

Benzimidazole resistant *Haemonchus contortus* in a wildlife park[#]

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Benzimidazol-resistente *Haemonchus contortus* in einem Wildpark

Die Resistenz gegen Anthelminthika (AR) von gastrointestinalen Nematoden (GINs) bei Wiederkäuern stellt weltweit ein grosses Problem dar. Unter den GINs ist der blutsaugende Labmagen-Parasit *Haemonchus contortus* besonders pathogen und kann gegen alle wichtigen anthelminthischen Substanzklassen Resistenzen aufweisen. In der vorliegenden Studie führte der Tod eines 1,5 Jahre alten Wisents (*Bison bonasus*) in einem deutschen Wildpark aufgrund einer Hämonchose trotz häufiger anthelminthischer Behandlung der Herde mit Fenbendazol sowie Doramectin zu einer Untersuchung der AR.

Gepoolte Kotproben wurden von vier verschiedenen Wisentgruppen, sowie von Mufflons (*Ovis orientalis*), Elchen (*Alces alces*), Rentieren (*Rangifer tarandus*), Sikahirschen (*Cervus nippon*), Persischem Damwild (*Dama mesopotamica*) und Rotwild (*Cervus elaphus*) aus dem Wildpark gesammelt und parasitologisch untersucht. Nach der koproskopischen Untersuchung wurden Larvenkulturen angelegt. *Haemonchus contortus*-positive Larvenkulturen wurden weiter auf genetische Polymorphismen im Zusammenhang mit Benzimidazolresistenz an den Codons 167 und 200 des β -Tubulin-Isotyp-1-Gens durch Echtzeit-Pyrosequenzierung untersucht.

Bei allen vier Wisentgruppen und bei den Mufflons wurden Infektionen mit *H. contortus* nachgewiesen. Die Häufigkeit des Resistenz-assoziierten Einzelnukleotid-Polymorphismus (SNP) bei Codon 200 betrug bei fünf Proben, von zwei Wisentgruppen und Mufflons, 100%. Dagegen wurden bei Codon 167 keine Resistenz-assoziierten SNPs nachgewiesen. Zusätzlich wiesen die Eizahlen pro Gramm Kot von zwei Wisents 14 Tage vor und nach der parenteralen Doramectin-Behandlung auf eine mögliche makrozyklische Lacton-resistenz hin. Der Nachweis von Anthelminthika-resistenten Nematoden war bei diesen Tieren angesichts der geplanten Wiederauswilderung besonders besorgniserregend.

Aufgrund der eingeschränkten Möglichkeiten in der prophylaktischen Helminthenbekämpfung, wie Rotationsweiden, sind die zoologischen Gärten und Wildparks

Summary

Anthelmintic resistance (AR) of ruminant gastrointestinal nematodes (GINs) constitutes a major problem worldwide. Among GINs, the abomasal blood-feeding parasite *Haemonchus contortus* is particularly pathogenic and may show resistance against all major anthelmintic substance classes. In the present study, the death of a 1,5 year-old European bison (*Bison bonasus*) from a German wildlife park due to haemonchosis despite frequent anthelmintic treatment of the herd with fenbendazole as well as doramectin prompted an investigation regarding AR. Pooled faecal samples were collected from four different bison groups as well as from mouflons (*Ovis orientalis*), elk (*Alces alces*), reindeer (*Rangifer tarandus*), sika deer (*Cervus nippon*), Persian fallow deer (*Dama mesopotamica*) and red deer (*Cervus elaphus*) housed by the wildlife park. After coproscopical examination, faecal larval cultures were established. *Haemonchus contortus*-positive larval cultures were further examined for genetic polymorphisms associated with benzimidazole resistance at codons 167 and 200 of the β -tubulin isotype 1 gene by real-time pyrosequencing. Infections with *H. contortus* were detected in all four bison groups, as well as in mouflons. In five samples, representing two bison groups and the mouflons, the frequency of the resistance-associated single-nucleotide polymorphism (SNP) at codon 200 was 100%. In contrast, resistance-associated SNPs were not detected at codon 167. In addition, faecal egg counts from two bison before and 14 days after parenteral doramectin treatment indicated possible macrocyclic lactone resistance. Detection of anthelmintic resistant nematodes in these animals was especially concerning in the light of planned reintroduction into the wild. As helminth control in zoological gardens and wildlife parks relies mostly on anthelmintic treatment due to restricted possibilities regarding management practices such as rotational grazing, care should be taken to avoid underdosing or unnecessary frequent treatments facilitating the development of AR.

