Mortality in a Group of Zoo Siamang Gibbon Monkeys *(Symphalangus syndactylus)* due to *Salmonella enteritidis*. First Report in Israel

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ABSTRACT

This case report describes *Salmonella enteritidis* in a zoo colony of Siamang Gibbon Monkeys (*Symphalangus syndactylus*), in Israel, where 3 out of 4 monkeys in the group died within 48 hours with severe gastrointestinal signs. Post mortem findings included fibrinonecrotic typhlocolitis, necrohemorrhagic colitis and acute necrohemorrhagic typhlocolitis. To the best knowledge of the authors, this is the first case report identifying *Salmonella enteritidis* as a fatal pathological gastrointestinal agent in Siamang Gibbon Monkeys.

Key words: Gibbon Monkey; Zoo; *Symphalangus syndactylus*; *Salmonella enteritidis.*

INTRODUCTION

Symphalangus syndactylus is an arboreal, black-furred gibbon monkey native to the forests of Indonesia, Malaysia, and Thailand. The largest of the gibbons, the siamang can be twice the size of other gibbons, reaching 1 m in height, and weighing up to 14 Kg. The siamang eats mainly various parts of plants. Siamang's are generally known to have monogamous mating pairs. Infants belonging to monogamous groups were found to receive more overall male care than infants in the polyandrous groups. As an arboreal primate whose survival absolutely depends on the forest, the siamang faces population pressure due to habitat loss, poaching and hunting.

Nonhuman primates, as well as humans, are susceptible to wide variety of bacterial agents. The bacteria that deserve the most concern are *Mycobacteria*, *Shigella*, *Salmonella*, *Campylobacter* and *Klebsiella* (1). Among the list of bacterial enteric pathogens that may infect monkeys kept in zoos, *Shigella* and *Salmonella* (typhoid or non-typhoid) may be considered among the most important agents (2). Both of these are frequently present in the alimentary tract of nonhuman primates (1), and may be a source of diseases causing gastroenteritis and sepsis in human (*Salmonella*-derived diarrhea or shigella dysenteries).

In the present study, *Salmonella enteritidis* was identified in a colony of Siamang Gibbon Monkeys, where 3 out of 4 Gibbon monkeys in the group died within 48 hours. To the best knowledge of the authors, this is the first documented report of *Salmonella enteritidis,* which is one a major serovar of *Salmonella* with zoonotic implications, causing severe enteric disease and death in Gibbon zoo monkeys.

CLINICAL FINDINGS AND CLINICAL REPORT

Clinical description

The group of Siamang monkeys consisted of a family of two adult apes (male and female), one adolescent male and a baby female. A few days prior to the clinical presentation, the group was seen devouring a carcass of a cattle egret, which

