



Multiple infestations of gastrointestinal parasites – Probable cause for high mortality of Spot-billed Pelican (*Pelecanus philippensis*) at Kokrebellur Community Reserve, India

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ABSTRACT

We witnessed mortalities of Spot-billed Pelicans *Pelecanus philippensis* between December 2017 and May 2018 in Mandya and Mysuru districts of Karnataka, especially at Kokrebellur Community Reserve in Mandya district. The region has experienced severe drought in recent years with negligible water in all the water tanks. A total of 67 Spot-billed Pelicans died in five locations, of which 55 adult birds died at Kokrebellur. We collected four dead pelicans along with 97 fecal samples of live birds at Kokrebellur, water samples from nine water tanks around Kokrebellur, and six fish samples. We isolated the endoparasite eggs by following sedimentation and flotation technique, and counted the eggs from the water and fecal samples, and identified at the genus level using light microscope. We approximately counted the endoparasites by dissecting the fish and conducting a necropsy on dead pelicans. Endoparasite eggs were detected in seven of the nine water tanks. Each fish sample had at least 50–100 L3 stage worms of *Contracaecum* sp., and $880.0 \pm 459.3_{SD}$ of *Contracaecum* sp., worms in the digestive tracts and $60.0 \pm 36.5_{SD}$ worms of *Echinostoma* sp. in the intestine of the four dead pelicans. The endoparasite prevalence was 84.5% (N = 83) with a mean abundance of $368.2 \pm 561.5_{SD}$ eggs/g in the fecal samples of live pelicans. *Contracaecum* sp., *Echinostoma* sp. and *Opisthorchis viverrini* were recorded in 51, 67 and nine fecal samples respectively. The high load of endoparasite eggs in the water tanks, an infestation of *Contracaecum* sp. in fishes and a heavy load of fully-grown worms of *Contracaecum* sp. and *Echinostoma* sp. in the adult pelicans are indicative of their high mortality in Kokrebellur Community Reserve. The coordinated program was initiated with the support of all stakeholders to control the endoparasites in water, fish, and pelicans.

1. Introduction

High mortality events within wild populations of various species are not uncommon including in birds. Amongst birds, in particular, a natural outbreak of parasites, pathogens, and human-induced catastrophes are widespread cause for high mortalities. Mass mortalities of birds also reported due to endoparasites, e.g. helminths in Eurasian Cranes *Grus* (Fanke, 2013), Dalmatian Pelican *Pelecanus crispus* (Pyrovetsi and

Papazahariadou, 1995), Brown Pelican *Pelecanus occidentalis* (Grimes et al., 1989; Dyer et al., 2002), helminths and acanthocephalan in Eider Ducks *Somateria mollissima* (Borgsteede et al., 2005), Brown Pelican (Courtney and Forrester, 1974) and American White Pelican *Pelecanus erythrorhynchos* (Kinsella et al., 2004) and nematodes in Great Blue Herons *Ardea herodias* (Wiese et al., 1977). Even though the consequences of such high mortalities are not clear, however, the loss of individuals may lead to population decline with occasional local

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extinction of a species, for example, vultures in India (Prakash et al., 2003). An elaborate understanding of the causes for high mortality events is therefore crucial to channelize management actions for the survivability of remaining individuals.

Mass mortality of water birds particularly Spot-billed Pelicans *Pelecanus philippensis* were reported from Mandya and Mysuru districts of Karnataka, specifically at Kokrebellur Community Reserve in Mandya district between December 2017 and May 2018. The Spot-billed Pelican from Pelicanidae family are piscivorous (Taher, 2007) and breeds in South Asia from southern Pakistan across India east to Indonesia (BirdLife International, 2019). Spot-billed Pelican is widely distributed in India, with no records of mass mortality. However, one dead bird each from Andhra Pradesh (Sreedevi et al., 2017), Assam (Islam and Talukdar, 2009) and Tamil Nadu (Pazhanivel et al., 2017), was reported to be infested with endoparasite *Contracaecum* sp.

