Department of Agriculture, Forestry and Fisheries
Directorate Animal Health

Notice No. VPN/53/2019-01

SUBJECT: **Standards for research facilities that want to import and/or maintain foreign strains of endemic companion animal helminth species for research and/or breeding purposes**

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Director: Animal Health

2019 -11- 05

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Date

PART I

DEFINITIONS

FOR THE PURPOSES OF THIS STANDARD DOCUMENT

Helminth	A general term meaning worm. The helminths are invertebrates characterized by elongated, flat or round bodies. For the purposes of this document parasitic helminths of veterinary importance, including Platyhelminthes or flatworms (flukes and tapeworms) and Nematoda or roundworms (Wakelin 1996; Castro 1996). This VPN will deal with foreign strains of species that are endemic to South Africa. This VPN pertains to helminth strains of companion animals (dogs and cats).
Helminth donor animal	An animal experimentally infected with the infective stage of a helminth species, with the aim of establishing a patent infection. The latter would allow harvesting additional infective stages, either for the purpose of maintaining the helminth strain as part of an ongoing breeding program, or as a means to obtain adequate infective material for use in a study. Normally death will not be an end point for donor animals, but may be (if for example necropsy is required to obtain fresh helminth specimens to verify species and ensure strain integrity).
Helminth study animal	An animal experimentally infected with the infective stage of a helminth species, with the aim of establishing an infection for the purpose of participating in a clinical study. Depending on the specific efficacy study design, timing of Investigational Veterinary Product (IVP) administration can target either immature or mature stages. However, apart from evaluation of efficacy, such studies can also have other objectives, such as experimental infection model validation. In efficacy studies death will typically be an end point, as helminths need to be recovered at necropsy to allow parasite counts.
Helminth breeding areas	Helminth breeding areas can be divided into animal donor areas where donor animals are maintained, and helminth breeding areas where infective helminth stages are harvested (for example from faecal cultures) and maintained. These two different types of helminth breeding areas will not necessarily be in the same building, but both will be indoors, vector proof and environmentally controlled (for temperature) in units with restricted access. Helminth breeding areas may be separate from research areas.
Helminth research areas	Areas where study animals participating in clinical studies will be housed, as well as the *post-mortem* facilities where necropsy will be performed for said studies. Helminth research areas will be indoors, vector proof, environmentally controlled (for temperature) and have restricted access. Research areas may be separate from breeding areas.
Helminth breeding	Maintaining parasite colonies and as needed propagating parasite numbers for use in studies, including the use and management of suitable host animals.
Helminth research	Studies performed on helminth parasites often evaluating the efficacy of anthelmintics against parasites in the experimentally infected target host animal.

DAFF	Department of Agriculture, Forestry and Fisheries
IATA	International Air Transport Association
PPE	Personal Protective Equipment

Red Cross Permit	A veterinary movement permit in terms of Regulation 20 (1) (a) of the animal diseases act, 1984 (Act No. 35 of 1984). It is used where animals or products to be moved are potentially infected and therefore subject to one or more restrictions en route or at destination. The animals or products must be loaded under the supervision of and sealed by a state veterinary official. The state veterinary official at origin must inform the state veterinarian or veterinary official at destination either telephonically or by facsimile of e-mail of the consignment and provide a copy of the red cross permit. The state veterinary official at destination is responsible for receiving of the animals or products and breaking of the seals prior to releasing the consignment.

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PART II

RISKS ASSOCIATED WITH THE IMPORTATION, BREEDING AND MAINTENANCE OF HELMINTHS AND PURPOSE OF THIS VPN

1. INTRODUCTION

Laboratories in which living helminths are cycled in suitable host donors, with the relevant infective stages (larvae or embryonated eggs) maintained for research purposes, have been in existence for decades, with few reports of harm to their workers or to the communities in which they are located. In addition, such laboratory strains are also maintained under appropriate biosafety conditions (US Department of Health and Human Services, 2009), including implementation of laboratory safety and containment recommendations. The latter also ensures protection of the environment, resulting in the risk of environmental contamination and infection of non-target animals (i.e. other than donors or study animals) to be reduced to negligible levels. Those of veterinary importance, however, are associated with potential risks as they are pathogens of animals with some species also posing a zoonotic threat.