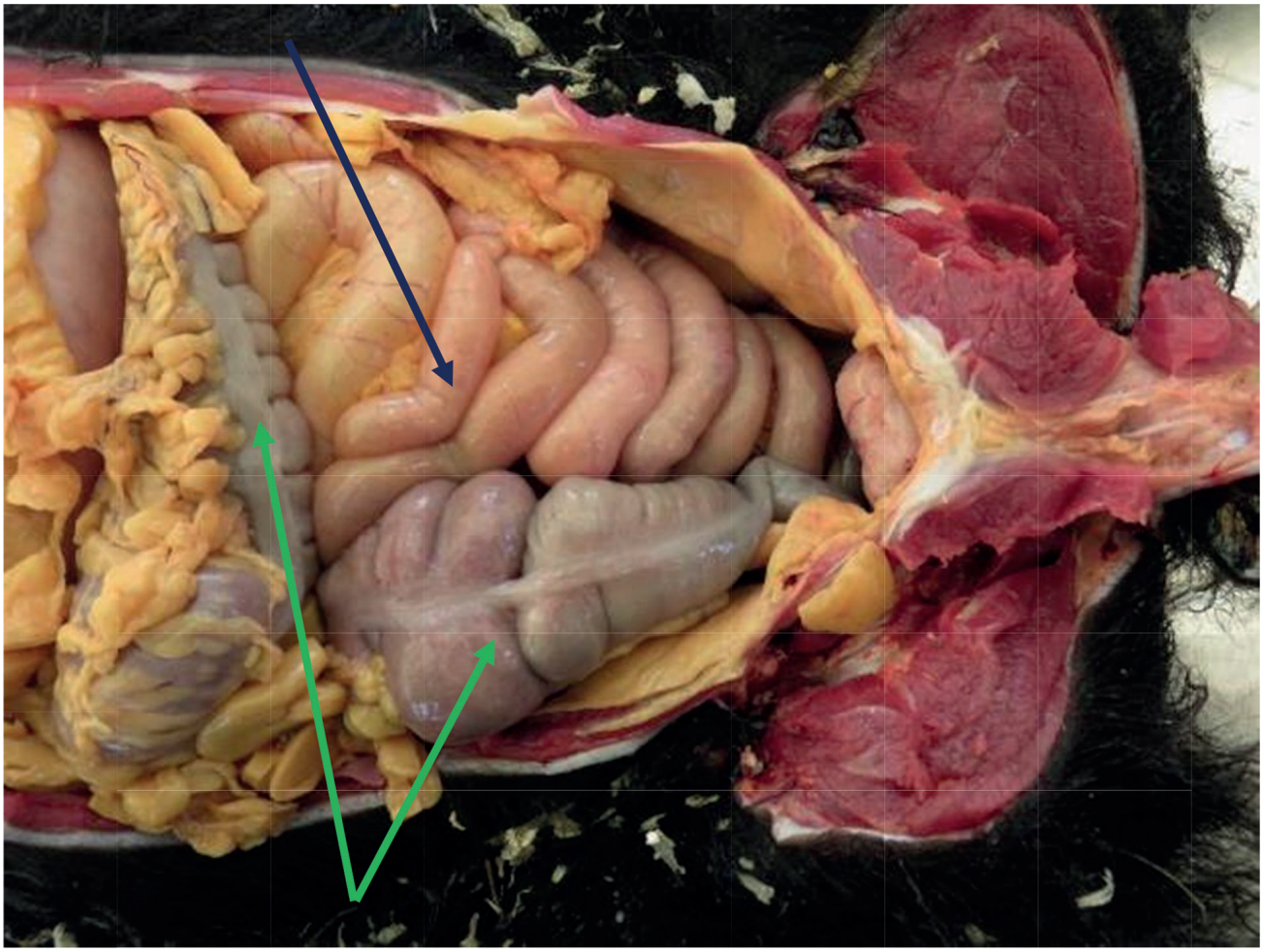

Figure 1: The colonic and the cecal serosa was markedly hyperemic (green arrows). The small intestine was normal (blue arrows)

was a member of a migrating flock of birds, considered a common sight at the zoo during the migration season. The adult female, the adolescent male and the baby exhibited vomiting and watery pale non-bloody diarrhea along with lethargy and anorexia. They were treated with a restricted diet (of rice and prickly pear), antibiotics and supplemented with intravenous fluids. Following a poor response to the therapy, the young male died after about 24 hours and the mother and baby died the following day within 48 hours from presenting clinical signs. The adult male presented milder clinical signs responding well to the therapy and had a full recovery within 72 hours.

Gross pathology

Out of three monkeys, two were submitted for a full necropsy followed by ancillary tests.

Both (the adult gibbon and the juvenile gibbon) were in a good post mortem condition and excellent body condition. There were multiple petechiae on subcutis tissue, particularly in the region of the neck and the inner aspects of the thighs. The lungs were dark red, slightly rubbery but floated in water and oozed large amounts of foamy fluids on cut section, indicative of pulmonary edema.

The liver was moderately congested. The spleen was markedly enlarged (Fig. 2) (suggesting a septicemia). The kidneys were congested and showed a slightly bulging cut surface.

