Orangutan Conservancy 2010 Veterinary Workshop

August 2-6 2010

The Hill Hotel & Resort, Sibolangit, Deli Serdang, Northern Sumatra, Indonesia

Participating Organizations:

Orangutan Conservancy, United States
Chester Zoo / NEZS, United Kingdom
Liverpool School of Tropical Medicine, United Kingdom
Murdoch University, Perth, Western Australia
Sumatran Orangutan Conservation Programme (SOCP), Medan, Indonesia
Borneo Orangutan Survival Foundation, Nyaru Menteng, Palangkaraya, Kalimantan, Indonesia
Borneo Orangutan Survival Foundation, Samboja Lestari, Samboja, Kalimantan, Indonesia
Orangutan Foundation International (OFI), Kalimantan, Indonesia
Orangutan Foundation United Kingdom (OFUK), Kalimantan, Indonesia
Syah Kuala University, Aceh, Sumatra, Indonesia
Gadjah Mada University, Jogyjakarta, Indonesia
International Wildlife Rescue, Indonesia (GPOCP)
BOS Switzerland, Switzerland
ABAXIS Europe, Germany
Bogor Agricultural University/Primate Center for Wildlife Studies (IPB/PSSP) Java, Indonesia
Putra University, Kuala Lumpur, Malaysia
Frankfurt Zoological Society/Jambi SOCP Orangutan Release Site, Sumatra, Indonesia
Veterinary Society for Sumatran Wildlife Conservation, Sumatra, Indonesia
VESSWIC
Supporting Organizations:

Orangutan Conservancy, United States
Chester Zoo/NEZS, United Kingdom
American Association of Zoo Keepers (Birmingham, AL), United States
ABAXIS Europe, Germany
Cleveland Metroparks Zoo / Cleveland Zoological Society, United States
Murdoch University, Australia
Chembio Diagnostics, Inc., United States
Liverpool School of Tropical Medicine, United Kingdom

Hosted by:

Sumatran Orangutan Conservation Programme(SOCP)/Yayasan Ekosystem Lestari (YEL)
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Orangutan Conservancy 2010 Veterinary Workshop

Workshop Report

August 2 - 6, 2010

Section 1
Executive Summary

Collectively, they care for the largest captive population of orangutans in the world. Yet the handful of veterinarians and healthcare staff who work at orangutan rehabilitation centers across Sumatra and Borneo face nearly impossible odds, and often find themselves short of medicine, equipment, money, space, support staff and time.

But those same dedicated men and women do not lack for skill. Or commitment. And that is why the Orangutan Conservancy was proud to be able to stage the Orangutan Conservancy (OC) 2010 Veterinary Workshop, which was held August 2-6 in Medan, Sumatra. The workshop series, which was inaugurated in 2009 in Borneo, gathered together the veterinary teams that work at the frontlines of the orangutan conservation crisis, and gave them a rare opportunity to hone skills, discuss issues and ideas, and renew friendships that could some day mean the difference between life and death for endangered apes.

Orangutans are in severe crisis. The largest of the great apes found in Asia, their natural range is limited to the islands of Borneo and Sumatra, and their rainforest homes are disappearing quickly. More than 80 percent of the orangutans’ habitat has been destroyed over the last 20 years, and no more than 63,000 orangutans are thought to exist. At the current rate of decline, experts believe that orangutans may become extinct in the wild within 25 years.

The primary threats to orangutans are illegal logging and habitat destruction, human encroachment, the conversion of rainforests to oil palm plantations, and the pet trade. As a result of such intense pressures, an extremely large number of orphaned orangutans exist in rehabilitation centers across Borneo and Sumatra. These orangutans – which number approximately 1,600 – arrive bearing a host of physical and emotional wounds, and require intense veterinary care to recover.

The orangutans that are judged fit to return to the wild are reintroduced through a long, complex process, but the overwhelming majority continue to reside in the rehabilitation centers.

The OC 2010 Veterinary Workshop focused on all aspects of captive orangutan care, with a special emphasis on the detection and treatment of tuberculosis (TB). A joint program between OC and Chembio Diagnostics Systems Inc. provided large caches of PrimaTB STAT-PAK test kits to each of the facilities as part of a large-scale tuberculosis study, and each delegate was instructed in the proper use and interpretation of the results. The PrimaTB STAT-PAK testing kits are considered useful in the detection of tuberculosis in primates, a severe respiratory disease that can prove deadly.

The OC 2010 Veterinary Workshop was sponsored by the Birmingham (U.S.) chapter of the American Association of Zoo Keepers (AAZK), which once again directed the proceeds of its annual Zoo Run to support the workshop. Other sponsors included a Cleveland Metroparks Zoo / Cleveland Zoological Society Asian Seed Grant, the Chester Zoo, and the Orangutan Conservancy, in association with the Liverpool School of Tropical Medicine, Chembio Diagnostics Systems Inc., Murdoch University, Abaxis (Europe) and the Sumatran Orangutan Conservation Program (SOCP).
The OC 2010 Veterinary Workshop included 26 participants from the orangutan rescue and rehabilitation centers in Indonesia and Malaysia, along with experts and facilitators from the United States, the United Kingdom, Australia, and Germany. The OC 2010 Veterinary Workshop was designed and facilitated by Dr. Steve Unwin of the Chester Zoo, in partnership with Dr. Raffaella Commitante of OC, the same team that helped create the format a year ago.

In addition to presentations, practical demonstrations and roundtable discussions, the delegates made site visits to the SOCP facility and the Bukit Lawang orangutan feeding station, and tested their own fortitude by engaging in a durian-eating contest.

But the focus of the OC 2010 Veterinary Workshop remained the practical sessions, presentations, roundtables, and break-out groups that make the workshop so valuable. There, veterinarians who often work alone under extreme duress got a chance to pose questions and tackle hypothetical scenarios that might otherwise get overlooked. They also established friendships and alliances that strengthened the orangutan conservation community as a whole.

At the OC 2009 veterinary Workshop, the delegates took the bold step of forming the Orangutan Veterinary Advisory Group (OVAG), which quickly became a forum for issues such as contraception, reintroduction, diseases, and other hot-button topics. In Sumatra, the OVAG continued to tackle tough issues, such as euthanasia, laboratory politics, veterinary aspects of eco-tourism, field diagnostics, fundamentals of environmental enrichment, disease case studies and tuberculosis testing. In this way, the OC Veterinary Workshops have helped build a community of veterinary healthcare experts that stands strongest when it stands together.
Orangutan Conservancy 2010 Veterinary Workshop

Workshop Report

August 2-6, 2010
## Workshop Budget

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Orangutan Conservancy 2010 Veterinary Workshop

Workshop Report

August 2-6, 2010

Section 2
June 1, 2010

RE: Orangutan Veterinary Advisory Group Workshop 2010
Lokakarya Kommunitas Dokter Herwan Orangutan 2010

To Whom It May Concern:

This letter shall serve as an invitation to attend the Orangutan Conservancy Veterinary Advisory Group Workshop 2010 sponsored by the Orangutan Conservancy (OC), a United States not-for-profit organization and its Orangutan Crisis Coalition (OCC), and hosted by The Sumatra Orangutan Conservation Program (SOCP) to be held at The Hill Hotel and Resort in Medan.

This, our second workshop, will bring together experts working closely with orangutans in Indonesia and Malaysia and in the international community to allow for the sharing of information and the creation of long lasting friendships and contacts. It will be held:

August 2 – August 6 2010

Participants should plan to arrive on August 1 and leave on August 7.

We thank you for your participation in allowing your staff to attend.

Travel expenses to the workshop and home again as well as accommodation will be paid for by the Orangutan Conservancy during the workshop.