Keywords: trichostrongyles, gastrointestinal nematodes, ruminants, European bison, *Bison bonasus*, anthelmintic resistance

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meist auf eine anthelminthische Behandlung angewiesen. Daher sollten Unterdosierungen oder unnötig häufige Behandlungen vermieden werden, da diese die die Entwicklung von AR begünstigen.

Schlüsselwörter: Trichostrongyliden, gastrointestinale Nematoden, Wiederkäuer, Wisent, *Bison bonasus*, anthelmintische Resistenz

Introduction

Since the 1980s, anthelmintic resistance of ruminant gastrointestinal nematodes (GINs) has become a major concern worldwide.¹ Among GINs, the abomasal parasite *Haemonchus contortus*, which frequently affects sheep and goats and occasionally other ruminants, is particularly pathogenic due to its haematophagous behaviour and high fecundity, resulting in rapid development of large parasite burdens.² Infection takes place via ingestion of third-stage larvae (L3) present on pasture, which develop into fourth-stage larvae (L4) during a histotrophic phase in the abomasal mucosa, before returning to the mucosal surface, where they start blood feeding and develop into adults. Clinical signs are primarily related to hypoproteinaemia and anaemia as a result of blood loss². In sheep, lambs are particularly susceptible to clinical haemonchosis and even death, whereas older animals develop partial immunity after continuous exposure.³

While *H. contortus* is considered the most important parasite of livestock in warm climatic regions, it shows a heterogeneous spatial distribution in Europe.⁴ Indeed, *H. contortus* is adapted to a milder climate than other Trichostrongyloidea, thus, free-living larvae stages usually do not survive the winter on pasture in central and northern Europe and overwintering mainly takes place via within-host arrested development.⁵ Nevertheless, climatic change is predicted to result in an altered epidemiological situation with an increased *H. contortus* infection pressure in northern Europe.⁶

Anthelmintic resistance against all major anthelmintic drug classes represents a global challenge in the control of *H. contortus*. In Europe, resistance against benzimidazoles (BZ) is particularly widespread, and macrocyclic lactone and monepantel resistance as well as multidrug resistance is also increasingly reported.^{7–11} Resistant *H. contortus* isolates have not only been detected in commercially farmed ruminants, but also in zoological settings, e.g. affecting giraffes¹² and European bison,¹³ and other wild ruminants.^{14,15} Three single nucleotide polymorphisms (SNPs) of the β -tubulin isotype 1 gene, at codons 167, 198 and 200, correlate with BZ resistance in several nematode species.^{16–18} Regarding *H. contortus*,

field investigations have shown that the substitution at codon 200 (TTC to TAC), resulting in a change of phenylalanine to tyrosine (F200Y), plays the most important role, while the mutation at codon 167 (F167Y) is less frequently and at codon 198 (E198A or E198L) only rarely detected.^{19–23} The present report describes the occurrence of BZ resistant *H. contortus* isolates, based on detection of the F200Y mutation by real-time pyrosequencing, in European bison and mouflons in a German wildlife park.

Materials and Methods

Epidemiological setting and clinical history

In March 2018, a 1,5-year old, female European bison (*Bison bonasus*) was translocated from a zoological garden to a wildlife park, where it joined a herd of young bison intended for future release into the wild. The wildlife park of about 90 ha also kept a variety of other ruminant species, including red deer (*Cervus elaphus*), Persian fallow deer (*Dama mesopotamica*), sika deer (*Cervus nippon*), elk (*Alces alces*), reindeer (*Rangifer tarandus*) and mouflons (*Ovis orientalis*). Red deer and Persian fallow deer shared the same enclosure, whereas all other species were kept in separate enclosures. Parasitological management in ruminants consisted of regular coproscopical monitoring and metaphylactic anthelmintic treatment with fenbendazole (Fenbendat 5%, aniMedica GmbH, Im Südfeld 9, 48308 Senden-Bösensell, Germany; 2 mg/kg bodymass p.o., daily mixed in pelleted feed), due to previous problems with parasitic bronchitis.²⁴ With regard to the bison, metaphylactic treatment had been changed to doramectin (Dectomax®, Elanco Animal Health, Werner-Reimers-Str. 2–4, 61352 Bad Homburg, Germany; 0,2 mg/kg estimated bodymass, i.m. via teleinjection) at the beginning of 2018, due to a perceived decline in fenbendazole efficacy. Bison < 4 years of age were scheduled for four doramectin treatments, older animals for two treatments per year.