The small intestines were unremarkable (Fig. 1). The colonic and cecal serosa was hyperemic and contained multifocal petechiae (Fig. 1). The colonic and cecal mucosa was dark red to black (more severe at the ascending colon and the cecum, particularly the base of the cecum which was necrotic

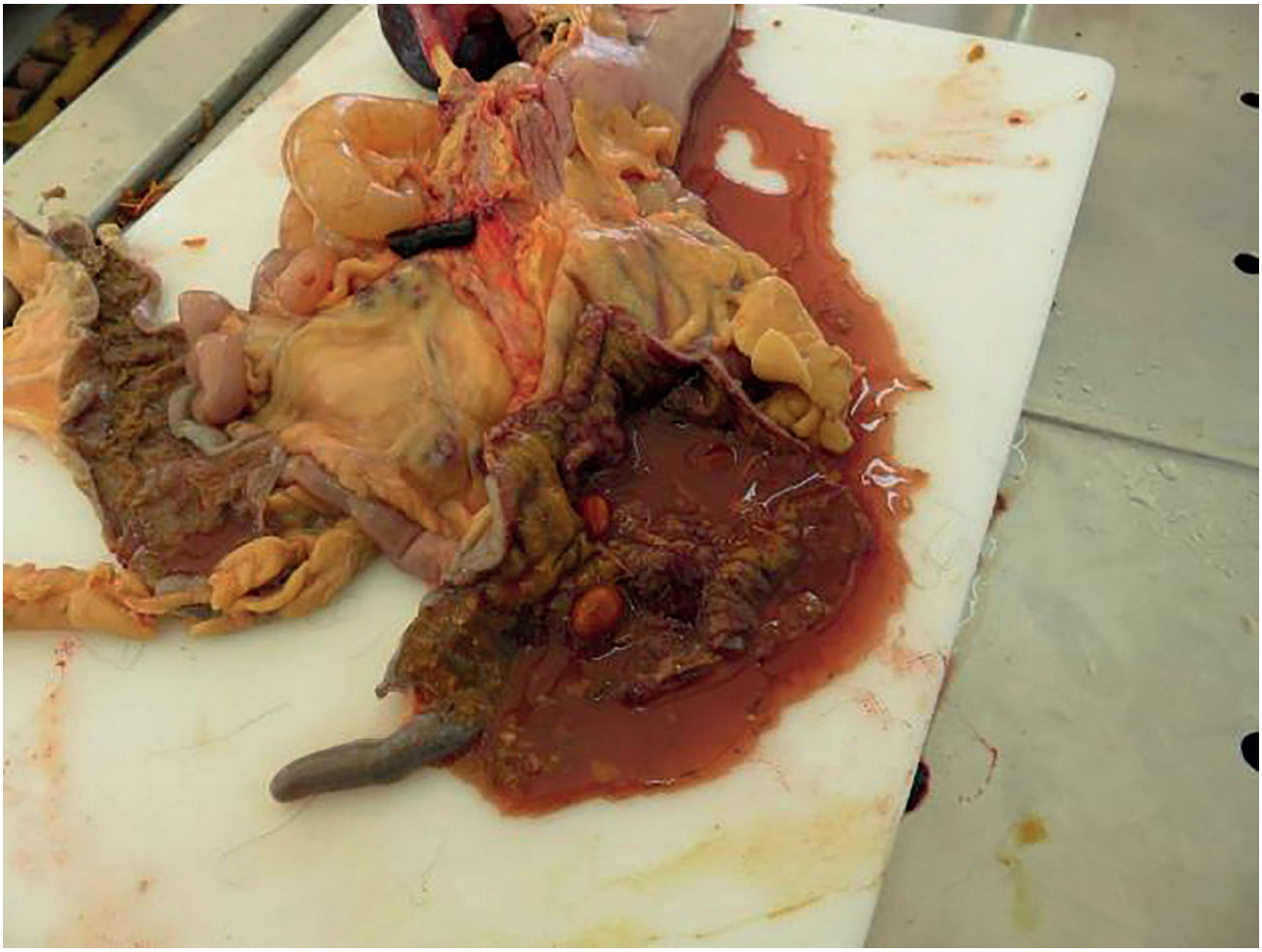

Figure 2: The cecum contains large amounts of bloody murky contents admixed with a few non-digested whole grains.

in the adolescent male). The mucosa was eroded, contained multiple pinpoint hemorrhages and was multifocally covered by thick yellow pseudomembrane (fibrinonecrotic typhlocolitis) (Fig. 4). The colonic lumen contained large amounts of bloody foul smelling viscous murky fluid, admixed with flakes of fibrin, whole undigested grains and necrotic debris (necrohemorrhagic colitis) (Fig. 2). The spleen was markedly enlarged (Fig. 3).