To address the risks associated with maintaining helminth colonies for research purposes, certain requirements regarding the management and use of these colonies must be complied with. DAFF will consider granting permission for the importation and maintenance of laboratory bred foreign strains of indigenous species for research purposes, provided that the requirements of this VPN are adhered to. DAFF will also consider granting permission for local (South African) field strains to be sourced to establish a resident laboratory helminth strain provided that the requirements of this VPN are adhered to.

The section below is not intended to be exhaustive, but highlights potential risks associated with common helminth species often associated with companion animals.

2. HELMINTH SPECIES AND ASSOCIATED RISKS

2.1. Helminth species where the infective stage is a hatched third stage larvae (L3)

Hookworm

Ancylostoma caninum

Ancylostoma braziliense

Ancylostoma tubaeforme

Uncinaria stenocephala

Modes of infection: *Ancylostoma* spp. infection in dogs and cats is endemic worldwide, and can occur either by ingestion or through the skin (US Department of Health and Human Services, 2009). Note that *A. caninum* can also infect puppies through the milk of infected bitches (http://www.msdvetmanual.com/digestive-system/gastrointestinal-parasites-of-small-animals/hookworms-in-small-animals).

The same modes of infection apply to *Uncinaria stenocephala*, but application of larvae to the skin results in lower infection rates (http://www.aavp.org/wiki/nematodes/strongylida/ancylostomatoidea/uncinaria-stenocephala/).

Risk to personnel: Skin penetration by infective larvae is the primary hazards to laboratory staff and animal care personnel. Infection with hookworm of animal origin can result in cutaneous larva migrans or creeping eruption of the skin.

Risk to the environment: Contamination of the environment with infective third stage larvae (L3) that can infect non-target animals (i.e. other than donor or dedicated study animal dogs or cats). This can occur through:

i. Spreading of eggs or larvae with water during cage washing procedures;

ii. Spreading of eggs or larvae when processing faeces or parasite stages in the laboratory;

iii. Indirect mechanical transmission (arthropods such as flies may transmit pathogens picked up from substrates contaminated by secretory and/or excretory products of infected hosts) [Balla *et al.* (2014) and Sarwar (2015) specifically mentions house flies as a potential vector for hookworm].

2.2. Helminth species where the infective stage is a larvae developing within the egg

Roundworm

Toxocara cati

Toxocara canis

Toxascaris leonina

Modes of infection: For the purpose of this discussion, the life cycle of *Toxocara canis* will be considered, as it is complex and presents with a number of potential modes of infection (http://cal.vet.upenn.edu/projects/merial/ascarids/asc_05a.html):

i. Hosts become infected when ingesting the larvated egg containing an ensheathed second stage larva (L2).

ii. However, mice can act as paratenic hosts when they ingest the eggs, with the final host (dogs) infected when they consume the paratenic host.

In addition, puppies can become infected in two ways:

i. Firstly, nursing pups may ingest L3's in their mothers' milk.

ii. Secondly, pups may be born infected as a result of L2's migrating from tissue reservoirs in the pregnant bitch.

Risk to personnel: Working with infective eggs of ascarids such as *Toxocara* spp., poses significant risk because of the potential for visceral migration of larvae, including invasion of the eyes and central nervous system (US Department of Health and Human Services, 2009).

Risk to the environment: Contamination of the environment with larvated eggs that can infect non-target animals (i.e. other than donor or dedicated study animal dogs or cats). This can occur through:

i. Spreading of larvated eggs with water during cage washing procedures;

ii. Spreading of larvated eggs when processing faeces or parasite stages in the laboratory;

iii. Ingestion of the larvated egg by a rodent (mouse or rat) paratenic host;

iv. Transmission of parasites from bitch to offspring;

v. Indirect mechanical transmission (arthropods such as flies may transmit pathogens picked up from substrates contaminated by secretory and or excretory products of infected hosts) [Oliveira *et al.* (2002) specifically mentions house flies as a potential vector for *Toxocara* spp. and *Toxascaris* sp.].