Before translocation, a parasitological examination had revealed a low level of strongyle egg excretion and the bison had been treated with ivermectin (Ivomec®, Boehringer Ingelheim Vetmedica GmbH, Binger Strasse

173, 55216 Ingelheim, Germany; 0,2 mg/kg bodymass s.c.). Two months after the translocation, the animal showed diarrhoea, weight loss, general weakness and pale mucous membranes. Haematological examination revealed a haematocrit of 6,3% (reference range: 22,5–39,9%), erythropenia (1,53 million/ μ l; reference range: 4,47–9,35 million/ μ l), low haemoglobin (3,38 g/dl; reference range: 11,9–20,6 g/dl), hypoproteinaemia (3,9 g/dl; reference range: 6,0–8,5 g/dl) and uraemia (32 mg/dl; reference range: 6–22 mg/dl). Despite treatment with doramectin (0,2 mg/kg bodymass s.c.), penicillin (Procain Penicillin G, aniMedica GmbH, Germany; 20 mg/kg i.m.), dexamethasone (Dexatad[®], aniMedica GmbH, Germany; 0,06 mg/kg i.m.) and menbutone (Menbutil[®], aniMedica GmbH, Germany; 7,5 mg/kg i.m.), clinical signs did not improve, and the animal was euthanized three days after initial presentation because of its inability to stand. *Post-mortem* examination revealed cachexia, serous fluid in the abdominal and thoracic cavities as well as in the pericardium, and a diffuse, lymphoplasmacellular and granulomatous abomasitis as the main findings.

The remaining members of the bison herd did not show any clinical signs of disease but were nonetheless treated with doramectin (0,2 mg/kg estimated bodymass i.m., via teleinjection).

Parasitological investigations

Abomasum content of the euthanized bison was sent to the Institute for Parasitology, University of Veterinary Medicine Hannover, where it was washed through a 50 μ m sieve. Recovered helminth specimens were microscopically examined.

Faecal samples taken from three herd members of the deceased individual before and 14 days after doramectin treatment were examined semiquantitatively using approximately 5 g faeces by a combined sedimentation-flotation technique²⁵ with zinc sulfate as flotation medium (specific gravity [SG]: 1,3). Level of egg/oocyst excretion was classified semi-quantitatively as follows: +: low (1–5 parasite stages detected), ++: moderate (5–10 parasite stages), +++: high (11–20 parasite stages), ++++: very high (>20 parasite stages). Furthermore, faecal egg counts (FEC) were determined using a modified McMaster technique²⁵ with sodium chloride as flotation medium (SG: 1,2). The remaining samples of the two coproscopically positive individuals were subjected to larval culture²⁶ at 26 °C for ten days.

Furthermore, pooled faecal samples were obtained from all four bison groups as well as from further ruminant species in the wildlife park and analysed by combined semiquantitative sedimentation-flotation. Samples positive for strongyle eggs were subjected to larval culture as described above.

Molecular parasite identification and pyrosequencing

Obtained L3 were subjected to DNA isolation in pools of 1000 (two bison samples from the affected herd) or 100–200 larvae each, depending on the number of available larvae. DNA isolation of the samples containing 1000 larvae was carried out using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Valenciennes Str. 11, 52355 Düren, Germany) according to the manufacturer's instructions, with prior homogenization by bead-beating in the Precellys[®] 24 (PEQLAB Biotechnology GmbH, Carl-Thiersch-Str. 2b, 91052, Erlangen, Germany; 2 x 10 s at 5000 rpm). For the remaining samples, the DirectPCR[®] Cell Lysis reagent (PEQLAB Biotechnology GmbH) was used. Larvae were pelleted by centrifugation (10 min at 3,000 g), the supernatant removed, and 90 μ l reagent as well as 10 μ l proteinase K were added to the pellet, followed by incubation at 55 °C for 16 hrs and 85 °C for 45 min.