The lesions were consistent with an acute necrohemorrhagic typhlocolitis with septicemia.

Preliminary differential diagnoses included: *Salmonella* (typhoid or non-typhoid) , *Shigella* (necrohemorrhagic colitis but usually with no systemic septicemia), *Campylobacter* (usually affects both small and large intestine), *Yersinia* (usually causing enteric and serosal microabscesses), enteropathogenic *E.coli*-EPEC (attaching and effacing *E. coli*), *Clostridium perfringens*, *Entamoeba histolytica/Balantidium coli* (amoebae), stress , inflammatory bowel disease and enteric neoplasia (colonic carcinoma or lymphoma).

Bacteriology

Samples of organs (liver, spleen, lungs) were inoculated onto Blood Agar, MacConkey Agar (BD Pharmaceutical Systems, New Jersey, USA) and Nutrient Agar plates, and incubated aerobically at 37°C for 24 h. Intestinal contents were thoroughly mixed and homogenized and 1 ml was placed in 9 ml Tetrathionate enrichment broth (BD Pharmaceutical Systems, New Jersey, USA) aerobically at 37°C for 24 h, before culturing 10µl onto MacConkey (BD Pharmaceutical Systems) and Brilliant Green Agar plates. (Oxoid Limited, Hampshire, UK) which were incubated at 37°C for 24 h (3).