Whipworm

Trichuris vulpis

Modes of infection: Hosts are infected when ingesting embryonated eggs.

Risk to personnel: Although there are reports in the medical literature of human infections with *T. vulpis*, these reports lack sufficient validation to consider *T. vulpis* a zoonotic parasite at this time. (https://www.capcvet.org/capc-recommendations/whipworms)

Risk to the environment: Contamination of the environment with larvated eggs that can infect non-

target animals (i.e. other than donor or dedicated study animal dogs or cats). This can occur through:

i. Spreading of larvated eggs with water during cage washing procedures;

ii. Spreading of larvated eggs when processing faeces or parasite stages in the laboratory;

iii. Indirect mechanical transmission (arthropods such as flies may transmit pathogens picked up from substrates contaminated by secretory and or excretory products of infected hosts) [Oliveira *et al.* (2002) specifically mentions house flies as a potential vector for *Trichuris* spp.].

2.3. Cestode species

Tapeworm for which breeding models making use of both intermediate and final hosts can be implemented in the laboratory

Dipylidium caninum

Taenia taeniaeformis

Modes of infection: Both helminths make use of intermediate hosts, namely fleas (*Dipylidium caninum*) and rodents (*Taenia taeniaeformis*). The final host must ingest the infected intermediate host to become infected.

Risk to personnel: Only cats have been reported to become infected by *T. taeniaeformis*, but human infections with *D. caninum* have been known to occur.

Risk to the environment: Contamination of the environment with infective eggs that can infect non-target animals (i.e. other than donor or dedicated study animal dogs or cats). This can occur through:

i. Spreading of gravid proglottids or eggs with water during cage washing procedures;

ii. Spreading of gravid proglottids or eggs when processing faeces or parasite stages in the laboratory;

iii. Ingestions of eggs by intermediate hosts (fleas or rodents, as applicable);

iv. Indirect mechanical transmission (arthropods such as flies may transmit pathogens picked up from substrates contaminated by secretory and or excretory products of infected hosts) [Sarwar (2015) specifically mentions house flies as a potential vector for both *Taenia* spp. and *Dipylidium* spp.].

Tapeworm for which breeding models making use of a final host shouldbe implemented in the laboratory with extreme caution

Echinococcus multilocularis

Echinococcus granulosus

Modes of infection: Both helminths make use of intermediate hosts, namely rodents (*E. multilocularis*) and a wide range of other mammals (*E. granulosus*). The final host must ingest the infected intermediate host to become infected. However, infective material can also reproduce by asexual budding within the intermediate host in the case of *E. multilocularis*.

Risk to personnel: Both parasite species pose a severe zoonotic threat to humans if infective eggs are ingested, as the parasite will then assume the human is the intermediate host and cyst formation will ensue. For breeding/cycling strains of *E. granulosus* in the laboratory, sheep have to be infected with eggs from gravid proglottids (increasing the infections risk to research personnel). For cycling of *E. multilocularis*, cycling through a final host is not required (rodent intermediate hosts can be infected through injection of infective material into the peritoneal cavity).

Risk to the environment: Contamination of the environment with gravid proglottids and infective eggs. All study animals should be subjected to euthanasia prior to patency being reached (i.e. prior to mature proglottids being present). For breeding/cycling strains of *E. granulosus* in the laboratory,

use of gravid proglottids is a necessity and specific precautions will be required as detailed elsewhere in this VPN (Parts III and IV).

3. HELMINTH RISK ASSESSMENT SYNOPSIS

Personnel:

Some of the helminth species mentioned pose a zoonotic threat, either though ingestion or through contact with the skin.