To confirm the nematode species assignment, two samples from the clinically affected herd were subjected to amplification of the ITS1-5.8S-ITS2 rDNA region using pan-nematode primers NC5 and NC2²⁷ in a 25 μ l reaction mixture containing 0,5 μ l DreamTaq polymerase (Thermo Fisher Scientific, ABgene House, Blenheim Road, KT19 9AP Epsom, United Kingdom), 2,5 μ l 10x buffer, 0,5 μ l dNTP mix (10 mM each), 0,5 μ l of each primer (10 μ M each) and 5 μ l DNA template. Thermocycling conditions comprised 3 min at 95 °C, 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 90 s, and final extension at 72 °C for 10 min. After visualisation by gel electrophoresis, PCR products were Sanger-sequenced (Microsynth Seqlab Sequencing Laboratories, Maschmühlenweg 36, 37081 Göttingen, Germany) and compared to publicly available sequences using NCBI BLAST. These samples as well as the remaining larval DNA samples were also amplified by *H. contortus* species-specific PCR,²⁸ allowing the detection of *H. contortus* even in mixed-species samples. The reaction mixture and thermoprofile were as described above, except that the annealing temperature was adjusted to 58 °C.

Real-time pyrosequencing of codon 167 and 200 of the β -tubulin isotype 1 gene was carried out as previously described.¹⁹ Briefly, 10 μ l template was added to 40 μ l reaction mixture containing 1 μ l DreamTaq polymerase, 5 μ l 10x buffer, 1 μ l dNTP mix (10 mM each) and 1 μ l each of primers HcPy2PCR-Forw and biotinylated HcPy2PCR-Rev (10 μ M each) to amplify both β -tubulin isotypes.¹⁹ The thermocycling protocol comprised 3 min at 95 °C, 40 cycles of 30 s at 94 °C, 30 s at 53 °C, 1 min at 72 °C, and 10 min at 72 °C. Real-time pyrosequencing of 40 μ l PCR product with sequencing primers Hc167PySeq1 and Hc200PySeq1 was carried out using the PSQ 96 MA instrument (Biotage Sweden AB, Vimpelgatan 5, 753 18 Uppsala, Sweden), PyroMark[®] Gold Q96 reagent kit (Qiagen, Qiagen Str. 1, 40724 Hilden, Germany) and Streptavidin Sepharose[™] beads (GE Healthcare, Beethovenstrasse 239, 42655 Solingen, Germany).

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Results

Several dozen nematodes, 1.5–3.0 cm in length, were recovered from the abomasal content of the euthanised bison. In total, 50 adult specimens (18 males, 32 females) were examined in detail and identified as *Haemonchus contortus* based on body length, bursa copulatrix characteristics (males) and shape of cervical papillae (Figure 1).^{29,30}

Faecal samples from two herd members of the euthanised individual contained strongyle eggs, with FEC values of 2233 eggs per gram faeces (EpG) and 133 EpG, respectively (pre-treatment with doramectin). After treatment with doramectin, the FEC decreased to 800 EpG in the first animal (64.2% reduction), whereas in the other animal an increase to 533 EpG was observed. The third animal examined coproscopically was negative pre- and post-treatment. L3 obtained from the pre-treatment faecal samples were identified as *H. contortus* based on morphological characteristics, which was confirmed by PCR and sequencing of the ITS1–5,8S-ITS2 rDNA region. The obtained sequences from both animals were identical and showed 99.6% identity (100% query cover) to various publicly available *H. contortus* sequences. Real-time pyrosequencing revealed 100% frequency of the F200Y SNP in both samples, while the resistance-associated mutation F167Y was not detected (Table 1).