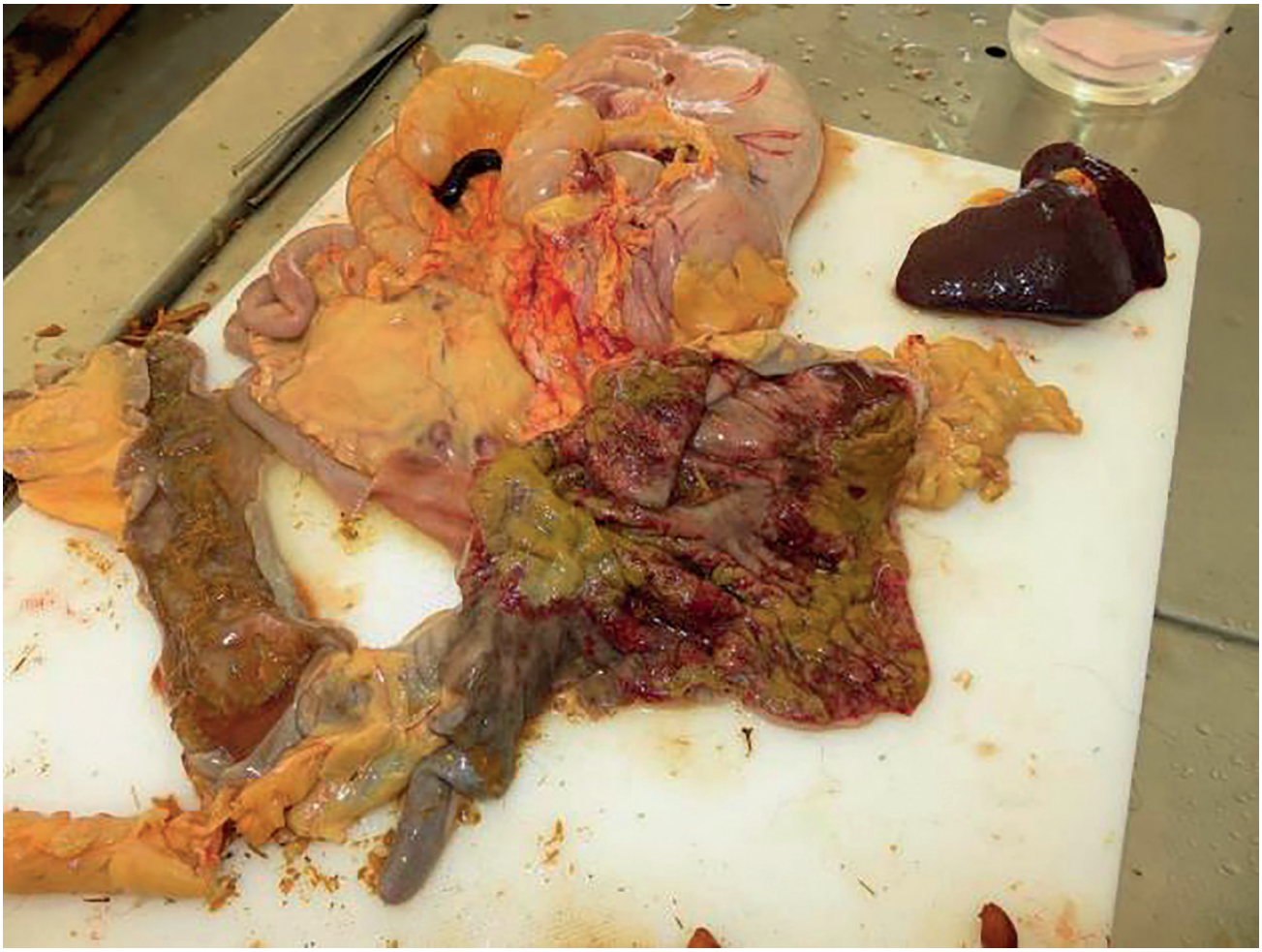

Figure 3: The cecal and colonic mucosa is covered by abundant fibrin mottled with multifocal hemorrhage. The adjacent small intestine maintains a normal appearance. The spleen is markedly enlarged, consistent with septicemia.

Suspected colonies cultured from intestines were tested by slide agglutination with polyvalent *Salmonella* antiserum and tested for biochemical properties, i.e., Triple Sugar Iron, Lysine Iron and Urea slant agar tubes. Positive *Salmonella* isolates tested serologically with serogroup specific antisera (B to E). Samples from three of the four monkeys were positive to *Salmonella* by culturing (in all three, from all cultured organs, and in one, also from intestines), that was identified serologically as group-D *Salmonella*.

Molecular identification

Putative Salmonella colonies were submitted to Triplex real-time PCR (*Salmonella* spp., *Salmonella typhimurium* and *Salmonella enteritidis)*. For the detection of *Salmonella* spp., the primers were designed according the ttrRSBCA locus,

Table 1.

	Forward primer	Reverse primer	Probe
Salmonella spp.	CTCACCAGGAGATTACAACATGG	AGCTCAGACCAAAAGTGACCATC	CACCGACGGCGAGACCGACTTT
Salmonella typhimurium	CATTACACCTTCAGCGGTAT	CTGGTAAGAGAGCCTTATAGG	CGGCATGATTATCCGTTT CTACAGAGG
Salmonella enteritidis	GGTTGCTAACACGACACTG	TGGGGCATTGGTATCAAAG	CTCCTCCCATTCCACATTTGCG