Respectfully,

Raffaella Commitante, PhD

Orangutan Conservancy / P.O. Box 513 / 5001 Wilshire Blvd. / #112
Los Angeles, CA / 90036 / USA /
www.orangutan.com / info@orangutan.com
Orangutan Conservancy 2010 Veterinary Workshop
Orangutan Veterinary Advisory Group Workshop (OVAG)
August 2-6, 2010

**Agenda**

**Sunday, August 1**
Delegate Arrival / Set Up of Sessions

**Monday, August 2**

08:00  Welcome to delegates
09:00  Veterinary Aspects of Reintroduction Part 1
       Risk Analysis Recap / Mapping the Pathway (Steve Unwin)
10:30  Coffee/Tea
11:00  Veterinary Aspects of Reintroduction Part 2
       Mapping The Pathway Exercise (all delegates)
13:00  Lunch
14:00  Veterinary Aspects of Reintroduction Part 3
       Disease Risk Assessment Exercise (all delegates)
15:30  Coffee/Tea
16:00  Reintroduction Round Table
18:00    Dinner/Ice Breaker

Tuesday, August 3
08:00    Tuberculosis and ChemBio Report (Steve Unwin / Citra Kasih Nente)
09:30    New Technologies in Diagnostics (Wendi Bailey / Steve Unwin)
10:30    Coffee/Tea
11:00    Veterinary Aspects of Reintroduction Part 4
Disease and Contingency Planning (all delegates)
13:00    Lunch
14:00    Disease Contingency Planning – TB Case Study (Steve Unwin)
15:30    Coffee/Tea
16:00    Case Studies: Viral Pathogens In Orangutan (Pak Joko/Nyaru Menteng Vets)

Wednesday, August 4
08:00    Bus to SOCP Quarantine
09:00    Practicals / Diagnostics / Parasitology (Wendi Bailey)
Primate Enrichment (Steve Unwin w/ materials from Sabrina Brando)
13:00    Lunch
14:00    Group Photo
Local Fruit Taste Testing
16:00    Bus to Bukit Lawang (National Park/Orangutan Release Area) for Overnight Stay
19:00    Dinner at Bukit Lawang Eco Lodge

Thursday, August 5
08:00    Forest Walk Through National Park (Taman Nasional Gunung Leuser) to Orangutan
Feeding Site
Observation of Orangutan / Visitor Interaction
11:00    Meeting with National Park Rangers and local Guides
Ways to Improve Orangutan / Visitor Interaction

13:00  Lunch with National Park Rangers
15:00  Visit to Eco-Farming Facility, Yayasan Ekosystem Lestari (YEL)
16:00  Bus Back To Hill Hotel, Sibolangit
19:00  Dinner

Friday, August 6

07:30  Q and A from Exercises of Day 1 (Reintroduction)
09:30  Coffee/Tea
10:00  Risk Assessment Quiz/Review
11:30  Friday Praying Time / Lunch
14:00  Review of Medical Tests (Wendi Bailey)
       Evaluating Tests, Equipment and Labs (Wendi Bailey)
15:00  Euthanasia Discussion and Recommendations
17:00  Overview of the past year/Veterinary Needs/Diagnostics
18:00  Closing Dinner / Presentation of Certificates
# Participant Contact List

<table>
<thead>
<tr>
<th>Name</th>
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<td>21</td>
<td>Dr. drh. Joko Pamungkas</td>
<td>IPB/PSSP</td>
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<td>Murdoch University</td>
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<td>drh. Muhammad Wahyu</td>
<td>VESSWIC</td>
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<td>24</td>
<td>Dr. Reuben Sharma</td>
<td>Putra University, KL</td>
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<td>25</td>
<td>Dr. Sumita Sugnaseelan</td>
<td>Putra University, KL</td>
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<td>26</td>
<td>drh. Winny Pramesywari</td>
<td>Frankfurt Zoo/Jambi</td>
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Orangutan Conservancy 2010 Veterinary Workshop

Workshop Report

August 2-6, 2010

Section 3
Orangutan Conservancy 2010 Veterinary Workshop

Workshop Report

August 2-6, 2010

Proceedings

Veterinary Aspects of Reintroduction

Steve Unwin

Introduction.

The main focus of this year’s workshop was veterinary aspects of primate reintroduction programmes. The CBSG has put together a risk analysis workbook entitled Animal Movements and Disease Risk, itself based on discussions at several workshops. We have put together a basic workbook on Veterinary Aspects of reintroduction based on the CBSG notes.

Opening message to the delegates: This section of the workshop intends to provide you with the analytical tools to return to your centre and create a comprehensive animal health plan for your reintroduction programme. The methodology used here is DISEASE FOCUSED. Health risk assessments can also be TAXON FOCUSED or AREA FOCUSED. The strategy used will depend on what questions you are trying to answer. It has been found for a reintroduction programme, concentrating on diseases makes the most sense. If however you are trying to assess your quarantine area, a different approach could be used. The following process is generic enough to allow adaptation to a variety of different scenarios. The IUCN Great Ape Reintroduction guidelines will be referenced and followed throughout our discussions.

Can wildlife reintroduction programmes be used as a conservation tool? PASA’s programme logic for reintroduction (Lucas 2007 incorporated in the PASA Operations Manual Farmer et al 2009) highlights how a properly conducted reintroduction programme will promote and assist conservation biodiversity. Even if the reintroduction programme never gets to the stage of animal release, this programme logic
outlines how you will have contributed to protecting and enhancing biodiversity and ecosystem services. Two examples could be:

Conducting an environmental scan will help understanding local social and political systems, to provide valid inputs to decision making and policy processes.

Release site preparation could include revegetation to increase the extent and quality of the habitat, enhancing land stewardship and ecosystem health

Copies of the IUCN Best Practice Guidelines for the Re-introduction of Great Apes, the Animal Movements and Disease Risk Workbook, the Veterinary Aspects of Primate Reintroduction Workbook, and the PASA Operations Manual were provided as pdf’s to delegates during the workshop.

Fundamentals of Disease Risk Analysis

Please refer to the Veterinary Aspects of Primate Reintroduction Workbook, attached with this report. Risk analysis allows us to highlight areas of uncertainty or data gaps, which we need to fill if we are to make sound, scientific evidence based decisions.

There will always be areas of uncertainty, but knowledge of the big picture of your reintroduction, that is, the aims and long term plan, helps you concentrate on areas of most concern.

Does it require further investigation?

Potential pathogens are being discovered constantly, and there is still uncertainty as to their pathogenic nature. For example, Simian Foamy Virus (SFV) has been isolated in captive monkeys and apes, cows, people as well as wild primates. No clinical signs have been noted. Do we test for a potential pathogen like this for our reintroduction programme? Why or why not?

We are data deficient in this area. The more data that is collected the better we are able to answer these questions.
You may decide that if there are no pathological findings recorded, as with SFV, there is no reason to investigate further. But it is important to keep up with current research as new data is becoming available constantly that may have implications for your animals’ health.

Chimpanzee reintroductions in The Republic of Congo and Guinea were discussed. **IT IS IMPORTANT TO PUBLISH RESULTS POSTIVIE or NEGATIVE to assist future primate release programme decision makers.**

Some relevant papers were discussed (all provided as pdf’s to delegates – see below). A central database of relevant abstracts is being put together online by Columbus Zoo (USA) and PASA. Details for access to follow.


Ko`ndgen S, Ku`hl H, N’Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Ma`tz-Rensing K, Schweiger B, Junglen S, Ellerbrok H, Nitsche A, Brieze T, Lipkin I, Pauli G, Boesch C and Leendertz FH (2008). Pandemic Human Viruses Cause Decline of Endangered Great Apes. **Current Biology V18:** 1–5. Investigation in the field found that pneumonia outbreaks in chimpanzees were initiated by disease from field researchers. This highlights the need to enforce biosecurity measures, including maintaining the **seven meter distance between any human and primate**, as we now have proof that disease transmission occurs and can cause death


Lankester F, Kiyang J, Bailey W, and Unwin S (2010). Dientamoeba fragilis: Initial Evidence of Pathogenicity in the Western Lowland Gorilla (Gorilla gorilla gorilla). **Journal of Zoo and Wildlife Medicine 41**(2): 350–352. Case study of irritable bowel syndrome due to a protozoal infection that can affect any great ape. Conjecture about it’s ability as a pathogen? This case finds it in a gorilla where it was successfully treated, with resolutions of clinical signs. This lends evidence to the argument that Dientamoeba fragilis is pathogenic in gorillas, and so should be investigated, but a single case alone is not scientifically robust.


Conducting the first stages of a risk assessment a risk analysis

This will include risk mitigation strategies such as targeted biosecurity and disease contingency planning. This is a continually evolving process and involves ongoing communication – making sure that everyone understands what is going on.

Assessment steps:

**Step 1.** Tell the story – what is your project doing

PASA example. 3 sanctuaries – potential collaboration – risk assessment needed for 2 subspecies of chimpanzees as the centers did not want to mix subspecies. This is not of concern for your orangutans per se. So what is important to you?

**Step 2.** Define the questions – identify and define potential problems

What are they exposed to? What will they release into the environment? What is the likelihood that something will happen – what are the consequences/disease severity of that exposure?

What will be a threat from the environment to the release animals?

