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Evidence-based practice

The use of risk analysis methodology to generate evidence-based decision making in zoo animal disease management: using simian immunodeficiency virus (SIV) in De Brazza's monkeys (*Cercopithecus neglectus*) as a model

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Abstract
Difficult decisions regarding the management of disease in zoo animals are faced routinely. These may have a significant impact on the individual animal or a population of animals and therefore the best available evidence must be used. However, in zoos there are many situations where there is a lack of peer-reviewed papers, or significant uncertainty, controversy or confusion means that decision-making is hindered. This paper demonstrates how qualitative risk analysis techniques can be used to aide decision-making in circumstances where there is a lack of other evidence. Simian immunodeficiency virus in the De Brazza's monkey (*Cercopithecus neglectus*) has been diagnosed in the European population. Risk analysis was used to generate management guidelines to address the potential risks to other De Brazza's monkeys, other primates and humans.

Introduction

Risk is the likelihood that a hazard will cause its effects, together with a measure of its impact (MacDiarmid and Pharo 1997). Risk assessment is a tool intended to provide decision makers with an objective, repeatable and documented assessment of the risks posed by a particular course of action (MacDiarmid and Pharo 1997). It is a tool now routinely used to guide policy making and disease control planning by governments and international organisations such as the OIE (World Organisation for Animal Health). Risk assessment is intended to answer the questions:

- What can go wrong ?
- How likely is it to go wrong ?
- What would be the consequences of it going wrong ?
- What can be done to reduce the likelihood or the consequences of its going wrong?

This technique is rarely used to aide decision making in managed zoo captive breeding programmes. However, risk assessment has significant potential to help Taxon Advisory Groups formulate evidence-based policies for issues where there is an element of uncertainty, confusion or controversy, as this technique is designed to present information fully in a structured and transparent way. It is particularly useful as qualitative rather than quantitative techniques can be used where numerical or statistical data are not available or are of limited value – for example, because of small population sizes.

Simian immunodeficiency viruses (SIV) are lentiviruses that infect a wide variety of primate species (Ohta et al. 1988). Cases of SIV infection have been diagnosed in De Brazza's monkeys (*Cercopithecus neglectus*) (Bibollet-Ruche et al. 2004), and there was considerable concern about the risks these animals posed to other primates and humans. Policies thus needed to be developed for the management of these individual animals and the European Studbook (ESB) population as a whole, and this paper describes how risk assessment techniques were used to develop guidelines for the management of SIV in De Brazza's monkeys in European zoos.

Methods

There are a number of approaches to risk analysis; perhaps the most widely used and flexible is the OIE Risk Analysis Framework (1994). This is composed of four steps: (1) hazard identification, (2) risk assessment, (3) risk management and (4) risk communication.

The risk assessment process is constructed using the following steps:

Table 1. Risk terminology.

Term	Definition
Likelihood	Probability; the state or fact of being likely
Likely	Probable; such as might well happen or be true; to be reasonably expected
Negligible	So rare that it does not merit being considered
Very low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often

Table 2. Uncertainty definitions.

Level	Definition
Low	Solid and complete data available; strong evidence provided in multiple references; authors report similar conclusions
Medium	Some but no complete data available; evidence provided in small number of references; authors report conclusions that vary from one another
High	Scarce or no data available; evidence not provided in references but rather in unpublished reports or based on observations, or personal communication; authors report conclusions that vary considerably between them

- Define the unwanted outcomes and the relevant risk questions.
- Clarify the steps that are necessary to get from the hazard to the defined unwanted outcomes. This is usually achieved by producing a 'risk pathway'.
- Collect the information necessary to estimate the probability of each event in the pathway.
- Assess the risk.

Risk management is the process by which the risk manager uses the results of the risk assessment, balanced with the 'level of acceptable risk', to determine the risk mitigation measures to be put into place. Levels of acceptable risk are value-based and affected by many factors including costs, culture and perceptions, and will differ between different groups of those who are likely to be affected by the risk.

Risk communication is the exchange of information between risk managers, risk assessors and stakeholders during the development of the risk assessment and certainly before the policy is finalised. This often includes a peer-review process by experts both in risk assessment techniques (to review the methodology) and in the hazard that is being assessed. This is vital, to ensure acceptance of the risk assessment and implementation of the resulting decisions guided by it.