Furthermore, 10 pooled faecal samples from 4 different bison groups as well as 12 pooled samples from six further ruminant species, including mouflon, red deer, Persian fallow deer, sika deer, reindeer and elk, were screened for parasite stages (Table 1). Strongyle eggs were detected in all examined species, while *Trichuris* spp. eggs were noted in samples of fallow deer, sika deer and elk. Elk samples also contained *Nematodirus* spp. eggs. *Moniezia* spp. eggs were detected in one bison sample only. Most samples also contained *Eimeria* spp. oocysts. Positive larval cultures were obtained from 14 samples, and *H. contortus* L3 larvae were molecularly identified



Figure 1: (A) Anterior end (100× magnification) and (B) bursa copulatrix (50× magnification) of *Haemonchus contortus* from the abomasum of a European bison. The solid arrows indicate the cervical papillae and the arrowhead indicates the asymmetric dorsal lobe with y-shaped dorsal ray as distinguishing features of the genus *Haemonchus*.

in five of these, originating from all four bison groups, as well as the mouflons. By real-time pyrosequencing, a F200Y allele frequency of 100% was detected in two bison and the mouflon sample, while the pyrogram quality of two further bison samples was too low for interpretation. No resistance-associated SNPs were detected at codon 167 (Table 1).

Discussion

Control of parasitic gastroenteritis in ruminants kept in zoological gardens and wildlife parks can be challenging, because it mostly relies on anthelmintic treatment, which may favour AR. In the present case, detection of *H. contortus* isolates with the BZ resistance associated F200Y mutation was especially concerning in the light of the fact that the wildlife park regularly raises European bison for reintroduction into the wild. Although the species' conservation status has recently been changed from "vulnerable" to "near threatened"³¹ due to protection efforts, there are only few viable free-living subpopulations, and introduction of anthelmintic resistant parasites may represent a further threat to these populations.¹³ As shown by the present case, haemonchosis can cause severe clinical signs and even be fatal in European bison. Here, the affected animal may have been immunologically naïve to *H. contortus* or may not have developed sufficient immunity prior to its relocation, which may have led to a particularly severe disease course. Furthermore, stress due to the relocation may have induced immunosuppression and contributed to the severity of the disease. *Haemonchus contortus* has previously been detected in captive and wild European bison,^{13,32} although another highly pathogenic abomasal parasite, *Ashworthius sidemi*, which was introduced into European ruminant populations from Asia, is currently considered of higher concern for wild populations.³⁰ Furthermore, presence of anthelmintic resistant parasites in wild ruminants may also represent a risk for domestic livestock in cases of habitat overlap.^{14,15}

Application of sub-therapeutic doses represents one of the most important factors contributing to AR.³³ Due to previous problems with parasitic bronchitis and restricted possibilities regarding individual-animal treatment, e.g. via pour-on or injectable formulations, oral administration of fenbendazole mixed with pelleted feed at a low dose of 2 mg/kg bodymass was a common practice at the wildlife park. This subtherapeutic dose facilitated acceptance of the medicated feed by the animals, but likely represented a strong selection pressure favouring the resistant F200Y genotype. The F200Y resistance allele was present in *H. contortus* isolates from multiple bison groups as well as from mouflons, all kept in separate enclosures, indicating either cross-contami-

nation between enclosures or multiple occasions of AR selection. In line with previous studies, the F167Y mutation, which is generally rare in field settings,^{19,22} was not detected.

In addition, the FECs determined in two bison before and after doramectin treatment via teleinjection indicated possible macrocyclic lactone (ML) resistance. In fact, both MLs and BZs select for the F200Y and F167Y SNPs, however, these SNPs alone are not sufficient to lead to an ML resistant phenotype.³⁴ Alternatively, the lack of a FEC reduction after doramectin treatment may have been due to a subtherapeutic dose, as bodymass was only estimated instead of measured. Furthermore, it should be taken into account that the true therapeutic dose rate may differ between ruminant species due to differences in drug metabolism.³⁵ Comparative pharmacokinetics data from bison vs. other ruminants are lim-

ited, although one study indicated that ivermectin applied at the cattle dose rate effectively eliminated *Ostertagia ostertagi* from experimentally infected American bison (*Bison bison*).³⁶ So far, no molecular test for ML resistance in trichostrongylid nematodes exists,²³ but a larval development assay or larval migration inhibition assay³⁷ may shed further light on the ML resistance status of the *H. contortus* isolates in question.