Environment:

Contamination of the environment with infective stages that can infect non-target animals (i.e. other than donor or dedicated study animal dogs or cats) can occur through:

Route 1: Spreading of infective stages by donor animals when they are moved out of the helminth breeding or study units. This may potentially include shedding of infective material in faeces, or mechanical transmission of infective stages on the coats of animals even after effective deworming.

Route 2: Spreading of infective stages with water during cage/pen washing procedures.

Route 3: Spreading of infective stages when processing faeces or parasite stages in the laboratory. This may include procedures such as necropsy, washing, sieving, mechanical transmission by research and cleaning personnel and accidental spillage.

Of these potential containment risks, these three potential contamination routes are considered most important for all of the helminth species discussed.

Route 4: Indirect mechanical transmission by mechanical vectors (arthropods such as flies may transmit pathogens picked up from substrates contaminated by secretory and or excretory products of infected hosts);

With reference to route 4, Thyssen *et al* (2004) examined 700 insects (of which 275 were flies from the order Diptera. None of the Diptera representatives carried parasitic forms.

Furthermore, El-Sherbini and El-Sherbini (2011) found that flies only carried one to three eggs on the body surface. None the less, this route of transmission, though unlikely, remains a significant risk that needs to be adequately addressed.

Route 5: Ingestion of the infective stage by an intermediate or paratenic host (rodents, fleas and aquatic snails) is applicable to some of the examples mentioned.

Route 6: Transmission of parasites from bitch to offspring (applicable to *T. canis* and *A. caninum*, the latter through the milk of infected bitches).

Routes 5 and 6 are only applicable to certain helminth species.

Studies where both helminths and intermediate hosts are involved:

Note that for breeding programmes or studies where both intermediate hosts and definitive hosts are involved, dual control measures must be in place for both the relevant helminth and the intermediate host (for example *S. lupi* and beetles, *D. caninum* and fleas *et cetera*) involved in the breeding process. In such instances both must be maintained in the same insect/helminth breeding unit with the applicable dual measures in place. The same applies to *Echinococcus* spp. where animals are used as intermediate hosts. Such animals must be maintained in dedicated rooms within the relevant helminth breeding/research unit, with infection and recovery of infective stages also performed in dedicated rooms within said unit. No infected intermediate hosts may be allowed to leave the unit. Applicable measures will be discussed shortly (Parts III and IV).

4. PURPOSE OF THIS VPN

The purpose of this VPN is to provide guidance in terms of the Biosafety Level procedures to be in place for research facilities that wish to import and maintain laboratory bred foreign strains of endemic companion animal helminth species, for the purposes of breeding or use in research studies. Procedures to be followed to mitigate the risks will be addressed with reference to the zoonotic threat and the six routes discussed in point 3 on the previous page.

PART III
PRIMARY BARRIERS

1. PERSONAL PROTECTIVE EQUIPMENT (PPE)

1.1. Dedicated overalls must be worn at all times in the helminth breeding/research area.

1.2. In addition, disposable aprons and examination gloves must be worn at all times when handling potentially infective material or potential sources of such material.

1.3. Submersible protective work shoes must be worn at all times. Submersible shoes must be cleaned by stepping through a foot bath when leaving the area.

1.4. Where there is a danger of oral transmission of a zoonotic threat due to the research activity performed, for example when sieving gastro-intestinal content in cases where *Echinococcus* spp. is present, face masks must also be worn. Ingestion of *Echinococcus* spp. eggs poses the greatest zoonotic threat. When performing experimental studies, euthanasia and necropsy should be performed prior to the patency period being reached.

1.5. All personnel entering the helminth breeding/research areas must change into PPE that must be stored within the double door vestibule at the entrance to the helminth breeding/ research area.

1.6. The PPE must remain within the helminth research/breeding area. If the PPE washroom is not located inside the helminth breeding/research area, the PPE must be decontaminated with a method or disinfectant capable of, as far as possible inactivating adhered infective helminth stages and placed in double impervious plastic bags prior to removal to the washroom. Proof of the disinfectant's efficacy must be available.