What is the probability that an infected animal will be released?

**Step 3.** Map the pathway – completely outline the general pathway

What are the steps the individual will take to get to the wild?

**Step 4.** Potential Hazard Identification

Once you have a pathway, what are the diseases that we may be concerned about?

Knowing what is important will come – review steps in workbook

**Step 5.** Create list of hazards (rough assessment)
Establish ranking criteria

Rank of 1-5 for each question in the workbook. Stick to these questions for now, but the process means you can create your own questions to tailor them to your specific situation. When ranking, back up your ranking with either published/ anecdotal data/ evidence/ or whatever... but document it – and stay current as rankings may change. You will note that this is VERY subjective; hence this is only a rough assessment to make sure you don’t miss important diseases

Example discussed: Mountain Gorilla Veterinary Program – effects of disease on wild populations

Their questions: Disease/ Source / Is it found normally in population? / Justification / Follow-up required?

A disease example: Measles/In people in the area? – No. But - High mortality? - Yes

Step 6. Define specific question and policy/scenario

What is the likelihood of introducing diseases after following your model

Step 7. Build the model /ID critical control points

What can be the control points? Where could have control of the disease fallen

Step 8. Perform Qualitative assessment

Step 9. If required – quantitative assessment

Step 10. Describe uncertainty

Risk Management/ mitigation strategies can then be recommended because you can:

- Identify where better data are needed
- More data are always good as things may change
- Focus research goals
- Collect more relevant data

Keep communications open between:

- Local farmers
- Government
- Communication between field sites – this has been a problem in the past but without proper communication, sharing of information, and continuous contact – releases will fail

Where and how is the information kept? There needs to be continuity so that staff changes are smooth because information is clear and accessible
Everyone must communicate! Keep your contact continuous between all stakeholders

Breakout Sessions AIM: To provide delegates with the tools to create their own comprehensive veterinary programmes for their reintroductions utilizing risk analysis principles. It was acknowledged that this precise method was only one way of conducting this, and delegates are recommended to research other possibilities. It was also acknowledged that we would not have the time to complete the process within the workshop, thus one of the actions would be for presentations of completed programmes at the next workshop. Also, this was the first opportunity for most of the delegates to look at the ‘big picture’ of their projects and voice any concerns. As such, these notes should be viewed very much as a first draft.

Break Out Sessions

4 Groups. Case studies of 4 reintroduction programmes.

Group 1 – SOCP: Yenny, Rachmad, Ian, Wahyu, Winny

Group 2 – OFI: Popo, Fikri, Adi, Eriansyah, Melanie

Group 3 – Nyaru Menteng: Siska, Citra, Wendi, Barbel, Joko, Reuben

Group 4 – Samboja: Agus, Sumita, Anta, Hery, Karmele

Session 1. Step 1, 2 and 3. Tell the Story (what is happening now) and begin to highlight specific questions that need answering and then map your reintroduction pathways

NB, these pathways are reproduced, with critical control points, within session 3.

Group 1: SOCP-VESSWIC. Presenter: Ian Singleton

The project was under pressure to capture orangutans that are isolated in Palm oil plantations/confiscated from BKSDA. Since beginning there have been 9 deaths out of 198 orangutans. The project provides long term care for blind or other unreleasable individuals.

Jambi is the release site – fruiting phrenology key – into socialization cages in the release forest, some that are manageable can spend time in the forest, others are exposed to as many forest fruits as possible – then when ready they are taken several kilometers into the forest for release. Teams of field staff for long term monitoring when possible – some individuals are seen repeatedly, some are lost, when problems occur with illness or injury, they can go back to medical care at Jambi – most serious of those go back to SOCP.
A female orangutan from Perth (Tamara) was sent to SOCP for release through the efforts of Leif Cox — along with the female came funding support as well as political support which offset the problems of releasing a zoo orangutan.

QUESTIONS: What happens to unstable individuals? Are all individuals scheduled for release? Yes. However, each case is different. How do you know when an orangutan is ready for release? What is confidence level?

Group 2: OFI - Camp Leakey. Presenter: Drh Popo

Confiscations to quarantine: 3 day minimum for testing.

Post release monitoring for 10 days – release site Lemandau with feeding platform, sometimes wild orangutans come through. If babies are found without mother, they are paired with an adopted ex-captive mother – who acts as an auntie. Pre release quarantine for 30 days – there should be a 90 day quarantine for pre-release – monitoring is done of health condition, fighting, etc. 200 orangutans in 70 hectares – how many can the area hold? Carrying capacity?

Medical check with collected samples – Monitor faecal worms/eggs of released individuals.

Group 3: Nyaru Menteng. Presenter: Drh Siska

1999 start - 62 hectares, 597 ex-captives and 17 waiting for translocation – by 2013, 75% of the population will be released – 30% infants, 20% at least 7 yrs old, and 50% over 7 and ready for release

Difficulties are a secure forest location for release; site has been located with 300 orangutan carrying capacity.

Release 24 in a group – 3 groups per year until 2015

Quarantine: 30 days or until test results return, then to Forest schools, then to pre-release islands (soft release). From islands they are ready for release to a forest, they have inserted implants for post release monitoring – they will spend some time in the forest before going to actual release area

Anticipated problems:

Over population (they are currently at twice the capacity)

Behavioral problems on islands sometimes mean they must return to cages which impairs their forest skills. Check for Parasites, TB, Hep B, Typhoid, still no Standard Operating Procedures – is this enough? Are there problems that are being missed? About 10% are not releasable.

Group 4: Samboja/Wanariset. Presenter: Drh Anta

1991 start – two release sites Sungai Wain (30 to 1997) and Meratus – (300 since 2003) – limited post release monitoring – now a new place for a release has been located, 4 population categories:

1. Healthy – releasable
2. Hepatitis B – 35 individuals but not known whether human or simian – budget constraints

3. ‘Ex TB’ – had TB but were treated with human meds – un-releasable as they relapse or are re-infected – difficult to know which.

4. ‘Active TB’ – un-releasable – what are the possibilities/solutions for groups 3 and 4

Anticipated problems:

Finding release sites

Orangutan – confirm health status, behavior assessments to find orangutans for release, have preliminary data on releasable orangutans, survey has been done on carrying capacity, socio economic survey (local people surrounding area of release)

Budget – needs to be set in place

Government and local people for support for the area

Increase awareness / education in the area

Man power on the ground for post release monitoring required

Transport issues to the release area – difficult to get to which is why it is still pristine

High cost of testing – Pak Joko spoke on behalf of his lab in IPB – reagents and material is expensive as it is bought abroad – so difficult to cut costs even for sanctuaries/centers

Release sites – Ekosystem restoration Concession land available at the same cost as if you were a regular concession for 60 years renewable to a 95 year maximum

Session 2 – Steps 4 and 5.

Hazard Identification and rough assessment

Production of hazard list from the four groups, ranked in a rough assessment and questions that need answering for each hazard – following Notes on Disease Risk Analysis for Primate Reintroduction Programmes page 28)
Group 1 SOCP:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Susceptibility</th>
<th>Exposure</th>
<th>Infection</th>
<th>Transmission</th>
<th>Individual</th>
<th>Population</th>
<th>Programme</th>
<th>Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyloides</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>22</td>
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</tr>
<tr>
<td>Hepatitis B</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
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<tr>
<td>Respiratory Diseases</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>19</td>
<td>7</td>
</tr>
<tr>
<td>(Flu)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entamoeba</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>21</td>
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<td>Candidiasis</td>
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<td>2</td>
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<td>Tetanus</td>
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<td>1</td>
<td>4</td>
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<tr>
<td>Herpes (HSV)</td>
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<td>2</td>
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<td>Tapeworm</td>
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<td>Giardia</td>
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<td>Hookworm</td>
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<td>Hepatitis A</td>
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<td>Air Saccultis</td>
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### Group 2 OFI:

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<thead>
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<th>Disease</th>
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<th>Exposure</th>
<th>Infection</th>
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<th>Individual</th>
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<th>Programme</th>
<th>Placement</th>
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<td>5</td>
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<td>Soft tissue wounds (fights)</td>
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<td>Hepatitis C</td>
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<tr>
<td>Pinworm</td>
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<td>1</td>
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<td>1</td>
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</tr>
<tr>
<td>Tapeworm</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>10</td>
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<tr>
<td>Herpes</td>
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<td>Air sacculitis</td>
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### Group 3 NM:

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<td>TB</td>
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</tr>
<tr>
<td>Strongyloides</td>
<td>2</td>
</tr>
<tr>
<td>Balantidium</td>
<td>3</td>
</tr>
<tr>
<td>Malaria</td>
<td>4</td>
</tr>
<tr>
<td>Hookworm</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella</td>
<td>6</td>
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<tr>
<td>Enterobacter</td>
<td>7</td>
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<tr>
<td>Pseudomonas</td>
<td>8</td>
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<tr>
<td>Malnutrition</td>
<td>9</td>
</tr>
<tr>
<td>Trauma</td>
<td>10</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>11</td>
</tr>
<tr>
<td>Herpes</td>
<td>12</td>
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<tr>
<td>Cataract</td>
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<tr>
<td>Meliodosis</td>
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<tr>
<td>Influenza</td>
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### Group 4 Samboja:

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<th>Disease</th>
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<tbody>
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<td>TB</td>
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</tr>
<tr>
<td>Hepatitis B</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td>3</td>
</tr>
<tr>
<td>Salmonella</td>
<td>4</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>5</td>
</tr>
<tr>
<td>Malaria</td>
<td>6</td>
</tr>
<tr>
<td>Hepatitis A/C</td>
<td>7</td>
</tr>
<tr>
<td>Strongyloides</td>
<td>8</td>
</tr>
<tr>
<td>Balantidium</td>
<td>9</td>
</tr>
<tr>
<td>Entamoeba</td>
<td>10</td>
</tr>
<tr>
<td>Dengue</td>
<td>11</td>
</tr>
<tr>
<td>Hookworm</td>
<td>12</td>
</tr>
<tr>
<td>Ringworm</td>
<td>13</td>
</tr>
<tr>
<td>Rickets/Hypo Ca</td>
<td>14</td>
</tr>
<tr>
<td>Meliodosis</td>
<td>15</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16</td>
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<tr>
<td>Tetanus</td>
<td>17</td>
</tr>
<tr>
<td>Rota virus</td>
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<tr>
<td>Pasteurella</td>
<td>19</td>
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<tr>
<td>Pseudomonas</td>
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<tr>
<td>Strep pneumoniae</td>
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<td>Filariasis</td>
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<tr>
<td>Coccidiosis</td>
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<tr>
<td>Leptospirosis</td>
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<tr>
<td>Tapeworm</td>
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</tr>
<tr>
<td>Whipworm</td>
<td>26</td>
</tr>
<tr>
<td>Ascarids</td>
<td>27</td>
</tr>
</tbody>
</table>
List of 25 diseases of immediate concern

Each participant will research and investigate a disease from the list which will produce a rough assessment.

Diseases: Researchers:

- Mycobacterium complex: Citra
- Salmonella typhi: Sumita
- Shigella: Ian
- Strongyloides: Anta
- Balantidium: Siska
- Entamoeba: Siska
- Plasmodium: Reuben
- Hookworm: Yenny
- Hepatitis B: Winny
- Steptococcus: Agus/Melanie
- Haemophilus: Agus/Melanie
- Klebsiella: Agus/Melanie
- Pseudomonas: Agus/Melanie
- Pasteurella: Karmele
- Malnutrition: Popo/Fikri
- Clostridium: Rachmad
- Burkhodellia pseudomallei: Imung (Joko)
- STLV: Imung (Joko)
- SRV: Imung (Joko)
- EMCV: Steve
- Candida: Yenny
- Dengue Fever: Siska
- Micosporum spp: Popo/Fikri

2 examples below

**Rough Assessment: Disease example:** EMCV – Encephalomyocarditis virus. Family Picornaviridae, Genus Cardiovirus. Species: Orang-utans

**Likelihood of susceptibility:** 4. Susceptibility varies between species. Peracute mortality has occurred in orangutans.

**Likelihood of Exposure:** 4. Currently unknown due to lack of data, but with suspected prevalence being high in wildlife, take precautionary approach due to vermin issues in most sanctuaries. Biosecurity measures will mitigate this somewhat (vermin control and potential vaccination – this second IF have confirmed cases. Note however, severe local reaction to vaccination seen in bonobos).

**Likelihood of Becoming Infected:** 3. Depends on local biosecurity – is spread in urine and faeces from rodents. Also species dependant. Ro/ ID50 unknown, but highly virulent in African elephants, while Asian elephants appear to seroconvert. Sudden death has been seen in orangutans.
**Likelihood of Transmitting it to others:** 3. Depends on biosecurity as for above question.

**Severity for the individual:** 4. Species dependant – subclinical to per acute death

**Severity for the Population:** 4. Outbreaks confirmed in chimpanzees, bonobos and Bornean orangutans. Potentially disastrous, with mortality up to 10%.

**Zoonotic potential (extra question)? 2 (over 4 categories).** This is LOW directly from apes due to transmission method BUT, humans are susceptible to infection in the same way apes are. Infection is possible in humans, but disease is rare.

**Estimated Significance to the Programme?:** 23/35 = HIGH Requires risk assessment and management.

**References Used:** PASA vet healthcare manual Chapter 5.9 (and peer reviewed references contained therein); Vogelnest et al JZWM; Mclelland D Doctoral thesis, University of Sydney; see further reference listed within this thesis, personal experience

**Rough Risk Assessment:** *Pasteurella sp.*

According to Kawashima *et al.* (2010) and Ashley *et al.* (2003) *Pasteurella* is found in the nasopharinx and gastrointestinal track of domestic animals. It produces a secondary infection in humans with low pathogenicity in healthy individuals. Contact with domestic animals increase the likelihood of infection. Very occasionally produces infectious disease in humans. As reported by Ashley *et al.* (2004) most of the human *Pasteurella* infections usually manifest as local skin or soft tissue infection following an animal bite or scratch. Systemic infections are less common and are limited to patients at the extremes of age or those who have serious underlying disorders.

Escande and Lion (1993) found in a retrospective study of infections due to *Pasteurella* that among the 958 cases recorded, wound infections (bites, scratches and punctures) were the common forms of pasteurellosis (66%) caused by *P. multocida* (48%), *P. canis* (11%), *P. dagmatis* (5%), *P. stomatis* (4%). In human infections unrelated to animal wounds, respiratory tract diseases and bacteremia-septicemia were the predominant infections with respectively 19 and 11%, and caused by *P. multocida*. Next in importance were urogenital (2.5%), abdominal (1%) and central nervous system (< 1%) infections.

In a case study by Ashley *et al.* (2004) it is reported a fatal case of peritonitis and septicaemia caused by *Pasteurella dagmatis* in a patient with cirrhosis. The infection followed a scratch inflicted by a pet dog. Spontaneous bacterial peritonitis caused by *P. dagmatis* had not been reported previously. According to Ashley *et al.* (2004) *Pasteurella dagmatis* is a relatively recently described species, which is rarely reported as a human pathogen. This species may be misidentified unless commercial identification systems are supplemented by additional biochemical tests.

**Table by Kawashima *et al.* 2010:**

<table>
<thead>
<tr>
<th>Case/author</th>
<th>Age/sex</th>
<th>Animal Contact</th>
<th>Risk factor</th>
<th>Antibiotic</th>
<th>Neurological complication</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per <em>et al.</em></td>
<td>15/M</td>
<td>Rabbit</td>
<td>None</td>
<td>Cefazolin, penicillin, chloramphenicol</td>
<td>Epidural epyema</td>
<td>recovery</td>
</tr>
<tr>
<td>O’Neill <em>et al.</em></td>
<td>72/F</td>
<td>Dog</td>
<td>None</td>
<td>Penicilline</td>
<td>Meningoencephalitis</td>
<td>Recovery</td>
</tr>
<tr>
<td>Prulx <em>et al.</em></td>
<td>33/F</td>
<td>Dog</td>
<td>None</td>
<td>Penicillin G</td>
<td>ADEM</td>
<td>Recovery</td>
</tr>
<tr>
<td>Tjen <em>et al</em></td>
<td>72/F</td>
<td>NR</td>
<td>None</td>
<td>Penicilline, cefotaxime</td>
<td>ND</td>
<td>Recovery</td>
</tr>
</tbody>
</table>
**Likelihood of susceptibility:** 1. Susceptibility is low in humans. No data found in orang-utans.