Results

Hazard identification

The first stage in the process is hazard identification, which determines the hazard(s) that are to be assessed. In this case SIV virus infection in De Brazza's monkeys is the hazard of concern.

Simian immunodeficiency viruses (SIV) infect a wide variety of non-human primate species in sub-Saharan Africa. The evolution of the lentiviruses is very complex but there is some evidence to suggest that the viruses are ancient and co-evolved with specific species (Allan et al. 1990; Beer et al. 1999; Hirsch and Johnson 1994). The virus that naturally infects a specific species causes

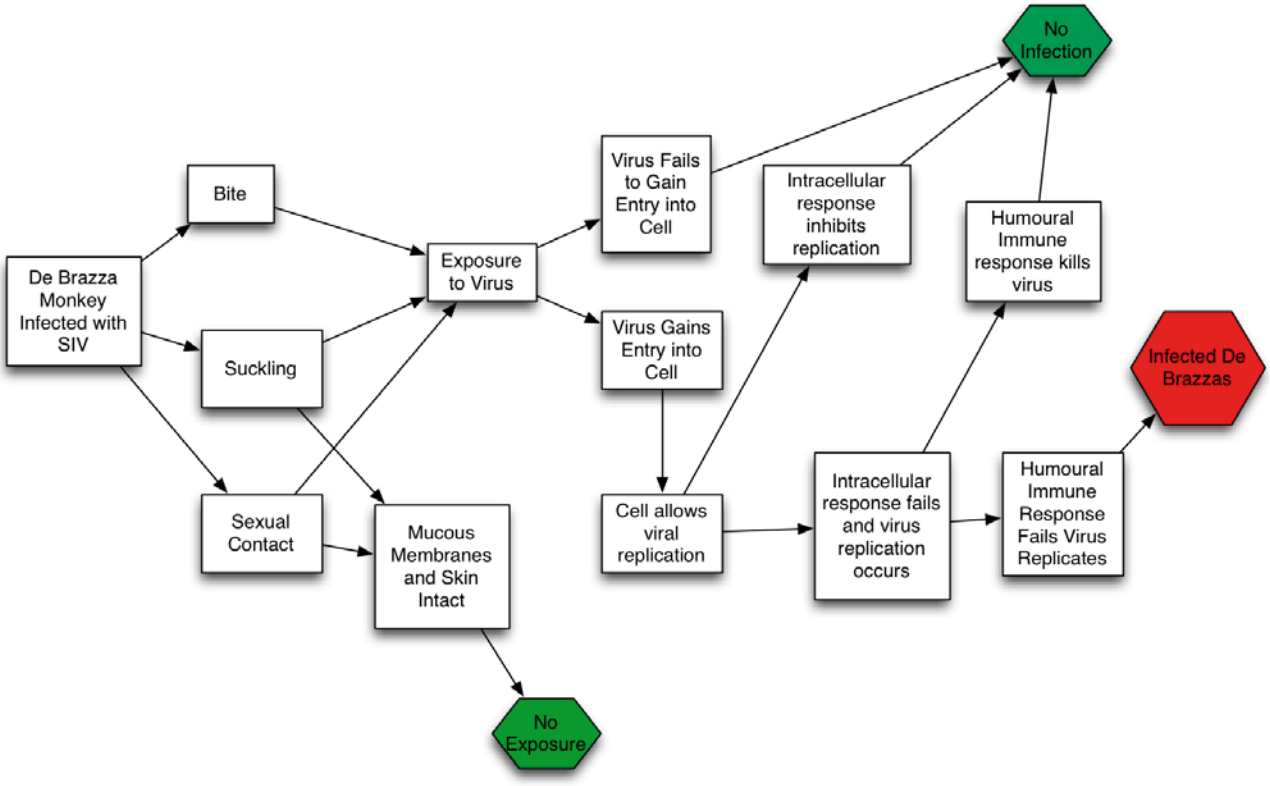

Figure 1. The risk scenario tree – the pathway of transmission between an infected De Brazza's monkey and an uninfected animal.