Veterinarians should be aware that administration of anthelmintics mixed in feed, as well as estimating instead of measuring bodymass, pose the risk of subtherapeutic doses. Likewise, unnecessary frequent treatments may accelerate selection for AR. In contrast, targeted treatment (TT) or targeted selective treatment (TST) strategies, as developed for small ruminants and cattle, can counteract AR by maintaining susceptible parasite populations in refugia, i.e. unexposed to anthel-

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A. Springer, P. Kloene, C. Strube

Table 1: Parasite stages detected in faecal samples from different ruminant species in a wildlife park and pyrosequencing results regarding benzimidazole resistance-associated SNPs of the obtained *H. contortus* larvae. Level of egg/oocyst excretion was classified semi-quantitatively as follows: +: low, ++: moderate, +++: high, ++++: very high.

Sample type	Host species	Strongyle eggs	Nematodirus spp.	Trichuris spp.	Moniezia spp.	Eimeria spp. oocysts	<i>H. contortus</i> in larval culture	F200Y allele frequency (%)	F167Y allele frequency (%)
Indiv.	European bison (group 1)	++++	-	-	-	+++	yes	100	0
Indiv.	European bison (group 1)	++++	-	-	-	-	yes	100	0
Pooled	European bison (group 1)	++++	-	-	+	+++	yes	100	0
Pooled	European bison (group 1)	++++	-	-	-	+	no	n.d.	n.d.
Pooled	European bison (group 2)	++	-	-	-	++	no	n.d.	n.d.
Pooled	European bison (group 2)	++	-	-	-	+++	no	n.d.	n.d.
Pooled	European bison (group 2)	++++	-	-	-	+++	yes	unsuccessful	n.d.
Pooled	European bison (group 3)	++	-	-	-	++	no	n.d.	n.d.
Pooled	European bison (group 3)	+	-	-	-	++	yes	100	0
Pooled	European bison (group 4)	+++	-	-	-	+	yes	unsuccessful	n.d.
Pooled	European bison (group 4)	-	-	-	-	+++	n.d.	n.d.	n.d.
Pooled	European bison (group 4)	-	-	-	-	-	n.d.	n.d.	n.d.
Pooled	Mouflon	++	-	-	-	-	yes	100	0
Pooled	Mouflon	+	-	-	-	+	no	n.d.	n.d.
Pooled	Mouflon	++	-	-	-	+	no	n.d.	n.d.
Pooled	Red deer	-	-	-	-	+	n.d.	n.d.	n.d.
Pooled	Red deer	-	-	-	-	-	n.d.	n.d.	n.d.
Pooled	Red deer	+	-	-	-	+	no	n.d.	n.d.
Pooled	Persian fallow deer	+	-	+	-	-	no	n.d.	n.d.
Pooled	Sika deer	+	-	+	-	-	no	n.d.	n.d.
Pooled	Reindeer	+++	-	-	-	+	no	n.d.	n.d.
Pooled	Elk	++	+	++++	-	++	no	n.d.	n.d.
Pooled	Elk	-	+	++++	-	+	no	n.d.	n.d.
Pooled	Elk	++	+	++++	-	+	no	n.d.	n.d.

Abbreviations: indiv.: individual; n.d.: not determined

Benzimidazole resistant *Haemonchus contortus* in a wildlife park

A. Springer, P. Kloene, C. Strube

mintics. The former strategy entails treating the whole herd or groups of animals based on diagnostic information or specific risks, while the latter directs treatment to individual animals, based on EpGs and/or production parameters and clinical indicators.^{38–40} A drawback of TST is the necessity for individual-animal diagnostics and treatment, which may not always be possible in wildlife parks, e.g. with regard to semi-free ranging cervids. In addition, use of combination products containing anthelmintics with different modes of action may delay AR, but only in the presence of sufficiently large refugia.⁴¹ Furthermore, pasture management is an important aspect of controlling GINs in domestic ruminants, e.g. by employing rotational grazing strategies.⁴² However, this is seldom possible in zoological gardens or wildlife parks with limited available space.