The other study (Muralidharan, SACON, Unpublished report) which explored the possible toxicological reasons for the current mass mortality of Pelicans in and around Kokrebellur, reported none of the heavy metals or pesticide contaminants were high enough to cause toxicity and mortality in Spot-billed Pelicans of the five heavy metals viz. Copper, Zinc, Cadmium, Chromium and Lead; and approximately 30 pesticides from the tissue samples of different body parts. Therefore, gastrointestinal parasites infestation is the only attributed reason for the cause of their mortalities. These findings prompted us to explore the gastrointestinal parasites in Spot-billed Pelican in Kokrebellur in light of mass mortality of them. We attempted to explore the possibilities of gastrointestinal parasites (henceforth called as endoparasites) infestation in Spot-billed Pelicans, fishes, and water of select water tanks around Kokrebellur Community Reserve.

2. Materials and methods

Study site: Kokrebellur Community Reserve (12° 30' 41.74" N and 77° 05' 18.67" E, elevation: 800 m asl) is at Maddur taluk, Mandya district, Karnataka, India (Fig. 1). Kokrebellur is the name of the village, which is derived from 'Kokre' meaning stork and 'Bellur' meaning 'white' village. This village is well known for the large colonies of Painted Storks (*Mycteria leucocephala*) and Spot-billed Pelicans nesting in ficus (*F. religiosa*, *F. bengalensis*), tamarind (*Tamarindus indica*), babul (*Acacia nilotica*), and Indian tulip (*Thespesia populnea*) trees and coexists with the villagers (Neginhal, 1977). We selected nine water tanks around Kokrebellur for water samples, namely, Deshahalli, Tylur,

Gurudevahalli, Iggalur, Kudiluvagilu, Madarahalli, Malavalli, Shimsha Barrage, and Sulekere Tank (Fig. 1).

2.1. Data collection

Population and Death Records: We counted the nests at Kokrebellur nesting site in October 2017 and December 2017–January 2018. We collected all the death records of Spot-billed Pelicans from the local forest watchers and naturalists for the Mysuru and Mandya district between December 2017 and May 2018. Every day, all the nesting trees were visited by a local person to monitor the birds and their deaths. Any notice of dropping neck in pelicans or decomposing smell of a bird was reported immediately to the local veterinary doctor and forest department personnel. Those birds were recovered from the nesting sites and taken to the feeding cage and provided the fish and treated them for the worms, however, none of the birds could survive from the treatment or food.

Sampling for endoparasites: We carried out endoparasite sampling in Spot-billed Pelican, fish, and water between February and March 2018. At each of the Spot-billed Pelican nest sites at Kokrebellur, we placed paper sheets on the ground to collect the fecal samples in the early morning hours. The usual quantity of pelican feces was about 1–1.5 g, and it used to be in semisolid condition. Efforts were made to collect only the pelican droppings upon confirmation, which were then stored in 15 ml centrifuge tubes. We weighed 1 g of fecal material from each dropping using 10 g-Pesola weighing balance, and collected a total of 97 fecal samples of Spot-billed Pelican and fixed in 10% of formalin solution.

We collected water samples from nine select water tanks frequently visited by the pelicans around Kokrebellur. We sampled water from four corners of each water tank at a distance of two to 3 m from the edge. We collected 250 ml of water by using 250 ml plastic bottles (250 ml disposable plastic bottle for a veterinary purpose: Shijiazhuang Xinfuda Medical Packaging Co., Ltd) and collected 36 water samples from nine water tanks. We added 1 ml of 10% formalin solution to the water samples.

We labeled all the centrifuge tubes with pelican fecal samples and plastic bottles with water samples with a specific identification number, date, and geo-coordinates, and entered the same information in data sheets. The samples were transported to the laboratory within three days of sample collection.

