Helminthological monitoring of introduced animals in the Steppe Research Station “Orenburg Tarpania”

P I Khristianovsky1,4 ORCID 0000-0003-3902-4379, D A Grudinin1 ORCID 0000-0002-1762-1116, V V Belimenko1 ORCID 0000-0001-8871-7863, S A Platonov1,2 ORCID 0000-0002-9806-412X, S S Maltsev1,4 ORCID 0000-0003-4796-3878

1Institute of Steppe of the Ural Branch of the Russian Academy of Sciences, Orenburg, Russia
2Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia
3Federal State Budget Scientific Institution “Federal Scientific Centre VIEV”, Moscow, Russia
4Orenburg State Agrarian University, Orenburg, Russia

Abstract. At the Steppe Research Station “Orenburg Tarpania” of the Institute of Steppe of the Ural Branch of the Russian Academy of Sciences (Orenburg region, Russia), we carried out work to study acclimatization of large ungulates of inhabiting arid ecosystems to the natural conditions of the South Ural steppe sub-region. The “Orenburg Tarpania” contains introduced animal species: Przewalski’s horse, kiangs, two-humped camels, domestic yaks and an indigenous breed of downy goats. The goal of the project is to return large phytophages to their natural steppe habitat. To prevent an increase in the infection of new populations with helminths to a clinically significant level and to study the adaptation to the existing territorial helminth-faunal complex, we monitor helminth infections of animals kept in the station and in the adjacent areas. Our survey revealed parasitism of nematodes of the suborder Strongylata (families Strongylidae and Trichonematidae) and the suborder Asagidata (Ragassagis equorum) was revealed in ungulates. Parasitism of nematodes of the suborder Strongylata (genera Chabertia and Nematodirus), and protozoa of the genus Eimegia, was detected in ruminants in the Steppe Research Station. The invasion intensity in all cases ranged from low to medium, with no clinical signs. Further studies are planned to determine the degree of biological equilibrium formation in the helminth-host system of the model plot.

1. Introduction

The Steppe Institute’ Research Station “Orenburg Tarpania” (SRS “Orenburg Tarpania”) which is located in Orenburg region (Russia) is a model platform for studying the processes of acclimatization of large ungulates of arid ecosystems to the natural conditions of the South Ural steppe sub-region [1]. The final goal of the project is the return of large phytophages to their natural steppe habitat and the formation of full-fledged, highly productive ecosystems that include all components of trophic chains [2]. The project’s ideology is in line with the principles of the environmental direction known as ecological rewilding [3-8]. In parallel, the project aims to preserve the Przhevalsky horse, as a rare, extinct species in nature. In addition to Przhevalsky horse, the SRS “Orenburg Tarpania” contains kiangs, two-humped camels, domestic yaks and downy goats. Only the downy goat is native species to the territory.
The success of the introduction of animal species is due to many factors. To predict the results of introduction at the community level of species, it is necessary to take into account the possible environmental and biological risks of project implementation. One of the significant risks that determine the health and viability of the animals being moved is the risk of spreading diseases and parasites, primarily helminths. Regular studies of the level of helminthic infections in introduced animals are necessary to prevent an increase in the infection rate of new populations to a clinically significant level and to monitor adaptation to the existing territorial helminthic-faunal complex [9-11]. In addition, in some cases, the introduced animals themselves can be sources of new parasites for the territory [9].

In order to determine the existing helminth fauna and assess the risk of spreading helminthiasis, coprological studies of animals of the SRS “Orenburg Tarpania” were conducted. Similar studies to determine the species community of helminths were conducted on domestic animals kept in the areas adjacent to our Research Station. Domestic animals can act as carriers and distributors of many parasites of wild animals [9, 12, 13, 14].

The aim of the study: to study the nosological composition and dynamics of helminthiasis of herbivorous animals at the SRS “Orenburg Tarpania”, and domestic animals of similar species in the adjacent area.

To achieve this goal, the following tasks were set:
1. Conduct coprological studies of ungulates and ruminants kept in the SRS “Orenburg Tarpania” at intervals of 1.5 months during the year;
2. Conduct similar studies of domestic ungulates and ruminants in the Sazan village located near the Steppe Research Station;
3. Determine the species composition of helminths in the examined animals;
4. Define the principles of ensuring biological balance in the helminth-host systems in animals of this model area.