**Likelihood of Exposure:** 3. Although currently in orang-utans is unknown due to lack of data, in humans it is mostly related to close contact (bite, scratches...) with domestic animals (dogs, cats...) therefore the possibilities of exposure in orang-utans is considered very low as the access to domestic animals in rehabilitation centres is quite limited. However, contact with dogs, cats and other domestic animals is possible while the orangutan in captivity. Biosecurity measures to avoiding the contact of orang-utans with cats and dogs would potentially reduced the risk to almost 0, unless this bacteria is also found as normal flora in orang-utans for what data has not been found in all searched literature.

**Likelihood of Becoming Infected:** 1. In humans the main via of transmission is through close contact (kissing, bite, scratch) with domestic animals. The likelihood of this happening in orangutans is very low.

**Likelihood of Transmitting it to others:** 0. No data has been found about direct transmission amongst humans therefore it is considered that likelihood of transmission amongst the orang-utans is 0.

**Severity for the individual:** 3. In humans only one fatal case has been found in the literature (Ashley et al. 2004). It is normally a treatable infection with the adequate antibiotherapy. Only concomitant diseases or association with underlying disorders and some cases of neurological complications have been found.

**Severity for the Population:** 0. Transmission amongst people has not been found in the literature. The probabilities of an outbreak are quite remote and mortality rate is very low.

**Zoonotic potential (extra question)? 1 (over 4 categories).** Potential zoonosis in humans from domestic animals. Zoonotic potential from orang-utans to humans is very unlikely.

**Estimated Significance to the Programme?:** 9/35 = very LOW risk. Does NOT Require risk management although more data specific for orangutans is needed. No data has been found for other species of *Pasteurella* (like *P. haemolytica* or *P. pestis*) in humans or other great apes.

**References Used:** Ashley et al. 2004; Escande and Lion 1993; Kawashima et al. 2010.

**Session 3. Steps 6 and 7**

Specific questions and policy/scenarios defined

Building the model /ID critical control points

Several diseases were worked on. Questions asked for these diseases: what could the possible disease path be?
Where could disease enter the pathway or exit from the pathway?

What can be done to mitigate these disease risks?

Then, review of the remaining steps in dealing with diseases of concern at the several control points

**Critical Control Points (CCP) charts following Notes on Disease Risk Analysis for Primate Reintroduction Programmes page 32**

Group 1 SOCP: tracked CCPs for Salmonella

CCP1: Place of origin and transport to quarantine / possibly from owner/visitor/keeper/other animals/villagers

CCP2: Quarantine/possibly from other orangutans/staff

CCP3: Transport to reintroduction site/from food/from people associated with reintroduction

CCP4: Reintroduction site/from staff/other orangutans

CCP5: Transport to release site/from porters

CCP6: Release Site (Forest)/from villagers living near forest/illegal loggers and hunters/food/rubbish
Group 2. OFI – tracked CCPs for strongyloides

CCP1: As illegal pet/contact between orangutan and humans/Orangutan kept on the ground

CCP2: At reintroduction site/contact with other orangutan/during forest training/staff/keepers/other animals/environment

CCP3: At release site/contact with other orangutans/contact with other wildlife/ the forest/humans
Group 3. NM – tracked CCPs for malaria

Confiscation/Rescue → Reintroduction Centre → Release Site

CCP1: New Arrival Quarantine
CCP2: Forest School 1, 2, 3
CCP3: Pre-Release Island
CCP4: Socialization Cages
CCP5: Quarantine (Pre-release)
CCP6: Transport (Truck/Plane)
CCP6: Holding Cages
CCP6: Transport (Choppers)
CCP7: Point of Release
Group 4. Samboja – tracked CCPs for TB (as TB is endemic in human populations in KalTim as well as impacting orangutans)

Drh. Citra tracked the spread of TB through the population of Samboja from 1998 through to 2010. What should be done with ex Tb and TB orangutans? Once you treat Orangutans for TB they are virtually out of any reintroduction.
Step 8-10

ACTION: Enhance and complete work conducted within this workshop and conduct qualitative +/- quantitative assessments in time for presentation at the 2011 workshop.

Introduction to various computer programmes that may be of interest and use for disease risk (refer also to the Animal movements and disease risk workbook)

Useful Software

POPULATION MODELLING - Vortex [www.cbsg.org/cbsg/vortex](http://www.cbsg.org/cbsg/vortex). Models population viability

RISK ANALYSIS - @risk - [www.palisade.com/risk](http://www.palisade.com/risk). Most widely used risk analysis tool on the internet

DISEASE INVESTIGATION - Epi-info – [www.cdc.gov/epiinfo](http://www.cdc.gov/epiinfo). Database


Session 4.

Risk Mitigation Strategies Disease Contingency Planning

PRESENTATION Example - Infection with Tuberculosis complex (human/bovine). Steve Unwin

What’s affected? Primates. Hoofstock. Wildlife reservoir. This disease is a zoonosis

Is there legislation that controls the movement of animals with TB?

Knowing the law is part of risk assessment

What is the public perception of human health risk?

How do you handle media interpretation of disease events?

What is your current strategy for preventing TB?

- 90 day quarantine?
- Separate groups of suspected cases?
• Restrict human contact – bio security measures?
Is this a disease of concern that requires management?

No one currently has a disease contingency plan in place for TB

EXERCISE: Create a draft contingency plan for a disease of your choice. Can utilize the same diseases as conducted in Session 3. These can be discussed over the coming months, to make sure all aspects have been considered. You can then use this as a template to create other contingency plans.

Group 1. SOCP: Salmonella Contingency Plan / Winny presented

<table>
<thead>
<tr>
<th>Main Routes of transmission</th>
<th>Contingency Plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Arrivals</td>
<td>Screening</td>
</tr>
<tr>
<td></td>
<td>Quarantine: 60 - 90 days</td>
</tr>
<tr>
<td></td>
<td>If positive, treatment initiated and individual isolated</td>
</tr>
<tr>
<td>Infected Existing Animals</td>
<td>Isolation of active animals in treatment</td>
</tr>
<tr>
<td></td>
<td>Carriers: routine screening and isolation</td>
</tr>
<tr>
<td>Wild and Domestic Animals</td>
<td>minimize possible entry and contact in food preparation area, utensils, equipment, etc.</td>
</tr>
<tr>
<td>Food Source</td>
<td>Clean, reliable source</td>
</tr>
<tr>
<td>Fomites</td>
<td>Clean equipment regularly</td>
</tr>
<tr>
<td></td>
<td>No sharing between facilities/sectors</td>
</tr>
<tr>
<td>Waste Management</td>
<td>Proper disposal of waste food to prevent/discourage wild animals from gaining access</td>
</tr>
<tr>
<td></td>
<td>Proper routine cleaning and management of waste material especially from infected cages/sectors</td>
</tr>
<tr>
<td>Personnel</td>
<td>Routine screening of existing staff</td>
</tr>
<tr>
<td></td>
<td>Screening of new staff</td>
</tr>
<tr>
<td></td>
<td>Use of P.P.E. when handling potential cases of salmonelosis</td>
</tr>
<tr>
<td></td>
<td>Infected personnel must be given necessary support: i.e. treatment and time off for cure</td>
</tr>
<tr>
<td>Visitors</td>
<td>Control and regulate contact with animals at facilities</td>
</tr>
<tr>
<td>Proper Disinfection Protocol</td>
<td>Personnel/Equipment/Environment!</td>
</tr>
</tbody>
</table>
## Group 2. OFI: Entamoeba Contingency Plan / Adi presented

<table>
<thead>
<tr>
<th>Main Routes</th>
<th>Contingency Plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>New orangutans</td>
<td>Control/prevention - isolation, hygiene, fecal tests on arrival and 2 more</td>
</tr>
<tr>
<td>Infected humans</td>
<td>Control - hygiene (wash hands, protective clothing)</td>
</tr>
<tr>
<td></td>
<td>Isolate in restrictive area / Test staff / Prevent contact with visitors</td>
</tr>
<tr>
<td></td>
<td>Fecal contamination (waste)</td>
</tr>
<tr>
<td>Food</td>
<td>Control: clean water source, wash food items, control source fo food</td>
</tr>
<tr>
<td>Fomites</td>
<td>Control vehicles into quarantine/center</td>
</tr>
<tr>
<td></td>
<td>Control/restrict visitors</td>
</tr>
<tr>
<td></td>
<td>Foot baths: disinfect or change shoes / Coveralls, protective clothing, masks</td>
</tr>
<tr>
<td>Outbreak</td>
<td>(Positive fecal entamoeba/clinically sick)</td>
</tr>
<tr>
<td></td>
<td>Clean area with disinfectant / move to individual cage for isolation</td>
</tr>
<tr>
<td></td>
<td>Limit staff access / Must wear protective clothing which stays at isolation area</td>
</tr>
<tr>
<td></td>
<td>Foot baths - clean and change shoes</td>
</tr>
<tr>
<td></td>
<td>Appropriate treatment /fecal test for all other orangutans</td>
</tr>
<tr>
<td></td>
<td>NO movement of people, animals, or equipment between epidemiological units</td>
</tr>
<tr>
<td></td>
<td>(Clinic / Isolation cages / Quarantine / New Arrivals / Socialization cages or areas)</td>
</tr>
</tbody>
</table>