1.7. Eating, drinking or smoking in the dedicated breeding or research building must not be allowed.

1.8. Handlers and cleaners working with helminth donor or helminth research animals should preferably not work with other study/breeding animals or in colony buildings.

1.9. Disposable PPE such as gloves and plastic aprons must be decontaminated with a method or product capable inactivating adhered infective helminth stages, sealed in double impervious, insect proof bags and transported to the incinerator on site by hand or vehicle. Proof of the disinfectant's efficacy must be available.

1.10. For Echinococcus spp. the following additional specific PPE requirements will apply:

1.10.1. For tasks not involving necropsy (such as cleaning of cages/stanchions and the rooms containing them), housing laboratory strain mice infected with *E. multilocularis*, or sheep infected with *E. granulosus*, goggles and face masks need not be worn (little risk of ingesting eggs and no other infective material are expelled by the intermediate hosts).

1.10.2. However, for all tasks (not involving necropsy) including feeding as well as cleaning of cages (and the rooms containing them) housing dog donors infected with *E. granulosus*, goggles and face masks (covering nose and mouth) need to be worn (risk of ingesting eggs as proglottids will be expelled in the faeces of the final host once the parasite reaches patency).

1.10.3. Infected intermediate hosts have to be necropsied to remove infective *Echinococcus*

spp.cyst material. During all necropsy procedures, or procedures where any potentially infective material (notably proglottids and eggs with reference to a zoonotic threat, but also when working with cyst material/metacestodes) is involved, disposable coveralls (used once and then incinerated), goggles and face masks need to be worn, as well as examination gloves with longer sleeves (such as those used during artificial insemination procedures). For additional necropsy requirements, see Part III Section 4.

1.10.4. Furthermore, a regular deworming program employing a registered anthelmintic with known efficacy (such as Biltricide or Cisticide), must be in place to treat personnel working with *Echinococcus* spp. material, working with infected final hosts or working with infected intermediate hosts. In case of exposure to infective material, the relevant Safety Officer must be informed, the incident formally logged on company records and the required prophylactic treatment initiated.

PART IV
SECONDARY BARRIERS

1. LOCATION AND ACCESS CONTROL FOR HELMINTH RESEARCH AND/OR BREEDING AREAS WITHIN THE FACILITY

1.1. Proper access control to the entire research facility must be in place.

1.2. There must be additional access control to the helminth research / breeding areas to prevent any persons, animals or vectors gaining unnecessary access to these. This must include:

1.2.1. Physical separation of the helminth breeding/research area from the rest of the unit or building

1.2.2. A double door system with a vestibule for the foot bath and protective clothing at the entrance and exit to the helminth breeding/research area.

1.3. Foot baths (not mats) must be in place at the entrance and exit of all helminth research/breeding areas. Foot baths must contain a disinfectant such as industrial bleach or other product at a concentration suitable to rinse any potential infective stages from boots. Proof of the disinfectant's efficacy must be available.

Foot baths must be emptied into a closed drain system as in Part IV point 2.3 when cleaned, to avoid environmental contamination.

1.4. The doors to all research areas where helminth studies are conducted, as well as breeding areas where helminths are maintained, must be self-locking and access controlled.

1.5. Ideally both the helminth research/breeding areas must be within the same building to prevent the transport of infective material between buildings. In cases where helminth stages have to be transported between buildings, suitable containers must be used as described in Section 3.

2. ADDITIONAL BARRIERS AT HELMINTH RESEARCH / BREEDING BUILDING(S)

2.1. Storage and handling of helminths must be restricted to designated areas within the helminth research/breeding building, where studies are conducted.