2. Material and Methods

The object of the study is herbivores of various species. During the reporting period, Przhevalsky horses (4), kiangs (3), yaks (13), two-humped camels (4), domestic goats (7) were kept in the SRS “Orenburg Tarpania”. In Sazan villagers keep domestic horses, sheep, goats and cattle.

The material of coprological studies was fecal masses from these animals. Feces were collected both by collective samples from groups of animals, and by individual samples from individuals. They were packed in plastic bags, labeled and delivered to the parasitological laboratory of the Orenburg State Agrarian University. Here, the material was examined by Fulleborn ovoscopy. For species differentiation of helminths, larvae were cultured for 8-10 days at a temperature of 22-25°C, followed by larvoscopy according to Berman-Orlov.

During the second half of 2020, helminthiological studies in the Steppe Station were conducted at intervals of 1.5 months (table 1).

| Table 1. Dates of coprological studies in 2020. |
|-----------------------------------------------|
| Fecal sampling  | Ovoscopy  | Larvoscopy |
| 30.06            | 01.07     | 11.07      |
| 13.08            | 14.08     | 22.08      |
| 30.09            | 01.10     | 09.10      |
| 13.11            | 14.11     | 23.11      |

When performing ovo- and larvoscopy, microphotography was performed to create a photo archive of the research results.
3. Results and Discussion

The data of the ovoscopy of the collected material show that the eggs of the nematodes of the suborder *Strongylata* were found in all animals of the examined groups (tables 2, 3).

Table 2. Results of helminthioscopy of fecal samples from animals of the SRS “Orenburg Tarpania” for the 3rd quarter of 2020.

| №  | Animal species (sampling details) | 01.07.2020 | Research date | Amount             | Result                          | 14.08.2020 | Amount             | Result                          |
|----|----------------------------------|------------|--------------|--------------------|--------------------------------|------------|--------------------|--------------------------------|
| 1  | Przhevalsky horse (male Orgo)    | Strongylate eggs | Up to 10 in field of microscope | Negative           | -                                |            |                    |                                  |
| 2  | Przhevalsky herd sampling        | Strongylate eggs | Up to 10 in field of microscope | Strongylate eggs   | Single once in field of microscope |
| 3  | Kiang, male                      | Strongylate eggs | Single once in field of microscope | Negative           | -                                |            |                    |                                  |
| 4  | Kiang (female, herd sampling)   | Strongylate eggs | Up to 3 in field of microscope | Strongylate eggs   | Single once in field of microscope |
| 5  | Camel (herd sampling)            | Strongylate eggs | Single once in field of microscope | Eimeria oocysts    | Single once in field of microscope |
| 6  | Yak (herd sampling)              | Strongylate eggs | Single once in field of microscope | Strongylate eggs   | Single once in field of microscope |
| 7  | Domestic goat (herd sampling)    | Strongylate eggs | Up to 5 in field of microscope | Eimeria oocysts    | Single once in field of microscope |

The intensity of the invasion was low and medium: from single to 10-12 eggs in the field of view of the microscope. In several cases, helminth eggs were not detected in the samples.

In the samples of ruminant feces, in addition to strongylate eggs, Eimeria oocysts were detected at different periods with a low invasion intensity (from single to 5 oocysts in the field of view).

There were no clinical signs of these invasions in all animal species.

Thus, in all animal species kept in the SRS “Orenburg Tarpania”, nematodoses in the carriage form were detected, and in ruminants, in addition, eimeriosis also in the carriage form.

Coprological studies of the material from domestic animals of Sazan village were carried out in similar periods. The results of the studies are presented in tables 4, 5.

It follows from the tables that the domestic animals of the Sazan village are infested with the same species of parasites as the animals of the SRS “Orenburg Tarpania”. The intensity of invasion in domestic horses and cattle is low (from single to 3-5 in the field of view of the microscope). In addition, the eggs of cestodes of *Moniezia expansa* were found in the fecal samples of small cattle. Such a composition of parasitocenosis (strongylates, moniesia, eimeria) is very typical for ruminants of this region.

According to the local veterinary service, there were no clinical signs of these invasions in domestic animals in the Sazan village.