## Group 3. NM: Malaria Contingency Plan / Reuben presented

<table>
<thead>
<tr>
<th>Main Routes of Transmission</th>
<th>Contingency Plan (Reduce Risk Transmission to/from Center)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito (Vector)</td>
<td>Aim: to control vector population</td>
</tr>
<tr>
<td></td>
<td>Preventative measure: to reduce breeding site vectors</td>
</tr>
<tr>
<td></td>
<td>Plant anti-mosquito plants</td>
</tr>
<tr>
<td></td>
<td>Fogging (chemical control)</td>
</tr>
<tr>
<td>Wildlife (NHP/Macaque)</td>
<td>Aim: reduce contact/distract macaques from the center</td>
</tr>
<tr>
<td></td>
<td>How: Population control - Birth control/provide food outside of center (Ablenkw Manover)</td>
</tr>
<tr>
<td>Infected Human</td>
<td>Aim: reduce transmission from human to population in the enter</td>
</tr>
<tr>
<td></td>
<td>How: Health surveillance on community and staff around the center /restrictions for visitors</td>
</tr>
</tbody>
</table>

### Early Warning System

| Screening for malaria       | Aim: current status of malaria                             |
|                            | How: screen orangutans and staff routinely / collaborate with local health service (PUSKESMAS) |
| Data Analysis (OU and human)| Aim: to know the trend of malaria                          |
|                            | How: Improve medical records/special recording of malaria/Human data from PUSKESMAS |
| If outbreak...             | Isolate the center and positive orangutans - epidemiological units |
|                            | Stop orangutan movement / Stop receiving new orangutans/check entire population |
|                            | Treatment for the entire population                         |
|                            | Media communication (Public information) / Check current community situation (PUSKESMAS) |
### Risk Analysis QUIZ

1. Knowledge of animal behaviour is one of three areas that help you make an evidence based decision in a medical case. What are the other 2?
2. Define risk.
3. What are the three basic areas of a risk analysis? What are the 10 steps of a risk analysis?
4. List 5 diseases that you are concerned with in your orangutans. Use a sentence or 2 for each to explain why.
5. What is a critical control point?
6. In a drawing, illustrate the difference between precision and accuracy.
7. What is the difference between specificity and sensitivity in a diagnostic test?
8. Name three factors that influence outcome of diagnostic test results.
9. What information do you need to initiate a disease contingency plan?
10. What is biosecurity?
11. Define quarantine.


Summary of responses:

Animal behavior, clinical experience/findings, research (other scientists, literature review) – this will inform you on how to manage that situation, then you can inform your manager – of course it helps if that manager is a vet, but the more informed you are the better informed your manager (who will ultimately make the decisions?)

Know the meaning of specificity – tests that are highly specific for perhaps one disease may not give you all the information you are really looking for. One of the main things to discover is how a disease is transmitted.

Know how to use the test correctly. Understand prevalence. Reliability of the samples – be sure you are sending the proper sample for the test you are doing. The quality of the test itself – are you using the correct test for what you are looking for?

How do you relay information to the manager? Sometimes you need to visually demonstrate to keepers by actually doing the procedure as you want it done until they get into the same rhythm – for managers, an informative PowerPoint should give them a better understanding of what you as a vet need and are up against – include a chart of your collected data – with data, you can say you know something is happening rather than you think something is happening – clinical accurate findings is key to supporting your case - you also should give your manager options on dealing with the issue

RISK ANALYSIS ACTIONS:
Present completed plans at the 2011 workshop
Present this year’s workshop report to CBSG for input
FIELD TRIPS

**Wednesday August 4**

Trip to SOCP Quarantine

At quarantine, Wendi presented parasitology session (see below)

Group divided into 3 sub-groups: with Wendi for Parasitology session / with Steve for Enrichment session / with Rachmad and Ian for center tour

Durian fruit feast

Bus ride to Bukit Lawang

**Thursday August 5**

Day at Bukit Lawang – to Orangutan feeding station

Informal discussion with Taman Nasional staff/rangers to offer suggestions on how to improve conditions between orangutans and tourists in the Park

Lunch with Park Rangers

Visit to Eco-farming facility run by YEL

Return to Hotel

**Field Parasitology and New Technologies: Dr Wendi Bailey**

Parasitology: Recap from last year’s meeting – parasitological slide quiz (Available on request)

New tuberculosis test by QBC. Fluorescent test - Previously this was quite expensive and difficult to use but now there is a microscope add-on objective lens with an LED light that can be attached to pretty much any microscope. The unit can run off a solar battery or regular electricity and can come with several magnifications. It is used with a simple dye, Oramine O. The kit is being used by WHO and results look good. Its sensitivity appears high with even a few bacilli standing out and it is very fast to get results. The cost is about £1,000. Perhaps one can be shared by all? It can also be used for different tests that require a fluorescent dye. Wendi will bring this equipment next year to demonstrate.

Who decides what tests you use?

What is the reason for testing?
Is it population surveillance or individual testing?

Whatever the test, are you collecting the correct sample?

  - Sensitivity? is this information available? Ask!
  - Specificity?
  - Cost?
  - Reliability? (quality of method/reputation of laboratory)
  - Availability? (on site? Locally? Overseas?)
  - Time to obtain results?
  - Usefullness of results/interpretations?

Group Discussion

When choosing a lab: do not rely only on reputation – ask questions about their maintenance schedule, the staff, reliability – you must have this information or else you are endangering the orangutans in your facility.

Some human labs will not always give information when animal samples are involved – most labs in Indonesia are located on Java.

Normally the vets ask labs only if they can do the test – they usually do not ask about specificity or sensitivity, the lab technicians usually do not know those things either – they are just doing what they are told to do. To get this sort of information you may need to go to the head of the laboratory or the manufacturer of whatever test the lab is using.

Lab kit information is usually available on line.

What are the possibilities of creating a lab that all the centers can use?

There are items available that work in the field – it is expensive but the cost of sending everything out to other labs is in itself is a very high cost and the results may not be correct

However, maintaining equipment to its proper standard is difficult and moving specimens from island to island is also tricky.

Proper care of microscope – in a light box with silica – can preserve the life of your equipment

Practice taking blood smears, do not rely on equipment as it may fail.

You should stay informed about new tests coming out and also be able to compare them with older tests – new may not always mean better.

There are machines that are out there that are robust and do not break down easily.

Do you have a list of what equipment and tests you would want?
On page 61 in the risk handout – what is the ideal test? What can we use if that test is not available?

As vets, we tend to forget that we can do our own tests – you as vets can actually do many things in the field with a good microscope and some simple stains. Of course, you will need to use labs for more complex testing, but basic tests can be done simply in the field

**COLLECTING SAMPLES FOR SPECIFIC TESTS**

Dr Wendi Baily

1. **BLOOD**

*Filter paper spots (NO anticoagulant):* either measure sample onto paper and leave to dry or ensure calibrated punch is available when cutting out spots : AB tests (Whatman 3M chromatography paper); PCR (often special PCR paper/cards available ie Whatman)

2. **BLOOD (anticoagulated with EDTA)**

*Wet preparation, unstained drop:* motile parasites (mf; trypanosomes)

*Thin films Romanowsky stained:* RBC morphology (various haemoglobinopathies, Fe deficiency etc); WBC’s (? Eosinophilia, WBC abnormalities ; parasites plus buffered water pH7.2 (malaria, mf, tryps, borrelia) (Leishmania =same on marrow smears)

*Thick films Romanowsky stained (Field’s, Giemsa):* parasites as above

*Thick/thin films plus a fluorochrome (acridine orange (AO)- parasites as above

*Concentration techniques: QBC (commercial kit tubes containing AO): malaria, trypanosomes, mf, borrelia

*Knotts concentration: (1ml blood + 9ml 2% formol saline) centrifuged deposit for mf

*Membrane filtration (3.0um pore PC membrane + Swinnex filter holder, both 25mm diameter) mf

*AG detection: lateral flow tests (RTD’s ) HRP2 & pLDH: malaria, mf (W.b & Bm) or may use fingerprick blood straight on to test pad.

