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Effect of orally applied ivermectin on gastrointestinal nematodes in douc langurs (*Pygathrix* spp.)

Constanze Hartmann[1], **Jannis Göttling**[1], **Tilo Nadler**[2], **and Ulrike Streicher**[3]

[1] Centre for Artificial Insemination and Embryo Transfer, University for Veterinary Sciences, Veterinärplatz 1, 1210 Vienna, Austria.

[2] Endangered Primate Rescue Center, Cuc Phuong National Park, Nho Quan District, Ninh Binh Province, Vietnam. <t.nadler@hust.edu,vn>

[3] Wildlife Veterinarian / Wildlife Management Consultant, Eugene, USA.
Corresponding author: Constanze Hartmann <Constanze.Hartmann@vetmeduni.ac.at>

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Summary

The aim of the study was to test the orally admistered effectiveness of the anthelminthic drug Ivomec® (Merial) with the active ingredient ivermectin against gastrointestinal nematodes. The effectiveness was tested in seventeen grey-shanked douc langurs (*Pygathrix cinerea*) and seven red-shanked douc langurs (*Pygathrix nemaeus*) at the Endangered Primate Rescue Center in Cuc Phuong National Park, Ninh Binh Province, Vietnam. The number of eggs in the faeces of each douc langur was counted three times every second day, and then the drug was given orally at a dosage of 0.2 mg/kg. Ten to sixteen days after drug administration the eggs were counted again three times every second day. The number of eggs was determined using a modified McMaster method. Applied at a dosage of 0.2 mg ivermectin per kg body weight Ivomec® did not significantly reduce the number of eggs of gastrointestinal nematodes in faeces of douc langurs.

Hiệu quả của thuốc ivermectin thông qua đường uống đối với giun tròn sống trong hệ tiêu hóa của các loài Voọc chà vá (*Pygathrix* spp.)

Tóm tắt

Mục tiêu của nghiên cứu này là nhằm kiểm tra hiệu quả của thuốc trị giun Ivomec® (Merial) với thành phần chính là ivermectin trong điều trị nhiễm giun tròn ở đường tiêu hóa. Đối tượng được kiểm tra là 17 cá thể chà vá chân xám (*Pygathrix cinerea*) và 7 cá thể chà vá chân nâu (*Pygathrix nemaeus*) tại trung tâm cứu hộ linh trưởng nguy cấp, vườn quốc gia Cúc Phương, Ninh Bình, Việt Nam. Số lượng trứng được xác định bằng phương pháp McMaster có hiệu chỉnh. Số lượng trứng giun trong phân của mỗi cá thể chà vá được đếm 3 lần mỗi ngày và thuốc được cho uống với liều lượng 0.2 mg/kg. 10 đến 16 ngày sau khi cho uống thuốc, trứng giun lại được đếm 3 lần mỗi ngày để so sánh. Kết quả cho thấy, với liều lượng 0.2 mg ivermectin cho một kg trọng lượng cơ thể, thuốc không có hiệu quả trong việc giảm số lượng trứng giun trong phân của các loài chà vá.

Introduction

Gastrointestinal parasites pose a severe threat to wild animals in captivity. Due to the close

phylogenetic relationship to humans infectious diseases in non-human primates have a great zoonotic potential and deserve special attention. Furthermore keeping primates in captivity facilitates the transfer of infectious agents among individuals. Compared to the life of free-roaming animals captive primates live in closer proximity and often at a higher density. And host density and the abundance of strongyloid nematodes are known to correlate significantly (Arneberg et al., 1998).

These risks make regular parasitological examinations a necessity for any primate holding facility. The present study was conducted at the Endangered Primate Rescue Center (EPRC) in Cuc Phuong National Park, Ninh Binh Province, Vietnam. Important aims of the EPRC are captive breeding and the reintroduction of endangered primates. To achieve these aims, animals must be in a very good condition and regular health checks are essential (Cunningham, 1996).

The work at the EPRC focusses on two genera of the cercopithecid subfamily Colobinae: these are the langurs which belong to the genus *Trachypithecus* and the douc langurs within the genus *Pygathrix*. The genus *Pygathrix* comprises three species which all are endemic to Indochina. The red-shanked douc langur (*P. nemaeus*) occurs in Laos and central Vietnam, the grey-shanked douc langur (*P. cinerea*) occurs in the Central Highlands of Vietnam and the black-shanked douc langur (*P. nigripes*) occurs in southern Vietnam and eastern Cambodia. All species are threatened with *P. nemaeus* and *P. nigripes* assessed by IUCN (IUCN, 2014) as “Endangered” and *P. cinerea* as “Critically Endangered”. The latter species is also listed among the “The World's 25 Most Endangered Primates” (Schwitzer et al., 2014). The EPRC is the only institution keeping *P. cinerea* thus the stock is of enormous conservation value.