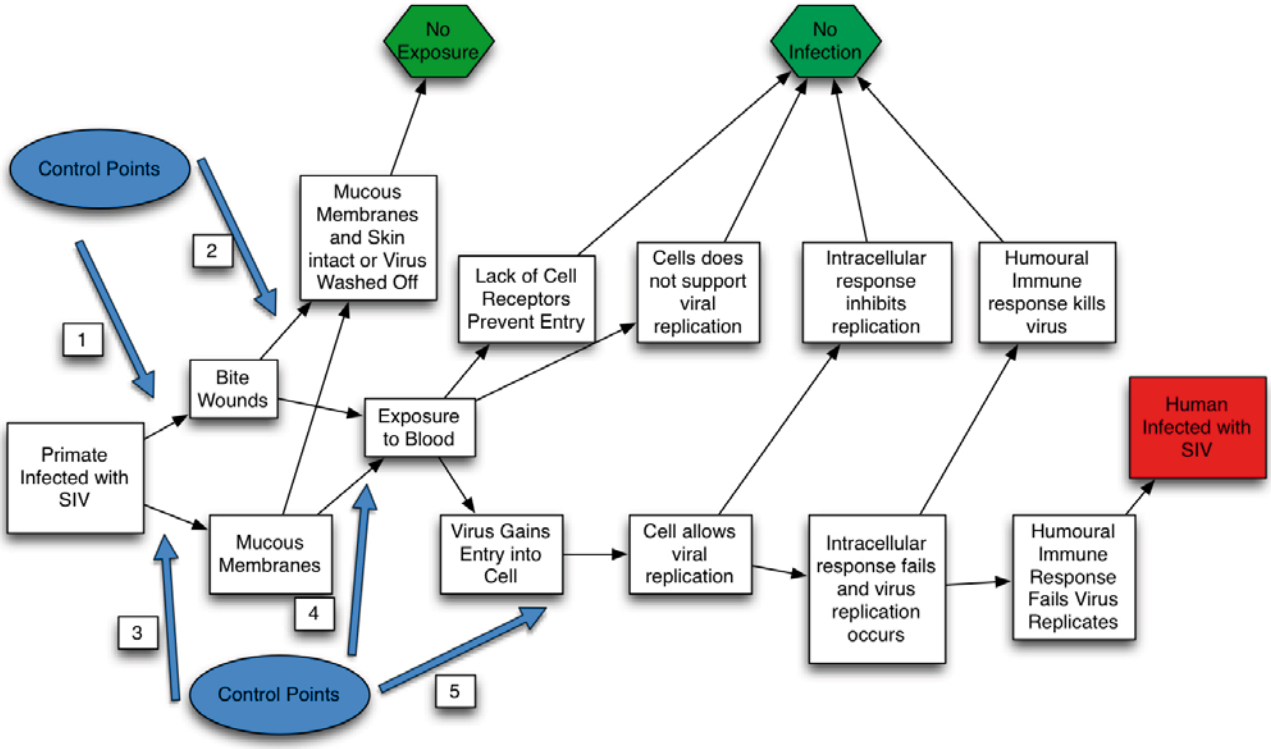

Figure 2. Transmission pathway from SIV-infected De Brazza's monkey to a human.

lifelong unapparent infection but not clinical disease. However, there is significant evidence of multiple cross-species infections (Ohta et al. 1988). In most instances these infections do not cause clinical disease but can on occasion result in immunosuppression, meningioencephalitis and lymphoproliferative disease.

The De Brazza's monkey is naturally infected with its own SIV virus, SIVdeb, which is very distinct from other guenon SIV viruses (Bibollet-Ruche et al. 2004). It is non-pathogenic to De Brazza's monkeys. Research suggests that up to 30% of this species are infected in the wild (Peeters et al. 2002).

Risk questions and pathways

The next stage in the process is to determine the risk questions. In this study these are:

1. What is the risk that an SIV-infected De Brazza's monkey will transmit the virus to another De Brazza's monkey?
2. What is the risk that an SIV-infected DeBrazza's monkey will transmit the virus to another primate that is housed in the zoo?
3. What is the risk that an SIV-infected De Brazza's monkey will transmit the virus to a human (either a keeper or zoo visitor)?