*PCR: research methods available for most organisms, equal volume of blood (EDTA) plus AL buffer (Qiagen ) OR marrow for leishmaniasis)

*Blood culture media: bacteria*
3. FAECES

**Direct smear (saline):** trophozoite stage of protozoan parasites

**Stained direct smear (Romanowsky stain):** D.fragilis

**Stained direct smear(ZN):** Cryptosporidium; Cyclospora & Isospora (can be seen without staining) (Gram’s) bacteria, morphology & staining properties; Romanowsky (blood cells)

**Flotation techniques:** NaCL (SG1.18); sugar(SG 1.27); Zn So4 (SG1.20); NaNo3 (SG 1.18 to1.20) – obtain different sensitivities depending on parasite eg cryptosporidium (sugar best) & taenia needs SG of 1.24 or>. So for diagnosis of unknown samples ideally a combination of solutions would need to be used.

**Concentration (formalin+solvent):** all ova,cysts & larvae

10% formol saline works well as general preservative for all ova,cysts and larvae- if doing field surveys useful to fix part of sample in SAF and part in formol saline.

**Saline swab-** Enterobius

**Culture (HW/strongyloides):** charcoal, Baermann, agar plate, Harada-Mori (basic method: Stool left in pot plus extra clean water) all in dark for up to 5 days at RT (26⁰C).

**Culture (bacterial):** type of media dependent upon suspected organism

**Faeces in transport swab: check media** (specific for aerobe/ anaerobe)

**AG detection:** fresh (max 24hrs)/fresh frozen or fixed 10% formol saline,SAF: Giardia, Cryptosporidium; for E.histolytica (must NOT fix sample).

**PCR:** viruses, bacteria : collected fresh (up to 24hrs) stool to cryovial if very dry add 1 drop water and mix well – freeze.) For parasites sieve stool to remove large debris, if no freezer add approx 0.1g stool/ml 96% ethanol, may be stored at RT (preferably 4⁰C) for up to 3 months NB (PCR still a research tool for faecal parasites)

4. URINE

**Fresh centrifuged deposit:** WBC’s/pus etc

**Filtration:** for S.haematobium total 10am-2pm urine collection (maximum time female worm releases eggs): 12.0um PC membrane with Swinnex filter holder.

5. SERUM/PLASMA

If PLASMA label bottle as such as many labs. heat treat sera prior to testing and plasma will clot! Always separate serum from the clot (centrifuge/stand) to avoid haemolysis if sample delayed during transit.

**AB detection tests:** viruses ; parasites- ? usefulness,
AG detection tests: viruses, bacteria, (see faeces or blood for parasites)

6. SKIN SRAPES

Fungal infections: collect into clean, dry (sterile) container: (NAOH microscopy), culture (charcoal transport swab)

mites (microscopy)

Insects: store in dry container (short term), 70% ethanol

7. SKIN SNIPS

Onchocerciasis: skin snips into small (200ul) volume of saline, examine at 30 min & 24 hrs if necessary.

NB if snip is contaminated with blood and POS mf should be stained to ensure they are not a blood inhabiting species.

8. SPUTUM/BRONCHIAL WASH

TB: ZN stain for AFB’s; fluorochrome microscopy: (auramine phenol/rapid Auramine O*) - culture (6 weeks); PCR

* developed by QBC diagnostics may be used with ParaLens (x40 objective) fluorescence microscope attachment

9. SKIN BIOPSY: PCR (Cutaneous leishmaniasis) rice grain sized tissue snip in ATL buffer, or 10% ethanol or dry container for short term storage

Fixed in 10% FS – histopathological investigations- bacteria, fungal, parasite- staining of sections will depend upon organism suspected.

10. PUS Gram’s stained smear, (check sampling technique/area sampled as no organisms in middle of lesion); E.histolytica MAY be seen on fresh (up to 30 min) sample- don’t usually see a lot of macrophages/bacteria in sample at same time as amoebae trophozoites.

11. CYST FLUID: microscopy of well-mixed drop and of centrifuged deposit (hydatid? viable protoscolices use eosin exclusion test)

12. TISSUE SAMPLES

Tissue biopsies-use a rice grain sized piece of tissue: immediate freezing OR add to 10% ethanol in distilled water OR into tissue lysis buffer(ATL- guanidine thiocyanate) RNA-later-OK for 2 weeks RT then frozen.

WHOLE WORMS

Whole, small nematode worms-wash by shaking in bottle with normal saline, pour off and add fresh saline to just cover worm.
By adding warmed 70% alcohol worms will die straight! Once cool transfer for storage to 70% alcohol containing 5% glycerol.
Large nematodes- ideally 2% glycerol in 70 alcohol- otherwise 10% formol saline (F.S.) OK but over long term worms go brittle.
For trematodes- shake vigorously in saline, remove half saline and add fixative: 10% F.S. or 70% alcohol with 3% acetic acid added.
Cestodes- wash in saline then transfer to water to relax proglottides (may take 2 hours). Blot dry and place segments in between 2 glass slides & tie with string. Place in 10% formol saline for 24hrs- remove from slides and leave in formol saline.

LIMITATIONS OF TESTS

**Direct faecal smear for E.histolytica:** faecal sample should be examined “hot”- within 30 mins of voiding.

**Strongyloides:** faeces must not be allowed to **get cold** (NOT kept in cold room or fridge as this will inhibit larval development leading to **false negative results**)

**For a faecal antigen tests:** check whether the faecal sample can be fixed prior to testing and if so what fixative is permitted)

**Bacteriology cultures:** availability of culture media/facilities for performing culture locally/time restrictions for receiving results (diagnosis or retrospective)

**Filter paper blood collection:** ensure blood spots are completely dry before placing in plastic “jiffy” bag and adding a small amount of silica gel before closing.

**Antibody tests:** false positives may occur between similar organisms (leishmania/trypanosomes; strongyloides/filariae). A high sensitivity may give a low specificity and vice versa. It may take a long time (months/years for seroconversion following successful treatment; if repeating serology the TREND of the test may be more useful than the result.

**Malaria antigen detection tests:** HRP II type tests may stay positive for 7 days to several weeks following successful treatment (long clearance time of HRP protein from blood), blood film more reliable for test of cure. pLDH tests only detect LIVE parasites so if possibility of previous, recent treatment use an HRP2 test instead (it is possible to get NEG antigen test but POS blood film in treated case). If no previous treatment the pLDH test should revert to NEG 3-5 days following treatment- if NOT then the malaria parasites are probably resistant to the drugs being used.

**PCR testing for faecal parasites:** still at research tool stage- have not been properly evaluated as a diagnostic test- ? interpretation of result.

**Blood samples containing microfilaria:** if a sheathed species and sample kept in fridge for a few days before examining, mf will have ex-sheathed so possible mis-diagnosis of species
**Further discussion topics**
Floatation and direct smears can miss things. Each faecal floatation methods are designed for a different worm - getting a simple test that can identify many rather than one

Freeze fecal samples if they are less than 24 hours old –

Many antigen tests are on the market and they are reliable – might be worth thinking about – can be read without an Eliza

PCR – difficult to interpret results for fecal parasites and it takes ages to get result back

Various things can be used to preserve the integrity of the sample for proper testing

Build a relationship with the lab you use so that they understand the priorities of your samples – perhaps invite the lab people to your facility – involve them – give them a personal connection to your samples

The storage for preservation of samples is key for getting good results – know how to keep the and preserve samples

All centers must prepare a list for next year’s workshop of:

What do you have and use on site?
What issues are you up against?
What you can do?
What you can’t do?
What you would like to do?

**Tuberculosis minuted discussions: Chairs - Steve and Citra**

Steve: 2 years ago there was an outbreak of human TB in vervet monkeys in South Arica – Chembio Diagnostics offered a serological test to look for TB in primates (as well as other species) but it has not been validated in great ape species – so we are validating their test to see if it is useful in finding TB in great apes. By combining the chembio lateral flow stat-pak with the TST for Tb surveillance we can increase sensitivity and specificity of our diagnostics. As surveillance tests, they need further testing after a positive reading. Chembio was approached with a joint proposal between PASA and OVAG for free access to the statpak test to allow TB investigations in a large number of apes to assist Chembio with test validation, and PASA and OVAG with their diagnostics and staff capacity building.