In addition to the observations regarding *H. contortus*, the high *Trichuris* spp. egg counts in elk were also concerning, as this species is particularly susceptible to this parasite.⁴³ *Trichuris* eggs are highly resistant in the environment; therefore considerable infection pressure may readily build up in continuous grazing systems.⁴⁴

In conclusion, anthelmintic resistance of GINs does not only play a role in (intensive) livestock farming, but can also represent a challenge for the management of zoological collections. As the development of AR is not reversible, veterinarians dealing with such collections should be aware of sustainable strategies to conserve anthelmintic efficacy as long as possible. In the future, further accompanying measures may become available, including biological control measures such as nematophagous fungi, which are not yet available on the European market.⁴⁵

***Haemonchus contortus* résistant au benzimidazole dans un parc animalier**

La résistance aux anthelminthiques (RA) des nématodes gastro-intestinaux (NGI) des ruminants constitue un problème majeur dans le monde. Parmi les NGI, le parasite hématophage de la caillette *Haemonchus contortus* est particulièrement pathogène et peut montrer une résistance contre toutes les principales classes de substances anthelminthiques. Dans la présente étude, la mort d'un bison d'Europe de 1,5 ans (*Bison bonasus*) d'un parc animalier allemand en raison d'une hémonchose malgré un traitement anthelminthique fréquent du troupeau avec du fenbendazole ainsi que de la doramectine a incité une enquête sur la RA. Des échantillons de matières fécales regroupés ont été collectés auprès de quatre groupes de bisons différents ainsi que de mouflons (*Ovis orientalis*), de wapitis (*Alces alces*), de rennes (*Rangifer tarandus*), de cerfs sika (*Cervus nippon*), de daims de Perse (*Dama mesopotamica*) et de cerfs élaphe (*Cervus elaphus*) hébergés par le parc animalier. Après examen coproscopique, des cultures larvaires fécales ont été réalisées. Des cultures larvaires positives pour *Haemonchus contortus* ont été examinées plus avant pour les polymorphismes génétiques associés à la résistance au benzimidazole aux codons 167 et 200 du gène de l'isotype 1 de la β -tubuline par pyroséquençage en temps réel. Des infections à *H. contortus* ont été détectées dans les quatre groupes de bisons, ainsi que chez les mouflons. Dans cinq échantillons, représentant deux groupes de bisons et les mouflons, la fréquence du polymorphisme mononucléotidique (SNP) associé à la résistance au codon 200 était de 100 %. En revanche, les SNP associés à la résistance n'ont pas été détectés au codon 167. De plus, le nombre d'œufs par gramme de fèces de deux bisons avant et 14 jours après le traitement

***Haemonchus contortus* resistente al benzimidazolo in un parco faunistico**

La resistenza agli antielmintici (RA) dei nematodi gastrointestinali (GIN) dei ruminanti costituisce un problema importante a livello mondiale. Tra i GIN, il parassita abomasale che si nutre di sangue *Haemonchus contortus* è particolarmente patogeno e può mostrare resistenza contro tutte le principali classi di sostanze antielmintiche. Nel presente studio, la morte di un bisono europeo di 1,5 anni (*Bison bonasus*) di un parco faunistico tedesco a causa di una emonchosi, nonostante il frequente trattamento antielmintico della mandria con fenbendazolo e doramectina, ha spinto ad indagare sulla RA. Campioni fecali raggruppati sono stati raccolti da quattro diversi gruppi di bisonti, nonché da mufloni (*Ovis orientalis*), alci (*Alces alces*), renne (*Rangifer tarandus*), cervi sika (*Cervus nippon*), daini persiani (*Dama mesopotamica*) e cervi nobili (*Cervus elaphus*) ospitati nel parco naturale. Dopo l'esame coproscopico, sono state stabilite le colture larvali fecali. Le colture larvali risultate positive di *Haemonchus contortus* sono state ulteriormente esaminate per i polimorfismi genetici associati alla resistenza al benzimidazolo ai codoni 167 e 200 del gene isotipo 1 della β -tubulina mediante pirosequenziamento in tempo reale. Le infezioni da *H. contortus* sono state rilevate in tutti e quattro i gruppi di bisonti, così come nei mufloni. In cinque campioni, che rappresentano due gruppi di bisonti e i mufloni, la frequenza del polimorfismo a singolo nucleotide (SNP) associato alla resistenza al codone 200 era del 100%. Al contrario, gli SNP associati alla resistenza non sono stati rilevati al codone 167. Inoltre, il conteggio delle uova fecali di due bisonti prima e 14 giorni dopo il trattamento parenterale con doramectina ha indicato una possibile resisten-

parentéral à la doramectine a indiqué une possible résistance aux lactones macrocycliques. La détection de nématodes résistants aux anthelminthiques chez ces animaux était particulièrement préoccupante à la lumière de la réintroduction prévue dans la nature. Étant donné que le contrôle des helminthes dans les jardins zoologiques et les parcs animaliers repose principalement sur un traitement anthelminthique en raison des possibilités limitées concernant les pratiques de gestion telles que le pâturage en rotation, des précautions doivent être prises pour éviter un sous-dosage ou des traitements fréquents inutiles facilitant le développement de la RA.

