189/ncr. 2019.030
[13] Shamin V L 1996 Extrosis of sheep and goats in the Orenburg region (epizootology, phenology, therapy and prevention) auth. diss. ... candidate of Veterinary Sciences (Tyumen)
[14] Terentyeva Z Kh, Shishkin A P and Trutnev V A 2006 Diseases of young animals of small ruminants in Orenburg region Izvestiya Orenburg State Agrarian University 2(10) pp 155-156