The species composition of strongylate complex of ungulate and ruminant animals of the Steppe Research Station and Sazan village is currently being clarified. According to preliminary data, representatives of the family *Strongylidae* (*Alfortia edentatus* and *Strongylus equinus*) were found in
the Research station and private sector in ungulates, and Chabertia ovina in ruminants (camels, yaks, domestic sheep and goats).

Table 3. Results of helminthioovoscopy of fecal samples from animals of the SRS “Orenburg Tarpania” for the 4th quarter of 2020.

| № | Animal species (sampling details) | Research date | 01.10.2020 | Result | Amount | 14.11.2020 | Result | Amount |
|---|-----------------------------------|---------------|-------------|---------|---------|-------------|---------|---------|
| 1 | Przhevalsky horse (male Orgo)     |               |             | Negative| -       | Strongylate eggs | Up to 3 in field of microscope |
| 2 | Przhevalsky horse (herd sampling) |               |             | Strongylate eggs | Up to 12 in field of microscope | Strongylate eggs | Single once in field of microscope |
| 3 | Kiang, male                       |               |             | Negative| -       | Strongylate eggs | Single once in field of microscope |
| 4 | Kiang (female, herd sampling)     |               |             | Strongylate eggs | Single once in field of microscope | Strongylate eggs | Single once in field of microscope |
| 5 | Camel (herd sampling)             |               |             | Strongylate eggs | Up to 10 in field of microscope | Strongylate eggs | Single once in field of microscope | Eimeria oocysts | Single once in field of microscope |
| 6 | Yak (herd sampling)               |               |             | Strongylate eggs | Single once in field of microscope | Strongylate eggs | Single once in field of microscope | Nematodirus eggs | Single once in field of microscope | Eimeria oocysts | Single once in field of microscope |
| 7 | Domestic goat (herd sampling)     |               |             | Negative| -       | Strongylate eggs | Up to 3 in field of microscope | Eimeria oocysts | Single once in field of microscope |

Table 4. Results of helminthioovoscopy of fecal samples from domestic animals in the Sazan village of the Belyaevsky district for the 3rd quarter of 2020.

| № | Animal species                  | Research date | 01.07.2020 | Result | Amount | 14.08.2020 | Result | Amount |
|---|---------------------------------|---------------|-------------|---------|---------|-------------|---------|---------|
| 1 | Horse                           |               |             | Strongylate eggs | Up to 3 in field of microscope | Strongylate eggs | Up to 5 in field of microscope |
| 2 | Cattle                          |               |             | Eimeria oocysts | Single once in field of microscope | Single once in field of microscope | - |
| 3 | Small cattle                    |               |             | Strongylate eggs | Up to 3 in field of microscope | Strongylate eggs | Up to 3 in field of microscope | Moniesium eggs | Single once in field of microscope | Eimeria oocysts | Single once in field of microscope |
Table 5. Results of helminthioscopy of fecal samples from domestic animals in the Sazan village of the Belyaevsky district for the 4th quarter of 2020.

| №  | Animal species | Research date | Result                          | Amount                                 |
|----|----------------|---------------|---------------------------------|----------------------------------------|
| 1  | Horse          | 01.10.2020    | Strongylate eggs                | Single once in field of microscope    |
|    |                |               |                                 |                                        |
|    |                | 14.11.2020    | Strongylate eggs                | Up to 7 in field of microscope        |
| 2  | Cattle         | 01.10.2020    | Strongylate eggs                | Single once in field of microscope    |
|    |                |               | Nematodirus eggs                | Single once in field of microscope    |
|    |                | 14.11.2020    | Negative                        | -                                      |
| 3  | Small cattle   | 01.10.2020    | Strongylate eggs                | Single once in field of microscope    |
|    |                |               |                                 |                                        |
|    |                | 14.11.2020    | Strongylate eggs                | Single once in field of microscope    |

In general, the invasion of helminths and eimeries in animals of the SRS “Orenburg Tarpania” and domestic animals of Sazan village during the reporting period was stable. There were slight fluctuations in the invasion within the low invasion intensity. Longer observations are needed to determine the seasonal dynamics of the process.