Mots clés: trichostrongylidés, nématodes gastrointestinaux, ruminants, bison d'Europe, *Bison bonasus*, résistance anthelminthique

Haemonchus contortus resistentis encunter Benzimidazol en in parc da selvaschinas

La resistenza encunter anthelmintics (AR) da nematods gastrointestinals (GINs) tier remegliaders caschuna in grond problem mundial. Denter ils GINs ei il parasit tschetschasaung el magun da cuagl *Haemonchus contortus* particularmein patogens e sa mussar resistenzas encunter tut las classas substanzialas anthelminticas. El studi presentau ha la mort d'in bison europeic (*Bison bonasus*) en in parc da selvaschinas tudestg, caschunada dad ina hemonchosa e quei malgrad tractament dalla muntanera frequentau cun fenbendazol e doramectin, menau tier ina perscrutaziun sin AR.

Emprovas dad excrements mischedadas ein vegnidas rimnadas el parc da selvaschinas da quater differentas gruppas da bisons, plinavon da mufflons (*Ovis orientalis*), elans (*Alces alces*), renns (*Rangifer taradus*), tshieriv sica (*Cervus nippon*), tshieriv dama persic (*Dama mesopotamica*) e tshieriv tgietschen (*Cervus elaphus*) ed intercuretgas parasitologicamein. Suenter las examinaziuns coproscopicas ein culturas da larvas ord ils excrements vegnidas cultivadas. Culturas positivas cun *Haemonchus contortus* ein vegnidas examinadas plinavon sin polimorfissemes genetics en connex cun resistenzas encunter benzimidazol vid ils codons 167 e 200 dil gen β -tubulin-isotip-1 tras pirosequenzaziun en tems real.

Tier tut las quater gruppas da bisons e tir ils mufflons han ins demussau infecziuns cun *H. contortus*. La frequenzaziun dil polimorfissem singul-nucleotid assoziatus cun resistenzas pertuccava 100% tier codon 200 en tschun emprovas, duas gruppas da bison e ils mufflons. Perencunter ein vegni demussai negins polimorfissemes

za ai lattoni macrociclici. Il rilevamento di nematodi resistenti agli antielmintici in questi animali è particolarmente preoccupante alla luce della prevista reintroduzione in natura. Poiché il controllo degli elminti nei giardini zoologici e nei parchi faunistici si basa principalmente sul trattamento antielmintico a causa delle limitate possibilità relative alle pratiche di gestione come il pascolo a rotazione, si dovrebbe fare attenzione ad evitare il sotto dosaggio o trattamenti frequenti non necessari che facilitano lo sviluppo della RA.

Parole chiave: trichostrongyle, nematodi gastrointestinali, ruminanti, bisonte europeo, *Bison bonasus*, resistenza antielmintica

assoziai cun resistenzas tier il codon 167. Suplementar mussavan las dumbraziuns dad ovs els excrements da dus bisons 14 dis avon e suenter il tractamen parenteral cun doramectin ina lacton-resistenza macrociclica. Il mussament da nematods cun resistenzas anthelminticas era particularmein inquietonts tier quels animals en vesta alla intenziun da schar liber els el selvadi.

Pervia da las pussevkladads limitadas da cumbatter preventivamein ils helminths, sco pastira da rotaziun, ein ils curtgins zoologics ed ils parcs da selvaschinas il bia dependents dad in tractament anthelmintic. Perquei ston sutdosas ni tractaments memia frequentai vegni evitai, pertgei quels favoriseschan la derasaziun dad AR.

Plaid-clav: Trichostrongylids, nematods gastrointestinals, remegliaders, bison europeic, *bison bonasus*, resistenza anthelmintica

Benzimidazole resistant *Haemonchus contortus* in a wildlife park

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