Risk questions 1 and 2

Pathways for the risk questions are then developed. For questions 1 and 2, these are presented in Figures 1 and 2 respectively.

The risk pathway is broken down into its components and the risk for each step is assessed. Risk assessment uses the risk terminology shown in Table 1, while uncertainty is categorised as shown in Table 2.

The virus is primarily transmitted horizontally through bite wounds and less commonly through sexual contact and breast milk. Indeed the virus can rarely be isolated from semen, cervical secretions or breast milk (CDC 1998). This does vary between species, with research suggesting that SIV in sooty mangabeys is definitely spread sexually, whilst this is less frequent in mandrills (George-Coubert et al. 1996). No experimental infections to further investigate transmission of SIV in De Brazza's monkeys have taken place.

Once the virus is transmitted to the new host it must enter the cells via cell receptors. The host's immune system will try to prevent this. There is evidence that intra-species and inter-species exposure does occur but an effective immune response prevents infection, as animals have been found to be serologically positive for SIV infection but not infected with the virus (VandeWoude et al. 2010).

To infect the animal the virus must successfully enter the cells and interact with cell organelles in order to replicate (VandeWoude et al. 2010). As the SIV viruses are very species-specific, it is likely that there will be incompatibility and the virus will not be able to replicate and therefore be unable to infect the animal. Experimental cross-species infection of SIVs among different species of primates has shown that in many cases the virus is harmless or cleared by the new host's immune system.

Table 3 summarises the analysis of questions 1 and 2. The overall risk assessment is of low to medium risk with medium uncertainty.

Risk question 3

A study of people with occupational exposure to primates was conducted by the USA Centers for Disease Control and Prevention Switcher et al. 2004, cited in Weston Murphy et al. 2006). Three thousand samples from people potentially exposed to SIV were tested. Only two demonstrated antibodies cross-reactive to SIV, a prevalence of less than 1%. One of these people handled known (experimentally) SIV-infected material without gloves whilst having a severe dermatitis of the hands and forearms. The second person had suffered from a needle-stick injury whilst handling

Table 3. Analysis of stages in risk pathway for questions 1 and 2.

Stage in risk pathway	Bite wound/suckling/sexual contact	Virus infects cell	Virus replicates in cell	Virus causes active disease in another primate
Mitigating actions	Avoid conflict in groups so that aggression is low. Hand raise infants of infected mothers. Do not allow infected monkeys to mate with uninfected monkeys.	Post-exposure prophylaxis	None possible but the SIV virus are very species-specific and so there is likely to be cell receptor incompatibility.	
Risk	Medium	Medium	Medium in other De Brazza's. Very low in other primates	Low
Uncertainty	Medium	Medium	Medium	Low

known experimentally infected blood. Both of these people had virtually undetectable levels of virus; this explains the lack of AIDS-like symptoms as a high circulating viral load is required for disease and transmission in HIV-infected humans. Evidence of SIV infection in zoo keepers has not been reported (Weston Murphy et al. 2006).

Epidemiological surveys of 1800 people from nine villages in Cameroon suggested very high (>60%) exposure to nonhuman primate blood and body fluids and demonstrated that 1% of exposed individuals were seropositive for SIV with three different nonhuman primate origins (Wolfe et al. 2004). Despite the fact that these events clearly demonstrate that human–primate contact occurs commonly, and can result in nonhuman primate to human retroviral transmission, human exposure to SIVs resulting in patent infections has been extremely rare. Therefore, exposure of humans to SIVs does not *a priori* result in successful cross-species infection; seropositivity merely demonstrates exposure to SIV and a subsequent immune reaction, not infection.

Cross-species infection from the natural host to other species can occur, however, and can result in pathological disease. Cross-species transmission of the specific chimpanzee and sooty mangabey SIV viruses to humans has been linked to the origin of the HIV-1 and HIV-2 virus respectively. It is thought that the SIVs entered human cells and underwent genetic changes, which then allowed human-to-human transmission. This is supported by the fact that humans in Africa have been exposed for centuries to SIVs and yet the HIV epidemic has only apparently emerged in the second half of the last century, which suggests that some other factor influenced the virus. This suggests that viral cross-species transmission is in itself not the only factor required for development of pathological disease (Wolfe et al. 2004).