4. Conclusions

1. During the research period in the SRS “Orenburg Tarpania”, parasitism of nematodes of the suborder Strongylata (families Strongylidae and Thishonematidae) was detected in ungulates. The species composition of strongylates is being specified.
2. The intensity of strongylatous invasion ranged from low to medium (from single to 10-12 helminth eggs in the field of view of the microscope). There was a higher invasion intensity in Przhevalsky horses than in kians.
3. In Przhevalsky’s horses, in addition, nematodes of the species Ragassis equorum (suborder Asagidata) were found with a low intensity of invasion.
4. Parasitism of nematodes of the suborder Strongylata (genera Chabertia and Nematodirus), and also protozoa of the genus Eimeria with a low invasion intensity was revealed in ruminants of the Steppe Research Station. Helminth infection in camels was higher than in yaks.
5. In domestic horses of the adjacent area (Sazan village), the same helminths species were detected as in ungulate animals of the SRS “Orenburg Tarpania” with low and medium invasion intensity.
6. In ruminants of Sazan village, helminths of the same species as in ruminants of the Research station were found, and also oocysts of Eimeria. The invasion intensity was low.
7. For all these helminthiases, no clinical signs were observed during the research period, invasion in all animals occurred in the form of carriage.

During the acclimatization period (2014-2020), herbivores of the SRS “Orenburg Tarpania” developed a certain helminthocenosis corresponding to the existing helminth-faunal complex of the model territory. At the same time, the species composition of helminths of ruminants and ungulates of the SRS “Orenburg Tarpania” is similar to that of domestic animals of similar species in private farms located 3 km away, from the Steppe Research Station of the village. There is a low level of extensiveness and intensity of helminthic invasion in wild and domestic animals.

Perhaps, the formation of biological equilibrium in the helminth-host systems in the specified model area occurs in our time. Further studies will show how stable it will be.

Acknowledgments

This work was done as part of the Steppe Institute Theme (#GP AAAA-A21-121011190016-1).
References
1. Chibilev A A 2017 Steppe Eurasia: regional review of natural diversity (Moscow; Orenburg: Institute of the Steppe of the Ural Branch of the Russian Academy of Sciences; RGO) p 324 ISBN 978-5-94162-122-4
2. Levykin S V, Kazachkov G V, Yakovlev I G, Chibileva V P and Grudinin D A 2019 History and prospects of Orenburg Tarpania as a key object of the concept of socio-ecological rehabilitation of steppes Voprosy stepevedeniya 15 pp 175-179
3. Laetitia M Navarro and Henrique M Pereira 2012 Rewilding Abandoned Landscapes in Europe Ecosistems 6 pp 900–912
4. Meredith Root-Bernstein, Mauro Galetti and Richard J Ladle 2017 Rewilding South America: Ten key questions Perspectives in Ecology and Conservation vol 15 pp 271-281
5. Kozorez A I 2014 Pleistocene park in Belarus Forest and hunting economy 10 pp 42-47
6. Peter A Lindsey, Guillaume Chapron, Lisanne S Petracca, Dawn Burnham and Amy Dickman 2017 Relative efforts of countries to conserve world’s megafauna Global Ecology and Conservation vol 10 pp 243-252
7. Donlan C J et al Pleistocene 2006 Rewilding: An Optimistic Agenda for Twenty-First Century Conservation The American Naturalist vol 168 5 pp 660-681
8. Vonselev M Yu and Podoshvelev D A 2018 Rewilding of Belarusian natural landscapes: reintroduction of large herbivorous animals Proceedings of BSTU Series 1: Forestry, nature management and processing of renewable resources 2 pp 128-132
9. Pelgunov A N and Maklakova L P 2013 Parasitological aspects related to acclimatization and introduction of wild ungulates Russian Parasitological Journal 3 pp 67-75
10. Zvegintsova N S, Zharkikh T L and Kuzmina T A 2019 Parasites of Przewalski’s horses (Equus ferus Przewalskii) in Askania Nova biosphere reserve (Ukraine) and Orenburg State Nature Reserves (Russia) Nature Conservation Research vol 4 S2 pp 83-88
11. Zharkikh T L, Khristianovsky 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. Baitursynov K K 2008 Factors of formation of the community of helminthofauna of wild and domestic ungulates of Kazakhstan Russian Parasitological Journal 4 pp 5-12
13. Govorka Ya V, Maklakova L P 1988 Helminths of wild ungulates of Eastern Europe, (Moscow: Aquarium) p 85
14. Muromtsev A B 2014 Parasitocenoses of domestic and wild ruminants in the Kaliningrad region Russian Parasitological Journal 1 pp 9-13