2.2. Helminth breeding and research areas must be designed, constructed, and maintained to facilitate effective cleaning and housekeeping. This includes appropriately coloured, smooth waterproof floors and walls to allow proper cleaning. These should not be covered or obscured;

2.3. Excessive "splashing" of water onto walls and other equipment during cleaning of cages and the helminth research/breeding area should be avoided;

2.4. These areas must have a closed drain system. Waste water from cages where donor or research animals are housed must be channeled into a closed drain system without crossing or flowing onto other areas or buildings. Prior to entering a water treatment plant, the waste water must be disinfected using suitable means (either chemical in a closed channel or storage tank, or by other means such as using heat with methodology proven effective against the relevant helminth species under investigation);

2.5. The cleaning and disinfection protocol must be approved by DAFF;

2.6. Rodent pest control stations must be in place at all buildings where helminth research/breeding will take place. The rodent control programme must documented and records must be archived for a period of five years.

2.7. All buildings where helminth research/breeding will take place must be insect vector protected and include:

2.7.1. Either no windows, sealed windows resistant to breakage or windows covered with mesh fine enough to prevent vector access.

2.7.2. Wind curtains at the entrance/exit vestibules to the buildings.

2.7.3. Electrical control measures (e.g. UV lights with electrified grid) in the helminth breeding/research areas, at vents and at the entrance/exit vestibules to the buildings.

2.8. The helminth breeding/research areas must be cleaned daily.

2.9. Faeces of donor/research animals must be incinerated daily.

2.10. Logs must be maintained where such actions are recorded, and must be archived for a period of five years.

3. REQUIREMENTS FOR HELMINTH STORAGE, TRANSPORT AND CONTAINER CONSTRUCTION

3.1. Storage and handling of helminths must be restricted to designated areas within the helminth breeding/research building where studies are conducted

3.2. Containers used to hold helminths of any stage must be leak-proof and break-resistant.

3.3. Helminths of any stage, any potentially infectious material and faecal material must be transported between buildings in leak-proof, break-resistant, properly labelled containers that are placed within a sealed hard outer carry case or impervious plastic bag for additional security

3.4. Containers used to store larvae or eggs in the incubator must also be break- resistant (e.g. plastic culture flasks), yet prevent excessive spillage in case of accidentally tilting or dropping a container (e.g. very small holes in the lid covered by fine gauze or sieve);

3.5. Containers must be clearly labelled.

3.6. Disposable containers are recommended to allow incineration after use.

3.7. An inventory must be kept of all the helminths present within the helminth breeding area. These records must be stored for at least five years for auditing purposes.

3.8. Work surfaces and equipment must be decontaminated daily after completion of work with an appropriate disinfectant. In all rooms where animals infected with *Echinococcus* spp. are housed (final and/or intermediate hosts) care must also be taken to disinfect examination gloves prior to opening doors or using taps (i.e. to avoid potential contamination of surfaces such as doorknobs or taps). Gloves should be sprayed with disinfectant. Taps should be opened using the elbow if at all possible (i.e. if tap handle ergonomics/shape allows) with gloved hands then rinsed under running water. The tap should again be closed preferably using the elbow.

3.9. Any spillage of any material potentially containing any stage of the helminth must be

immediately decontaminated and cleaned with a disinfectant, with suitable contact times effective against the helminth stage. Note that in helminth breeding areas it will be undesirable to have bleach fumes in the genera area. Inside the area, spray bottles with a bleach solution will be available, but will only be used on specific surfaces in case of spillage. All sprayed surfaces will be carefully cleaned with disposable laboratory paper, which will be disposed of as described in section 4 below

4. HELMINTH WASTE MANAGEMENT AND NECROPSY REQUIREMENTS

4.1. The helminth breeding/research areas must have a closed drain system.

4.2. All potentially infectious liquid waste (excluding chemical waste) to be discarded must either be:

4.2.1. Poured onto adequate quantities of laboratory tissue paper or similar material, decontaminated with method or disinfectant capable of, as far as possible, inactivating infective helminth stages, placed in double impervious plastic bags and incinerated on site, or

4.2.2. Be disposed of directly in the closed drain system as described in Part IV, point 2.3;

4.3. All potentially infectious solid material must be decontaminated with a method or disinfectant capable of, as far as possible, inactivating infective helminth stages, sealed in double impervious plastic bags and placed into an insect proof waste bin located within the facility, until final disposal by incineration. All potentially infectious solid waste must be incinerated on site.