We also collected five fish samples from Sulekere tank along with

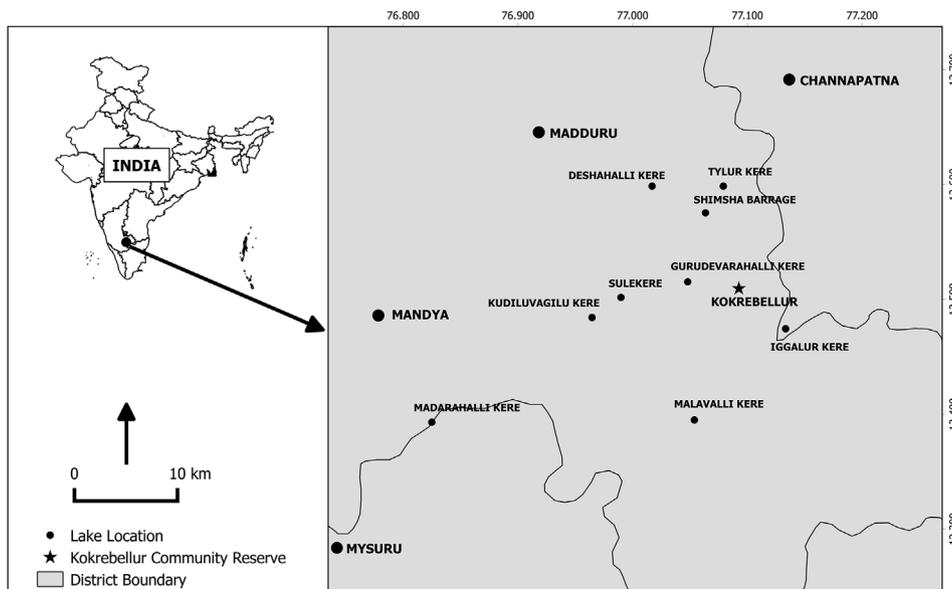


Fig. 1. Sampling locations of select water tanks that are frequented by Spot-billed Pelicans and Kokrebellur.

Table 1
Endoparasite load in different water tanks around Kokrebellur.

Tank Name	Geocoordinates	<i>Echinostoma</i> sp.	<i>Contracaecum</i> sp.
Deshahalli	E77.0171, N12.5984	0	0
Gurudevahalli	E77.0480, N12.5156	0	30.3 ± 47.4
Iggalur	E77.1334, N12.4756	0	6.3 ± 12.5
Kudiluvagilu	E76.9634, N12.4838	0	18.8 ± 23.9
Madarahalli	E76.8252, N12.3920	0	10.0 ± 20.0
Malavalli	E77.0534, N12.3947	0	53.3 ± 72.5
Shimsha Barrage	E77.0654, N12.5756	0	11.3 ± 13.1
Sulekere	E76.9898, N12.5020	13.3 ± 16.3	116.8 ± 233.5
Tylur	E77.0780, N12.5984	0	0

one fish sample which had dropped from a pelican nest while the adults fed the chicks. We dissected the fishes and counted the endoparasite worms. We conducted a necropsy of dead pelicans and roughly counted the endoparasite worms (Greenacre and Morishita, 2014). The necropsy of dead pelicans was done at local Veterinary Dispensary.

Isolation of endoparasite from water samples: We transferred each 250 ml water sample into five 50 ml centrifuge tubes. The tubes were then centrifuged for 10 minutes at 1800 rpm, and discarded the resultant supernatant and transferred the pellets of all the five 50 ml centrifuge tubes to a 15 ml centrifuge tube. This process of accumulation of pellet was repeated for all the 36 water samples. The obtained pellet is further subjected to isolation and detection of endoparasites using sedimentation and flotation techniques.

Isolation of endoparasites from fecal sample of pelicans: For each method, we took 1 g of fecal material in a 15 ml centrifuge tube, added distilled water and homogenized the mixture using a vortex mixer. We used cheesecloth to separate coarse material from the homogenized mixture, and centrifuged the filtrate for 10 minutes at 1800 rpm. After discarding the supernatant, we used the fecal pellet for further isolation and detection of the endoparasites using sedimentation and flotation techniques (World Health Organization, 1991; Garcia, 2009; Gillespie, 2005).

Sedimentation method: We resuspended the fecal pellet in 10 ml of dilute soap solution (two-three drops of liquid soap in 100 ml of distilled water) in a centrifuge tube. Since the specific gravity of soap solution is less than the specific gravity of most of the parasitic eggs or oocysts that ranges from 1.05 to 1.23 g/ml (Smear, 2009), this allowed the eggs to settle with the pellet. We again centrifuged the mixture for 5 minutes at 5000 rpm. We discarded the supernatant, leaving a few drops of suspension on the pellet. We transferred the pellet suspension from sedimentation to one of the McMaster's chambers (Cringoli et al., 2004). We observed each chamber under the microscope (Lynx PH-100, LM-52-1804/SL.No. 100044) for identification and counting of parasite eggs/oocysts (MAFF, 1979).

