

The prevalence of Salmonella and Shigella in Chlorocebus aethiops in Barbados

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Abstract

Since 1999, 1,608 stool samples from the Barbados Green Monkey *Chlorocebus aethiops* have been tested at the Veterinary Services Laboratory prior to exportation of the animals for polio vaccine production in France and Canada. All exported animals must test free of *Salmonella*, *Shigella* and *Yersinia* (Canada) or *Salmonella* and *Shigella* (France). Sixteen (16) of these samples have been positive for *Salmonella* species and there have been 32 isolates of *Shigella sonnei*.

Key words: Barbados Green Monkey, *Chlorocebus aethiops*, *Shigella sonnei*, *Salmonella*, polio vaccine,

Introduction

African Green Monkeys/Vervet monkeys, *Chlorocebus* (= *Cercopithecus*) *aethiops sabeus* have been exported to developed countries (mainly Canada and France) from Barbados for vaccine production and other biomedical research, since the early 1980s.

In Barbados the monkey is seen as an agricultural pest, but its value to laboratories involved in polio vaccine production was greatly enhanced when it was observed (Blakeslee *et al*, 1985)) that the Barbados green monkey was free of HIV, the virus which causes AIDS. Later work reported by Baulu *et al* (1987) shows that it is also free of Simian AIDS (SAIDS). Recent attempts to experimentally infect the Barbados green monkey with the HIV virus have failed. The Barbados population of African Greens seems to be resistant to the virus (Baulu, 2002)). In contrast, a high percentage of green monkeys/Vervets from Africa are AIDS and SAIDS positive. As polio vaccine is still being

produced in monkey kidney tissue cell cultures, the value of working with AIDS free cell cultures should be obvious. At the moment some 80% of the polio vaccine in the world is produced in Barbados green monkey kidney tissue (Baulu, 2002).

In the early years all disease testing of monkeys for export was carried out by laboratories abroad. To date testing for viruses is still done in this way. However, with the advent of the WTO and OIE imposed veterinary controls on animal movement world wide, testing for the required bacterial pathogens has been carried out by local national veterinary authority. The International Animal Health Code of the OIE (2002) mandates testing of monkeys destined for export for *Salmonella*, *Shigella* and *Yersinia*, which are serious human pathogens. Monkeys carrying these bacteria can infect entire primate colonies and animal handlers. All animals exported must therefore test negative for these pathogens. The monkeys, which are exported from Barbados, are captive bred and well housed and the colony is under veterinary supervision.

Materials and Methods

Surveillance is ongoing and this paper reports on the period 1999 to April 2003. Faecal samples or rectal swabs are submitted to the Veterinary Services Laboratory (VSL) from the Barbados Wildlife Reserve whenever a shipment is due. The swabs are individually labelled with each animal's identification number. Many more animals are tested than are required, in order to ensure a complete shipment even if some of the animals are disqualified.

Each sample is initially inoculated on to agar plates of Xylose Lactose Desoxycholate (XLD), MacConkey and Yersinia Selective media. The plates are examined the next day (Day 2) for suspicious colonies. The initial swabs are incubated in Selenite enrichment broth overnight (a selection process for isolation of *Salmonella* and plated out on to XLD and MacConkey media the following morning (Day 2), these are examined on Day 3, for suspicious colonies. All such colonies are inoculated on to Triple Sugar Iron agar (TSI) and Lysine Iron Agar (LIA) for further identification.

Colonies from TSI slants showing the following reactions: alkaline slant/ acid butt H_2S , and gas are further tested for *Salmonella*. Colonies from LIA slants showing the following reactions: alkaline slant / alkaline butt are further tested for *Salmonella*.

Colonies from TSI slants showing the following reactions; alkaline slant/acid butt no H_2S , no gas or Acid slant /acid butt, no H_2S , no gas, are further tested for *Shigella*. Colonies from LIA slants showing the following reactions:

alkaline slant/ acid butt (clearly demarcated) are further tested for *Shigella*.

All suspicious colonies from TSI and LIA are checked on API 20 E (BioMerieux) or API Rapid 20 E (BioMerieux) strips and identified on the API database. They are further tested on the Biolog Microlog 96 well Gram negative (GN) plate system and identified on the Biolog database. The testing plan for *Salmonella*, *Shigella* & *Yersinia* is shown in Figure 1.

All colonies identified by these two methods (API and Biolog) as *Salmonella* or *Shigella* at the VSL, are then sent to the Winston Scott Polyclinic Laboratory (the national *Salmonella* and *Shigella* centre) for *Shigella* serotyping or *Salmonella* grouping. All positive *Salmonella* (except the Group Bs) are sent by the Winston Scott Polyclinic laboratory to the Caribbean Epidemiological Centre (CAREC) laboratory, in Trinidad for serotyping. In the event that CAREC is unable to complete testing, cultures are sent to Canada for further serotyping.