Despite the large exposure of humans to SIV-infected primates in central and west Africa, through consumption of bushmeat, extensive molecular epidemiological studies have shown only 10 cross-species transmission events during the last century, and only four of these resulted in epidemic transmission (Apetrei et al. 2004).

There are over 40 species-specific SIVs, and only those from chimpanzees (SIVcpz) and sooty managabeys (SIVsm) have been shown to be associated with HIV. Indeed SIVdeb is one of the most genetically distinct viruses and is not similar to these two SIVs (Apetrei et al. 2004). The general experimental approach to determine this is to try and grow virus in human cells (human peripheral blood mononuclear cells, PBMCs) *in vitro.* Although many SIV viruses have been shown to grow in PBMCs, most of the cercopithecine SIVs do not grow in human PBMCs (Apetrei et al. 2004; Grimm et al. 2003) and none of the cercopithecine SIVs has been identified in humans (Apetrei et al. 2004).

Table 4 summarises the risk pathway for human exposure to De Brazza's monkey SIV. The overall risk assessment is very low to negligible with low uncertainty.

Risk management

By using the risk pathways it is possible to identify potential control points at which the risk pathway can be blocked and the likelihood of the pathway being completed reduced. In both pathways there are two control points; the first is preventing transmission and the second is preventing the virus from infecting cells. Once the virus has entered the cell there is little practical intervention possible to prevent infection.

There are several ways that transmission of SIV from an infected De Brazza's monkey could be prevented. SIV-infected animals could be euthanased or they could be housed individually in isolation facilities. In order to prevent infection of young born to SIV-infected mothers, infected animals could be contracepted or the young removed for handraising. Other options include

Table 4. Risk pathway for human exposure to De Brazza's monkey SIV virus.

Stage in risk pathway	Bite wound or mucus membrane exposure	Virus infects cell	Virus replicates in cell	Virus causes active disease in human
Mitigating actions	Handling precautions, gloves, goggles, face mask, washing hands, appropriate wound management	Post-exposure prophylaxis	Cells do not have correct receptors or cellular function to allow virus to replicate	None
Further evidence		In both occupational at-risk workers and bush meat hunters seroprevalence was less than 1%; infection in zoo keepers has not been reported	SIVdeb virus does not replicate in human PMBCs	Despite regular and widespread exposure for centuries, only 10 incidences of cross-species infection have been identified and only 4 of these have resulted in human disease
Risk	Low	Negligible	Negligible	Very low
Uncertainty	Low	Low	Low	Low

managing SIV-infected monkeys in groups composed only of infected animals, and enforcing management guidelines designed to reduce aggression and conflict in De Brazza's monkey groups with known infected animals.

The second control point is attempting to prevent infection in an animal exposed to the virus through the use of prophylactic drugs. This has not been attempted widely in naturally occurring exposure but has been effective in experimental infections.

Transmission to humans can be prevented through the use of protective clothing and management practices that reduce the risk of animal bites and bodily fluid transfer. Following a mucus membrane or bite wound exposure, copious lavage with chlorhexidine, which is virostatic, can be effective. Post-exposure prophylaxis with anti-retroviral drugs may be indicated following potential exposure. Medical intervention should be sought (Weston Murphy et al. 2006).

Risk communication

This risk assessment was reviewed in three ways as part of risk communication. The paper was reviewed by an expert in SIV to ensure technical and scientific completion and accuracy. The paper was also reviewed by members of the Old World Monkey Taxon Advisory Group and presented to this group in a formal meeting for ratification.

Risk mitigation and discussion

This risk assessment allowed a structured and objective evidence base to be presented to the European Association of Zoos and Aquaria (EAZA) Old World Monkey Taxon Advisory Group (TAG) for the development of a management strategy for SIV infection in the European studbook population of De Brazza's monkeys. The risks identified need to be balanced with the requirement to maintain and increase a genetically sound population of this species in European zoos.