4.4. Faeces from donor or research animals must be destroyed by incineration. Faeces must be decontaminated with a method or disinfectant capable of, as far as possible, inactivating infective helminth stages, sealed in double impervious plastic bags, and transported to the incinerator on site either by hand or by vehicle.

4.5. Sharp objects must be placed inside medical waste containers (sharps bin). These containers can then:

4.5.1. Be decontaminated with a suitable disinfectant and incinerated on site or;

4.5.2. Be decontaminated and stored inside the buildings prior to removal by an accredited medical waste company.

4.6. The facility must have waste water treatment capacity suitable for chlorination or other disinfection of helminths and able to treat the required amount of waste water effectively (e.g. a Liliput system which includes addition of chlorine). Proof of the disinfectant's efficacy must be available. Alternatively other disinfecting systems (e.g. employing heat) with proven efficacy against the relevant helminth species will be considered. Full details, including efficacy results, must be submitted to DAFF, and a DAFF inspection team must audit and approve the system/methodology.

4.7. Only treated waste water may be pumped into an evaporation pond and the area surrounding the pond must be adequately fenced off from animals and uninformed persons.

4.8. Necropsies and activities related to necropsies, such as sieving of intestinal content, must be performed in dedicated necropsy areas that comply with all the requirements for helminth breeding/research areas as contained in this VPN.

Said areas must preferably be in the same unit as breeding/research areas. However, if not possible, necropsy facilities must still adhere to all requirements specified for helminth breeding /research areas.

4.9. Equipment used for helminth necropsies, such as sieves, must be thoroughly cleaned and decontaminated with method or disinfectant capable of, as far as possible, inactivating infective helminth stages and must be dedicated for use in helminth related necropsies only.

4.10. Permission for storage of helminths removed at necropsy must be requested in the

relevant study Section 20 applications. These helminths should be fixed in 10% formalin for counting purposes or in 70% ethanol in cases where molecular work is required. Said samples may be stored according to facility SOP. If granted, conditions for storage will be stated on the relevant Section 20 approval letter.

4.11. For *Echinococcus* spp. the following additional specific necropsy requirements will apply:

4.11.1. Specific PPE requirements were mentioned in Part III Section 1.10. In studies, final hosts (cats and dogs) should be necropsied prior to patency being reached to minimize zoonotic risk (i.e. ingestion of eggs).

4.11.2. For breeding/strain cycling, intermediate hosts need to be necropsied to remove the infective material (metacestodes). For *E. granulosus* metacestodes are used to orally infect a final host (dog). For *E. multilocularis* metacestodes are used to inject other laboratory strain mice interperitoneally where the metacestodes then multiply through asexual budding.

4.11.3. Intermediate hosts must be dissected within a tray when removing the organs and intestinal tract and when harvesting the infective material. Any cyst material removed should be placed in smaller suitable trays or other suitable containers to allow processing whilst avoiding spillage. All carcasses, tissues and wastes must be sprayed with a suitable disinfectant, double-bagged and incinerated. Apart from disinfection measures mentioned earlier (e.g. footbath and disinfection of work surfaces and equipment), a container of suitable depth containing a suitable disinfectant must be available during necropsy, to allow personnel to routinely submerge their hand in forearms (covered in the long-sleeve examination gloves as per Part III Section 1.10) as needed, and also before removing the gloves after completion of necropsy. Also refer to Part IV Section 3.8.

5. USE OF ANIMALS AS HOSTS FOR HELMINTH BREEDING AND RESEARCH

5.1. As helminths are host species specific, the preferred small animal host species should be used as donor animals, depending on helminth species involved. For the final hosts this will be cats or dogs (for the helminth species listed in this VPN, see Part II Section 2). Intermediate hosts may include sheep (e.g. *E. granulosus*) or laboratory strain mice (e.g. *E. multilocularis*).