Flotation method: This method allows isolation and identification of helminth eggs and protozoan oocysts in the sample. For this, we resuspended the fecal pellet in 10 ml of saturated sucrose solution (specific gravity 1.3 g/ml) and mixed thoroughly. We increased the mixture up to the brim of the centrifuge tube and centrifuged for 10 minutes at 4000 rpm. We transferred the upper layer of the mixture (0.3 ml) to McMaster's chamber using transfer pipettes and allowed it to settle for 5 min for the eggs to float to the surface (Dryden et al., 2005).

We counted the eggs under a light microscope with a 10× objective. We separately photographed and measured the eggs/oocysts in each grid of the McMaster's slide using a microscope camera (ISH500) with the help of IS Capture 3.6.6 software (ISCapture.ink) and saved them with specific IDs.

Identification of oocyst/egg: We identified parasite prevalence through the photographs showing shape, colour, size, of the oocyst/egg or larvae unique to each species (Griffiths, 1978). We identified and counted the eggs or oocysts using identification keys (Jessee et al., 1970; Collet et al., 1986; Bowman et al., 1999; Arcari et al., 2000;

Chiodini et al., 2003; Taira et al., 2003).

Analysis: We compared the mean egg load in the samples using ANOVA in SPSS version 16.0 (SPSS Inc, 2007).

3. Results

Pelican death records: A total of 67 Spot-billed Pelicans died in five water bodies including 55 in Kokrebellur near Maddur, seven in Kukkarahalli tank in Mysuru, three in Sulekere and one each in Madarahalli near Maddur and Malavalli road. All the dead birds in different water bodies were adults.

Pelican population size: A total of 180 nests were counted in October 2017 at Kokrebellur, however, the number of nest count got reduced to 60 by December 2017 and January 2018. The initial pelican count in October 2017 was ca. 360, which got reduced to 120–140 by January 2018. Some pelicans abandoned the nests from the Kokrebellur nesting sites, and 55 pelicans died in the nesting site. The computed pelican population decline due to mortality was at least 15%.

Endoparasites in water tanks: Eggs of endoparasites were detected in seven of the nine water tanks (Table 1). The mean endoparasite eggs per 250 ml of water were 30.3 ± 47.4_{SD} in Gurudevahalli, 6.3 ± 12.5_{SD} in Iggalur, 18.8 ± 23.9_{SD} in Kudiluvagilu, 10.0 ± 20.0_{SD} in Madarahalli, 53.3 ± 72.5_{SD} in Malavalli tank, 11.3 ± 13.2_{SD} in Shimsha barrage, and 130.0 ± 246.8_{SD} in Sulekere tank. The mean endoparasite eggs across different water tanks did not vary significantly ($F_{27, 8} = 0.889$, $p = 0.539$). Of all the tanks Sulekere tank had the highest endoparasite egg load that is mostly of *Contracaecum* sp. The endoparasites were not detected in Deshahalli and Tylur tanks.

Endoparasites in fish and dead pelicans: All the fish samples had at least 50–100 L3 stage worms of *Contracaecum* sp. (Fig. 2A). Of the 55 pelicans died in the Kokrebellur nesting sites, the dead pelicans were recovered in different decomposing states. Of that, four birds were recovered just before or after the death. Thus the information from necropsy of those four birds was considered for identification of endoparasite and their count. Extent of post-mortem shows that no lesions in trachea and lungs, no abnormality in liver, spleen, heart, and kidney, but large number of ulcers and damaged mucosa was recorded in early part of intestine, proventriculus, and gizzard, and recorded clogged worms as spindle at proventriculus and gizzard was recorded in all the four samples. They had no food or fish in the digestive system, and the *Contracaecum* sp. count was 880.0 ± 459.3_{SD} worms in proventriculus and gizzard, and *Echinostoma* sp. count was 60.0 ± 36.5_{SD} worms in the early part of the intestine (Fig. 2B).