The first decision made was that it is essential to identify which animals in the population are SIV positive and which are not. This allows zoos to implement the management protocols devised and actively manage the low but potential risks to humans and other species of primates sharing mixed exhibits with De Brazza's monkeys. Accordingly the TAG has advised that De Brazza's monkeys of SIV positive or unknown status should not be housed in mixed exhibits with other primate species.

The risk assessment provides evidence to allow the following advice to be provided to keepers working with De Brazza's monkeys infected with SIV. The majority of these should be in use for routine contact with non-human primates in a zoo environment.

The risk of transmission from urine and faeces is negligible and SIV is susceptible to household bleach and disinfectants, which should therefore be used routinely for general cleansing.

Blood is the main risk to humans. As with all primates, latex gloves should be used when handing De Brazza's monkeys. Unknown status or SIV-positive De Brazza's should not be handled when conscious to avoid bite injuries, and should not be netted, but should be darted or a put in a crush cage and then examined under anaesthesia only. Should bite injuries occur they should be immediately and thoroughly washed and lavaged with chlorhexidine. During blood collection or other invasive procedures on unknown status or SIV-positive animals, goggles, gloves and face-masks should be worn to prevent mucous membrane contamination. If mucous membranes (eyes, mouth, nose, ears) are contaminated by SIV-infected primate bodily fluids, the area should be immediately washed with chlorhexidine.

The more challenging issue is to decide if the management of the De Brazza's monkey ESB should be changed in light of SIV status when SIV in De Brazza's monkeys is a naturally occurring infection and is non-pathogenic. However, as a result of perceptions and misunderstanding of the risks, some zoos are reluctant to hold SIV-infected animals and in some collections SIV status has contributed to a decision to euthanase animals (Redrobe, pers. comm.). There is also the moral quandary of placing an animal at risk of an infectious disease, albeit a non-pathogenic disease, by knowingly moving it into a group infected with SIV.

It was decided to collect further information on the status of the current ESB population and to avoid increasing the number of SIV-infected animals by not introducing SIV-infected animals to groups that were not infected or of unknown status.

Testing for SIV in primates is well established. However, there have been some problems with interpretation of the results of tests undertaken by different laboratories as the tests have differing sensitivities (i.e. ability to detect a positive result). This has resulted in animals previously testing negative to test positive when tested by a different laboratory. It is important to remember that once animals test positive they cannot revert to being negative. If an animal tests negative if could have been recently infected and the virus not yet replicated to detectable levels. As the amount of virus in the animal is so low it will not yet be able transmit disease. This animal can be considered negative, but at some point that cannot be determined will test positive when the virus reaches detectable levels. This is, however, rare as all the tests can detect virus at very low levels. If an animal tests negative and then at a later date tests positive it has been infected by the virus in the intervening period and the animals with which it has been in contact should be tested for SIV. In order to ensure consistency and expert interpretation of the results obtained, the De Brazza's monkey ESB has recommended using a single laboratory for all testing.

It was decided to undertake testing strategically and focus on groups of monkeys that were involved in movement transactions. This is for two reasons: firstly, these animals have the greatest potential to change the infected status of a group, and secondly, the potential conflict during introductions increases the risk of transmission.

Therefore the ESB instructed that when a movement recommendation has been made, both the animal that is being sent to the new zoo and the entire group the animal is destined to join should be tested for SIV. In this way we can ensure that an SIV-positive animal is not moved into an SIV-negative group or vice versa. Zoos are also being encouraged to submit samples opportunistically.

It was also decided that any animal that did test positive for SIV should not be euthanased but that groups of known positive animals would be established so that these animals could continue to play an important and full role in the ESB. It is important not to presume that offspring born to SIV-positive parents will also be positive, so using contraceptives in infected females was not considered appropriate.

Finally, it was also decided that due to the non-pathogenic nature of the virus, stable family groups do not need to be broken up if one of the animals tests positive. This positive result has no implications for the health of the group and indeed, the risk of SIV transmission will be increased by disrupting the group and increasing the likelihood of fighting. Ultimately, the long-term viability of the ESB could be threatened as a result.

The additional evidence obtained from the risk mitigation processes described above will be fed back into the risk assessment process. Regular review of the risk assessment in the light of new information and evidence ensures that management decisions are still appropriate.

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