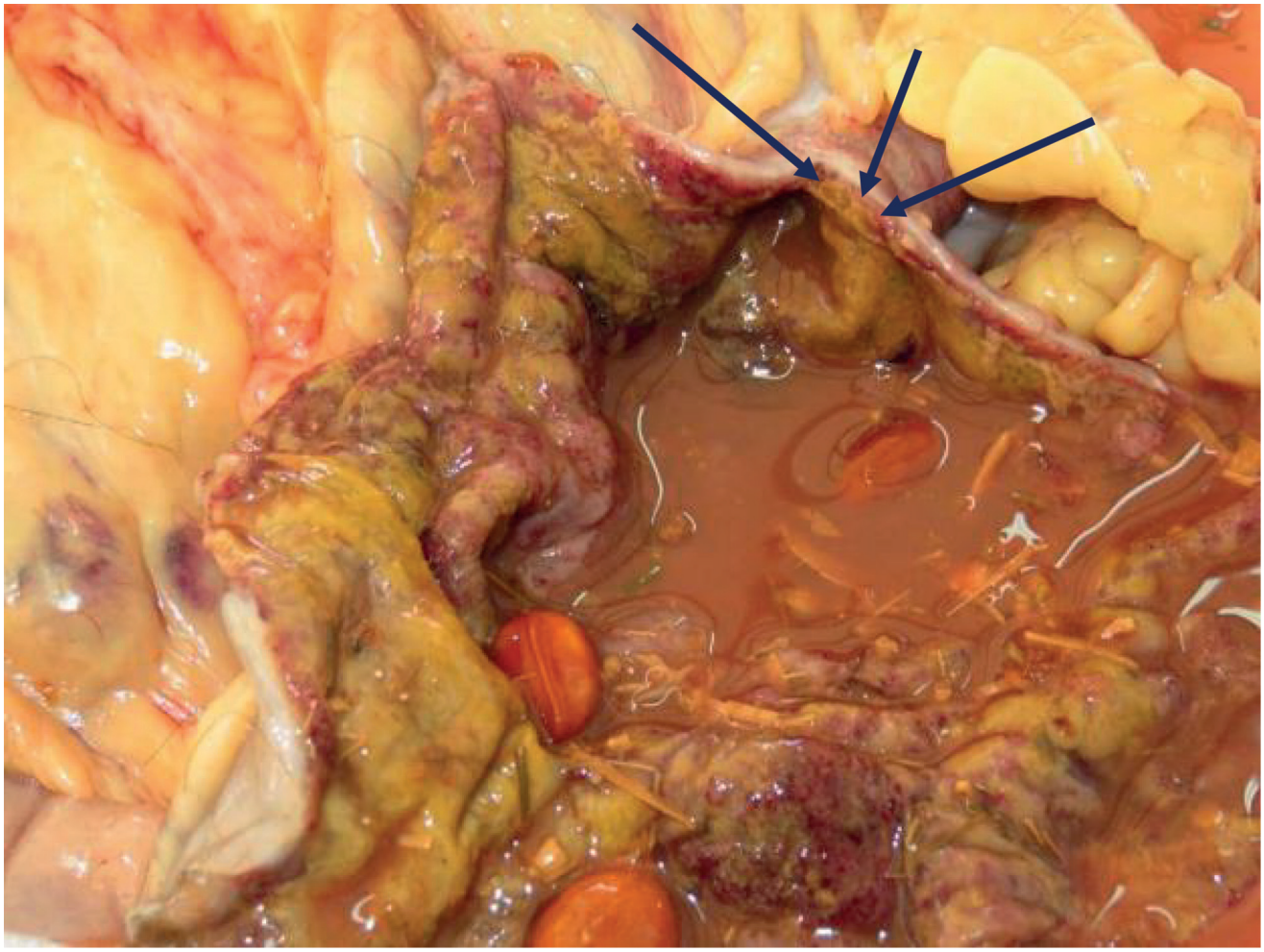

Figure 4: Higher magnification of the fibrinonecrotic mucosa, the bloody contents with non-digested whole grains. The necrosis extends into the deeper layers of the cecal wall (arrows)

required for Tetrathionate respiration in *Salmonella* (Table 1) (1). *S. typhimurium* was designed according fliA-IS200 gene. The primers for *S. enteritidis* were designed according safA gene, an outer membrane protein gene (4, 5). According this Triplex real-time PCR test, these isolates identified as *Salmonella enteritidis*.

DISCUSSION

Reports of *Salmonella* infection and diseases within primate colonies are rare (6). A survey of intestinal flora of 63 captured wild rhesus monkey revealed that none of them was infected with *Salmonella* (7), suggesting that *Salmonella* infection may be acquired and not a normal component of the intestinal flora of wild rhesus monkeys. In another survey of several species of monkeys, 12% were infected with *S.* anatum or *S. stanley* (2). In a further survey, approximately 20% of monkeys of the species *Presbytis enterllus* were infected with *Salmonella* spp. but it was rarely associated with clinical disease (2). However, in another study by Tribe and Fleming (1983), *Salmonella* was isolated from 0.2-2.7% of monkeys showing diarrhea (8). Salmonella infection and diarrhea was also reported in newly imported Cynomolgus monkeys (9).

Concerning Gibbon monkeys, a single case report documents septic abortion in a White-Handed Gibbon (*Hylobates lar*), caused by *Salmonella Heidelberg* (10). It appears that *Salmonella* infestations are rare in Gibbon Monkeys. Furthermore, an extensive literature survey did not reveal reported cases of *Salmonella enteritidis* in Gibbon Monkeys.

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