Endoparasites in fecal samples of pelicans: Of the 97 fecal samples, the endoparasite prevalence was in 84.5% (N = 83), with a mean abundance of 368.2 ± 561.5_{SD} eggs/g. The *Contracaecum* sp., *Echinostoma* sp. and *Opisthorchis viverrini* were recorded in 51, 67 and nine samples respectively (Fig. 2C, D, E). Unidentified nematode eggs were also recorded in a few samples, indicating the presence of a minimum of four endoparasite taxa in the pelicans. The highest egg count in a single sample was 3280, comprising chiefly of *Echinostoma* sp. The percent prevalence of *Contracaecum* sp., *Echinostoma* sp. and *Opisthorchis viverrini* was 52.6%, 69.1%, and 9.3% respectively. The mean abundance of *Contracaecum* sp., *Echinostoma* sp. and *Opisthorchis viverrini* were 97.2 ± 84.4_{SD}, 349.8 ± 553.1_{SD}, and 196.3 ± 241.8_{SD} eggs/gram respectively (Table 2). The mean abundance of the eggs of the three taxa differed significantly between the samples ($F_{2,122} = 5.299$, $p < 0.01$).

4. Discussion

We witnessed an unusual number of Spot-billed Pelican mortalities between December 2017 and May 2018 at Kokrebellur Community Reserve in Mandya district raising significant management concerns. Our findings reveal high infestation of adult *Contracaecum* sp. and

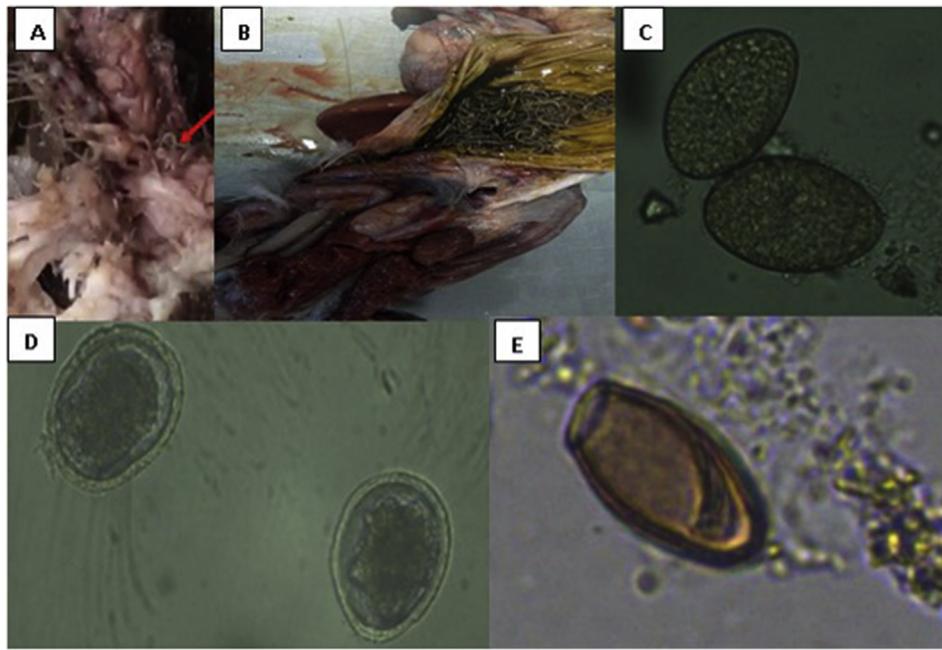


Fig. 2. A. Larvae of *Contracaecum* sp. in fish, B. Adult *Contracaecum* sp. worms in the pelican, C. Eggs of *Echinostoma* sp. in pelican fecal and water samples, D. Eggs of *Contracaecum* sp. in pelican fecal and water samples and E. Eggs of *Opisthorchis viverrini* in pelican fecal samples.

Echinostoma sp. worms in the digestive tracts of Spot-billed Pelicans. The same endoparasites have also infested the water tanks that were frequented by pelicans, along with the fishes residing in those water tanks. The high load of endoparasite eggs and fully-grown worms of *Contracaecum* sp. and *Echinostoma* sp. in the adult pelicans, water tanks and fishes are indicative of helminth infection in pelicans.