Anthelminthic treatment in non-human primates should be as gentle as possible. As some of the primates at the EPRC are intended for reintroduction, habituation and handling is kept at a necessary minimum. Regular drug administration by injection poses a challenge as animals are kept in groups and immobilization of each individual would be necessary to ensure proper drug application and dosage. Thus oral treatment is the most feasible option. In the past animals were usually dewormed twice per injection during the initial quarantine after arrival and after that deworming was continued roughly annually by oral administration. Animals born at the center however were not subjected to any initial treatment. During the years the primates at the EPRC were irregularly dewormed with 0.2 mg per kg bodyweight ivermectin (injectable solution IVOMEC-S®, ivermectin 10 mg/ml, Merial, United Kingdom).

Colobines have a multi-chambered stomach, comparable to the digestive system of a ruminant. The stomach consists of three compartments in *Trachypithecus* langurs and of four compartments in douc langurs (Caton,1998; Chivers, 1994). Bacteria in the first sections split cellulose, lignin and other substances, which are otherwise indigestible for vertebrates. Afterwards shorter carbohydrates can be resorbed in the more distal parts of the gastrointestinal system. An in-vitro study on the effectiveness of moxidectin and ivermectin in ruminal and abomasal content of sheep showed that ivermectin binds > 90 % to the solid ingesta of the fore-stomach (Lifschitz et al., 2005). Due to their special digestive system it is questionable whether orally administered ivermectin is effective in members of the subfamily Colobinae and to date no study on the effect of oral ivermectin in langurs is available. The aim of the study was to test the orally admistered effectiveness of ivermectin (Ivomec®, Merial) against gastrointestinal nematodes in langurs. We hypothesized that orally applied ivermectin in the dose of 0.2 mg/kg is not effective against nematodes.

Materials and Methods

Currently 15 species of primates are living at the EPRC. The langurs are housed in groups or solitary in outdoor enclosures under ambient weather conditions. The size of the larger cages is 10 m x 5,5 m x 3,5 m and the smaller cages measure 10 m x 5 m x 3,5 m. The larger cages have concrete floors whereas the smaller ones have natural soil. All enclosures are made of wire-mesh fence and the interior fitting consists of bamboo poles. The floors of the cages are cleaned two times per day, in the morning thoroughly with water and brush and in the afternoon faeces are collected and the cages are swept dry.

In preparation of the study faeces of all groups of primates at the EPRC were examined for gastrointestinal parasites. Pooled samples were collected two hours after the first cage cleaning in the morning, so the samples were not older than two hours. The samples were directly examined, using four different examination methods: direct smear, flotation, sedimentation and Baermann-Wetzel method. Detection of protozoa was not possible as no immersion oil was available. The preliminary control showed that the parasite load in the primates was high except in a few adult males that were kept solitary.

Eggs were identified according to their shape, color and content (Schnieder, 2006, Eckert et al., 2005). Measurement of the eggs was not possible. For the microscope at the EPRC (LABOVAL 4, Carl Zeiss Jena, Germany) an appropriate micrometer was not available.

For the study we selected 24 clinically healthy douc langurs, of which seven were red-shanked douc langurs and 17 were grey-shanked douc langurs (Table 1). The group size in which the

Table 1. Demographic data of the individuals.

Name	Number	Sex	Species	Date of Birth	Source
Butz	6-09	M	*P. nemaeus*	1997	Confiscated
Detlef	6-75	M	*P. nemaeus*	2.5.2012	Born EPRC
Orsa	6-60	M	*P. nemaeus*	2007	Confiscated
Julius	6-16	M	*P. nemaeus*	1995	Confiscated
Laura	6-55	F	*P. nemaeus*	2.2.2008	Born EPRC
Borsti	6-21	F	*P. nemaeus*	12.30.1998	Born EPRC
Halbfuss	6-46	F	*P. nemaeus*	~2002	Confiscated
Sung	7-25	M	*P. cinerea*	~2000	Confiscated
Mr. Ham	7-67	M	*P. cinerea*	5.14.2013	Born EPRC
Gordon	7-09	M	*P. cinerea*	~1996	Confiscated
Gin	7-46	M	*P. cinerea*	~2005	Confiscated
Cactus	7-55	M	*P. cinerea*	5.6.2010	Born EPRC
Falk	7-39	M	*P. cinerea*	~2003	Confiscated
Bummi	7-49	M	*P. cinerea*	2009	Confiscated
Seba	7-65	M	*P.cinerea*	2008	Confiscated
Ben	7-40	M	*P. cinerea*	10.10.2007	Born EPRC
Eric	7-52	M	*P. cinerea*	~2003	Confiscated
Cac Mac	7-16	M	*P. cinerea*	unknown	Confiscated
Eco	7-14	M	*P. cinerea*	1998	Confiscated
Sung	7-25	M	*P. cinerea*	2000	Confiscated
Pip	7-48	F	*P. cinerea*	March 2009	Born EPRC
Naomi	7-24	F	*P. cinerea*	1.15.2004	Born EPRC
Phu Cat	7-34	F	*P. cinerea*	unknown	Confiscated
Lychee	7-56	F	*P. cinerea*	4.17.2011	Born EPRC
Lucy	7-28	F	*P. cinerea*	6.6.2005	Born EPRC

animals lived varied between two and four members. 23 of the douc langurs live in family groups or in bachelor groups with other douc langurs. One male shared the cage with two Hatinh langurs (*Trachypithecus hatinhensis*). The youngest individual was one year and two months old, the oldest one 20 years. 16 douc langurs were male, eight were female.