Results

From 1999 to the present (April 2003) 1,608 rectal swabs have been cultured for *Salmonella*, *Shigella* and *Yersinia*, resulting in some 4,824 tests. There were no *Yersinia* isolates during the period under review. Please refer to Tables 1, 2 and 3 for details.

Testing Plan

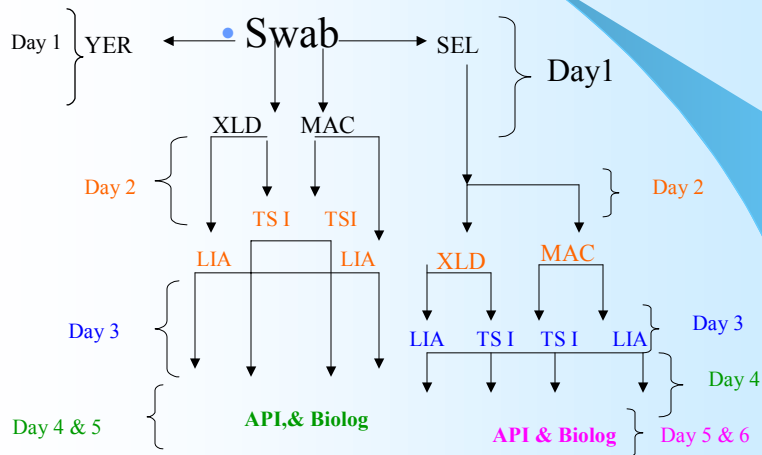


Figure 1- Testing Plan for *Salmonella*, *Shigella* & *Yersinia*

Table 1: Number of tests carried out on African Green Monkeys/Vervet monkeys from 1999 to April 2003

Year	No. of animals tested	No. of tests done
1999	68	249
2000	160	480
2001	578	1734
2002	552	1656
2003	235 (to date)	705 (to date)

Samples are initially tested for all three bacterial genera, and further extensive testing is required on any suspicious colonies before these can be confirmed as *Salmonella* or *Shigella*.

Table 2. Results of *Salmonella* testing between 1999 and April 2003

Year	Number of animals tested	Salmonella Positive	Isolates
1999	68	1	<i>Salmonella</i> oslo
2000	160	0	-
2001	578	4	<i>Salmonella</i> derby (2), <i>Salmonella</i> Group B (2)
2002	552	3	<i>Salmonella</i> Grp. B (2), <i>Salmonella</i> sp. (1)
2003	235	8	<i>Salmonella</i> Grp.B (7), <i>Salmonella</i> Grp.E (1)
Totals	1,608	16	

The Group B *Salmonellae* will be serotyped by the Winston Scott Polyclinic laboratory, the Group E will be serotyped at CAREC.

Table 3. Results of *Shigella* testing between 1999 and April 2003

Year	Number of animals tested	Shigella Positive	Isolates
1999	68	0	-
2000	160	6	<i>Shigella sonnei</i> (6)
2001	578	8	<i>Shigella sonnei</i> (8)
2002	552	10	<i>Shigella sonnei</i> (10)
2003	235	8	<i>Shigella sonnei</i> (8)
Totals	1,608	32	<i>Shigella sonnei</i> (32)

Discussion

Surveillance for *Salmonella* and *Shigella* is necessary in international trade because both of these organisms cause highly infectious zoonoses, which are spread by the faecal-oral route. Most infections are mild diarrhoeas with fever, but some strains of both organisms can cause septicaemic infections with serious sequelae, which may include septic arthritis and Reiter's syndrome in about 3% of cases. *Salmonellae* are found in the gastro intestinal tracts of mammals, birds and reptiles. True *Shigellae* are found only in humans and non-human primates (Quin *et al*, 1999). It takes only 10 colony

forming units (CFU) of *Shigella* or 20 CFU of *Salmonella* to cause disease in susceptible individuals. The comparable infective dose with another food borne bacterium (*Clostridium perfringens*) is 10⁸ CFU (FDA, 1997).

The reactor rates as found by the Barbados Veterinary Services Laboratory in healthy local *Chlorocebus aethiops* are 0.99% positive to *Salmonella* (at least four different serotypes) and 1.9% positive to *Shigella sonnei* as shown by Tables 2 and 3. It has been observed that the *Salmonella* isolates have tended to be of a sporadic nature, while the *Shigellae* tend to be isolated in small

batches, which would indicate that there may be some spread of *Shigella* in specific cages.

Conclusion

Given the present reactor rates, an estimated monkey population size of >14,800 (Boulton *et al*, 1996) and with the export of only 300- 500 animals per year, most of which are captive bred, Barbados should be able to continue to supply healthy monkeys for polio vaccine and other medical research purposes, without endangering the population of *Chlorocebus aethiops* on the island.

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