A good number of water bodies in southern India is one of the strongholds for Spot-billed Pelican populations. The droughts in recent years (2011–2017) which continued until 2018 has affected the overall landscape, particularly fishery in south-eastern Karnataka (KSNDMC, 2017). Such natural calamities majorly disrupt food resources thereby increasing stress to the dependent species eventually causing starvation and deaths. For example, high mortality of Dalmatian Pelican in Greece was attributed to unavailability of fishes due to abnormally low temperatures that in concert with a high load of parasites in their digestive system (Pyrovetsi and Papazahariadou, 1995). The interaction between parasite and its host is complex in a given environment. The parasite transmission mode, lethality of the infection, and the life history traits are decided by the parasites (Poulin et al., 2011). Even when the parasite is in sub-lethal level, it can cause damage to the host by taking partial control over the behaviour such as changes in locomotion, light sensitivity, pain threshold, disruption of food intake, even affecting the immunological system of the host (Holmes and Bethel, 1972; Crompton et al., 1985; Huerta et al., 1992; Poulin, 1994; Thompson and Kavaliers, 1994; Corbin et al., 1996; Kavaliers et al., 1999). The parasitic infections in aquatic birds are one of the major threats to their populations

along with factors including human disturbances at the nesting sites and wetlands, deforestation (Subramanya, 2006; Taher, 2007), hunting (Talukdar, 1999), and poaching of eggs and chicks (Talukdar, 1999; Chandrasekhar, 2009). Since the aquafauna or fishes contributes to a significant proportion of food of aquatic birds, parasites in water find their way to the birds via infested fishes and vice-versa (Himonas, 1970). Among parasites, helminths are one of the common endoparasites reported from the aquatic birds (Huizinga, 1971; Liu and Edward, 1971), particularly *Contracaecum* sp. has a wide range of host specificity in the order Pelecaniformes with latter being definitive hosts (Huizinga, 1971).

In case of Brown Pelican, *Contracaecum* sp. is a potential parasite (Courtney and Forrester, 1974), playing a role in fluctuation of pelican populations combined with the influence of other ecological factors (Greve et al., 1986). The *Contracaecum* sp. in the pelicans attacks the proventriculus and gizzard regions causing hemorrhages and ulcers further accelerating the death of infested individuals. Similarly, the mass mortality of Dalmatian Pelicans in Kerkini lake in Greece was attributed to massive infection from multiple species of helminths including *Contracaecum* sp. and *Echinocasmus* sp. (Pyrovetsi and Papazahariadou, 1995). Although, what has triggered an increase in the endoparasites may not be clear, but, ulcers and damaged proventriculus and gizzard perhaps have affected the absorption of food in these birds. Further, the clogged worms would not have allowed the ingestion of food, possibly resulted in the mass mortality of the Spot-billed Pelicans in Kokrebellur.

Table 2

Endoparasite prevalence in Spot-billed Pelican from Kokrebellur (N = 97 fecal samples of pelicans).

Taxon	No. of positive samples	Percent prevalence	Maximum No. eggs in a sample	Mean abundance in positive samples (SD)
Nematodes				
<i>Contracaecum</i> sp.	51	52.6	357	97.2 ± 84.4
Unidentified nematode eggs	3	3.1	100	–
Trematodes				
<i>Echinostoma</i> sp.	67	69.1	3120	349.8 ± 553.1
<i>Opisthorchis viverrini</i>	9	9.3	640	196.3 ± 241.8
Total	82	84.5	3280	368.2 ± 561.5

The high endoparasite prevalence and egg load in the surviving pelicans make us to suspect mortality of them might continue. Thus, we suggest, the initiation of a coordinated program with all the stakeholders (local people, veterinary department, forest department, and irrigation department and fishing-animal husbandry) to take control over the infection of these endoparasites in water and fish, at first and later to treat the pelicans for endoparasites.

Author credit statement

Shanthala Kumar: conceptualisation, formal analysis, methodology, project administration, validation, review and editing.

A. Periyasamy: data curation, formal analysis.

Nitin Venkatesh Ranga Rao: data curation, project administration.

S. S. Sunil: data curation, project administration.

Honnavalli N. Kumara: conceptualisation, supervision, validation, visualisation, roles/writing, original draft, writing, review and editing.

P. Sundararaj: Mentoring and resources.

G. Chidananda: data curation, project administration.

A. Sathish: data curation, formal analysis.

Conflicts of interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2019.04.001>.

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