Sample collection

Sample collection for the study was carried out between June 10th and 28th June 2014. The faeces were collected from each animal three times every second day. The groups were observed until every animal defecated and a sample had been collected.Once three samples had been examined from every animal, all were treated orally with 0,2 mg/kg ivermectin on the 13 June. Twelve individuals were treated orally with the injection solution from Ivomec-S® for pigs (ivermectin 10 mg/ml). The other twelve were treated with the Ivomec-P® horse paste (ivermectin 18,7 mg/g). Both pharmaceutical products were imported from Germany. The drugs were administered in a piece of sweet potato (*Ipomoea batatas*). All animals in a cage were dewormed, also those animals that were not part of the study.

On day 10 to day 16 after drug administration faeces were collected again (23rd to 28th June 2014), three times from each animal every second day.

A total of 143 samples were analyzed. The samples were either analyzed the same day or the following day. If they were analyzed the following day, they were stored in the fridge over night at temperatures of about 10°C. The number of eggs was measured using a modified McMaster method. Therefore two grams of the sample were mixed with 60 ml of a saturated saline solution. After ten minutes the counting chamber was filled and the eggs counted. The eggs were always counted by the same person.

Statistical analysis

The data were analyzed using the statistics program RProject® (The R Foundation for Statistical Computing, Austria). A paired t-test was carried out. A p-level of 0.05 was considered significant.

Results

73 % of the samples were found positive for gastrointestinal nematodes, 8 % were positive for large gastrointestinal strongylids, 24 % were positive for nematodes of the genus *Trichuris* and 49 % for *Strongyloides* spp.

100 % of the animals were positive before administration of ivermectin and 100 % of the animals were positive after the administration. There was no significant decrease between number of eggs before and after drug administration ($t = -2{,}3405$, $p = 0{,}0283$). We were not able to show a pharmacological effect of ivermectin in the animals of our study when orally applied at a dosage of 0.2mg/kg, irrespective of the formulation of the drug (Table 2).

Discussion

Since the establishment of the center in 1993 the primates at the EPRC were more or less regularly dewormed with Ivomec-S® (10 mg ivermectin/ml) at a dosage of 0.2 mg/kg and sometimes with fenbendazole (Panacur®, MSD, United States). From 1998 to 2006 regularly controls by a veterinarian were performed. Nevertheless reinfection with gastrointestinal parasites

Table 2. Mean egg counts before and after administration of ivermectin of each douc langur.

Name	Number	Mean Egg Count I - III (EpG)	Mean Egg Count IV - VI (EpG)
Butz	6-09	2933	4567
Laura	6-55	1333	1700
Borsti	6-21	1233	1067
Halbfuss	6-46	767	533
Detlef	6-75	3067	4667
Julius	6-16	2867	2400
Orsa	6-60	667	1033
Cactus	7-55	1600	1167
Phu Cat	7-34	267	33
Gordon	7-09	333	433
Ben	7-40	1033	500
Eric	7-52	200	467
Bummi	7-49	967	833
Seba	7-65	1167	2800
Cac Mac	7-16	67	933
Eco	7-14	333	1000
Falk	7-39	600	5767
Lychee	7-56	633	1433
Gin	7-46	1367	1533
Pip	7-48	800	567
Sung	7-25	800	900
Naomi	7-24	267	1867
Lucy	7-28	1000	600
Mr. Ham	7-67	4667	10000

can be expected: the animals climb down to the ground for drinking, eating or playing or touch bamboo furnishings contaminated with faeces. Doucs also show coprophagia occasionally (Nadler, pers. obs.). Frequent contact with infectious parasite stages is thus probable.

However this study showed that at least in douc langurs the treatment with ivermectin in a dosage of 0.2 mg/kg is ineffective.

Though Ivomec S is a formulation made for injection its off-label use is common in many areas of veterinary medicine. Ivermectin at this dosage is successfully orally used in animals with a simple digestion system (eg. bears) or in hindgut fermenters (eg. horses).

After years of irregular deworming with a low dose of ivermectin a resistance of nematodes against ivermectin should be taken into consideration. Resistances against ivermectin are known for various parasite species (Geary, 2005).

Proper storage of drugs in remote tropical areas is a problem. The injectable solution was stored in a dark container several months in a room which was cooled down for some hours per day, but might in other times reach outside temperatures in particular during the almost regular power cuts. The paste for oral treatment in horses was stored in the same room for one week. Both formulations were imported from Germany and inappropriate storage during transport could have occurred.

Eggs of parasites survive a long time under the hot and humid climate conditions. Despite its limited technical possibilities the study showed that the parasite pressure on the primates is high with the exception in the males which are kept alone. It is important to note that none of the animals in our study showed any symptoms of a parasite mediated disease. Nonetheless to protect the

health of the extremely valuable stock and to minimize the zoonotic potential some of the parasites hold, an effective and practicable treatment of helminthes in douc langurs should be investigated. Furthermore the effect of oral ivermectin at higher dosages and in other colobines should be researched.

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