TB samples and data should be collected by end of 2011. Data analysis from mid 2011.

A proportion of positive PASA samples can be sent to the VLA lab in the U.K. This is because there is not (yet) the capacity within West and Central Africa to do TB PCR and culture. Potentials for PCR/ culture testing for the OVAG samples is still being investigated, but must take place within Indonesia.

Drh. Citra is contact person for all questions involving TB testing in Indonesia.
Serum or whole blood is needed for the Chembio statpak and results in 20 minutes.

Citra: Brief overview in bahasa Indonesia – further clarification of Steve’s introduction

224 Orangutans have already been tested in NM. Testing at Samboja will begin as close of workshop. When more kits arrive, testing can begin at other locations (SOCP)

PRESENTATION - Citra

Background and preparation

- OVAG CONFERENCE 2009: MTB COMPLEX IS ONE OF THE PROBLEMS IN REHAB CENTRES
- TO DIAGNOSE MTB COMPLEX IS DIFFICULT
- COLLATE INFORMATION (CURRENT SITUATION, PREVIOUS RESEARCH, OTHER GREAT APES)
- CHEMBIO PRIMA TB STATPAK
- RIGHT TIME
- DONATION (PRIMA TB STATPAK-CHEMBIO)
- COORDINATION (CENTRES; FUNDING; LABS; UNIVERSITY)

Research plan

SCREENING TEST (1ST ROUND)

- PRIMA TB STATPAK
- TST : AVIAN PPD & BOVINE PPD

CONFIRMATION TEST (2ND ROUND) (+VE OUs FROM 1ST ROUND)

- PCR-MTB COMPLEX
- CHEST RADIOGRAPH
- CULTURE MTB COMPLEX
- HISTOPATOLOGY
- AFB (?)

TST

- SEDATED OU
- APPD : RIGHT EYE
- BPPD : LEFT EYE
- READ 24, 48, 72 H
- GRADE 0 TO 5 : 0-2 (-VE); 3 (SUSPICIOUS), 4-5 (+VE)
Chembio Prima TB statpak

- SERUM. ALSO POSSIBLE: WHOLE BLOOD, PLASMA.
- 30 μl SERUM+3 DROPS SOLUTION
- READ IN 20 MINUTES
- POSITIVE: LINE IN CONTROL (C) AND TEST (T)
- NEGATIVE: LINE ONLY IN CONTROL (C)
225 OU HAVE BEEN TESTED - BOS NYARU MENTENG
PRIMA STATPAK : 15 +VE (6.67%)
TST : 115 +VE (51.11%)

Challenges

- LAB FOR PCR-MTB COMPLEX & CULTURE MTB-COMPLEX
- TST REAGENT
- OU's (ESCAPE, STAY OVERNIGHT OUTSIDE CAGE, etc)

Follow up: Pak Joko will speak to his team at Bogor Agricultural University as being the participating lab for samples in Indonesia

Reuben (Putri University) volunteered doing the sampling for free. He suggested a tracheal wash, drying the fluid and bagging it and sending to Malaysia. Problem is getting samples out of Indonesia.

Discussion of TB presentation and testing – Q and A with Steve and Citra
**Euthanasia Discussion**

**When Should Euthanasia be Considered?**

Some recommendation from participants:

- After first diagnosis, treated, then recovered but a relapse
- If the individual is a threat to the rest of the population and the quality of the individual’s life is poor
- If a clinically healthy orangutan, then you find it is positive for TB – you can treat, if it gets better it can never be released – but if it is culture positive but otherwise healthy, then long term monitoring - but if it is clinically ill and can be spreading it and it is confirmed (in isolation) that it has TB, then it should be euthanized immediately

Center managers want some guidance as to what to do with TB individuals

- Current law in Indonesia (280, which is in revision) states that Euthanasia can be done but it must be approved by the PHKA office – if they agree then it can be done
- A letter of endorsement is needed to provide to the managers
- We want to conserve the orangutans to the best of our abilities. How do cases of TB interfere with that?
- We have a confirmed disease, TB, if these cases can potentially devastate the entire population, then they need to be euthanized or must be separated from other individuals
- Remember, our goal is to reintroduce orangutan in protected areas.

Veterinarians working directly with TB individuals were asked for suggestions:

**Citra:** Would treat the first time – isolate, never to be released, if they relapse – then euthanize

**Siska:** Starting now, if an orangutan has active TB it should be euthanized

**Rachmad:** TB is in the top 3 diseases that cause human mortality, people are treated with a 3 combination drug. Rachmad’s suggestion for orangutans is that drug use must be monitored, then after treatment, they should have two chances – once they get a round of meds, if they do not respond, try a different round of medication to see if there is any response – if they do not respond to the second round of meds, then euthanize

**Popo:** Same as Citra
Yenny: Same as Citra

Adi: same as Siska

Ian: Recommendation of vet and PHKA – treat once then if relapse, euthanize

Karmele: If the center cannot provide proper isolation and bio security, then okay to euthanize

Winny: Same as Karmele

Elizabeth: This has to be a long term effort in order to maintain it

Winda: Same as Citra

Anta: Issue needs to be brought to PHKA – she has seen what has happened – agrees with Citra

Steve: How does the administration of drug treatments affect further testing – having latent orangutans is not a good thing – is the risk of treatment too big?

Reuben: Humans, can they be cured?

Wendi : No, not really, the load may go down and it is hoped they do not spread it – but cannot be said that they are ever completely clear

Steve: Will take this question to micro bacterial experts – just to be sure...

If animals are treated, never can be released: All Agreed

With Chimpanzees, and gorillas – chimpanzees were treated, they either did not respond or relapsed and died – PASA, if confirmed TB culture within a sanctuary –individual will be euthanized

With a suspect case, until the case is clarified – complete isolation for that individual –

Citra: If we only are going to euthanize new cases – we might as well decide to euthanize new and latent cases.

OVAG Joint Recommendation:

- All new active cases of definitely positive TB orangutans euthanized so as not to continue a TB population
- Previously diagnosed orangutans that have continuously relapsed – the recommendation is to Euthanize
- Orangutans that have been treated for TB and have responded well to the treatment, they can be put in a permanent separate sanctuary and managed to extinction

Before recommendation of the above, Steve will contact TB experts (medic and veterinarian) on the diagnostic regime after treatment to assess whether treatment will cause mis-reads on further testing
Rethink: Two recommendations

1. Orangutans in general
2. For centers with an existing ex-TB population, follow the second protocol

Positive results of new cases should be euthanized – to determine TB status follow the table in OVAG 2009 Report – also, if you get a culture, if it is positive – it is TB, if it is negative, you do not know – combined with a PCR and follow up tests as stated in the table from 2009 Report. Possible protocol of testing, skin test, Chembio StatPak, Acid fast, PCR, Culture, also possible X-rays (if the unit is good and interpretation is correct and should be done lying down and standing) if are all negative – then that individual does not have TB to the best of our knowledge

BOS – Samboja, a sanctuary for latent TB if they have responded positively to treatment. Because we do not yet know the trend – if they die we can investigate further – if they relapse – treat them again and if they relapse again then euthanize.

Viewing of TB Video from PASA: Dr. Lawrence Mugisha speaking on behalf of the African Sanctuaries and TB investigations there (Citra’s African counterpart)

PRESENTATION: Viral Pathogens in Orangutan (Pak Joko)

Covered several important primate retro viruses –

Simian retrovirus serotype-2 (SRV-2) – a natural aids in macaques
Simian T-lymphotropic virus (STLV) – naturally infects monkeys worldwide
Simian immunodiviciency virus (SIV) – naturally infects African monkeys, but Asian macaques is fatal
No evidence of SIV in orangutan – may be non pathogenic for humans
Natural infections of Hep B like virus occur in non-human primates
He called for a collaboration to research orangutan viruses
Recommendations and suggestions for 2011:

Timing: First week of July
Location: Gadjia Mada University, Jogjakarta

Nutrition and disease caused by malnutrition is a big concern from all delegates. Request that a nutritionist to join group next year. Steve suggested Dr. Andrea Fidgett (possible assistance from Chester Zoo), EAZA NAG chair.

In one month Raff to remind everyone of their disease research

Delegates asked to think about what test or group of tests can you do in the field? What would you like to be able to do?
What equipment do you need?

Rueben and Sumita – would travel to each center and evaluate what is available and possible at each center – Raff to coordinate – Wendi to oversee

Barbel: can get many things donated – will get list together to send to Raff to coordinate items to be hand carried in