5.2. Pregnant animals must not be used as donors.

5.3. Donor or research animals infected with helminths must be kept inside dedicated, clearly marked cages within the helminth breeding/research area that complies with the conditions in this VPN.

5.4. All personnel must be made aware of any potential zoonotic threat, as applicable to the helminth species in question, with specific attention paid to the procedures and PPE requirements stated in Part III, Section 1.

5.5. Cage construction and design must allow effective cleaning and decontamination and must prevent host escape or infection of non-infected hosts.

5.6. Cages where donor or research animals are housed must be designed, constructed, and maintained to facilitate effective cleaning and housekeeping. This includes appropriately coloured, smooth waterproof floors and walls to allow proper cleaning. These should not be covered or obscured and waste water used for washing must be channeled into a closed drain system as described in Part IV, point 2.3.

6. FATE OF ANIMALS FOR RESEARCH PURPOSES WITHIN HELMINTH RESEARCH AND/OR BREEDING BUILDING(S)

6.1. After use in the breeding or research with helminths, animals must preferably either be euthanized and incinerated, or must remain in the dedicated helminth breeding unit for the duration of their lifetime;

6.2. However, where euthanasia or such confinement is not ethically possible, the animal used

in helminth breeding or research must be handled as follows:

6.2.1. The animal must be treated with two commercially available anthelmintic products registered in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947 (No 36 of 47) for use against the parasite in question, at double the recommended dose, two to four days apart. The active ingredients in the two products must belong to two different anthelmintic groups.

6.2.2. A recognized diagnostic faecal flotation method, employing centrifugation to obtain the highest level of sensitivity possible, must be performed seven, ten and fourteen days after treatment with the first of the two anthelmintics.

6.2.3. Animals may only be returned to the animal colony when all three flotation examinations employing centrifugation indicate that the animal has a zero egg count (i.e. negative on all three occasions), and after they have been thoroughly bathed in the helminth breeding or research unit to remove any potential contaminated faeces or infective stages that may cling to fur.

6.2.4. These animals must never leave the research facility colony. They may be used for other Section 20 approved research projects as applicable, but must remain within the access controlled facility for the life time of the animal.

6.3. If an animal still excretes eggs after the process described above, alternative anthelmintic groups may be employed and the procedure repeated. If not possible or the positive status persists, the animal must be subjected to euthanasia and the carcass incinerated;

6.4. Note that for each research study a Section 20 application must be submitted, detailing the fate of research animals so that associated risk can be assessed on an individual study basis.

6.5. In addition to the above, the following specifically applies to *Echinococcus* spp.: No infected intermediate hosts may leave the parasite breeding facility. See Part IV Section 4.11 for necropsy considerations. All carcasses must be double-bagged and incinerated.

7. REMOVAL OF EQUIPMENT FROM THE HELMINTH RESEARCH AND/OR BREEDING BUILDING(S)

7.1. Dedicated equipment clearly marked as such must be located within the helminth breeding /research area and necropsy area. In addition, respective equipment sets specifically dedicated to the respective *Echinococcus* spp. species (*E. granulosus* and *E. multilocularis)* must be used.

7.2. The wash room must ideally be located within the helminth breeding and research area. Equipment must be decontaminated with a method or disinfectant capable of, as far as possible, inactivating infective helminth stages and placed in impervious plastic bags prior to removal to the washroom, if the washroom is not located inside the helminth breeding/research area

PART V

MONITORING RISK OF HELMINTH INTRODUCTIONS TO THE ENVIRONMENT

1. All logs associated with the helminth breeding process as well as facilities employed must be maintained and routinely monitored to ensure adherence with the VPN requirements and internal facility Standard Operating Procedures (SOP). Monitoring will be performed by designated personnel, such as Quality Control, Quality Assurance, Parasite Breeding Manager, or any other designated role as defined by facility SOPs.
2. This must include regular inspections of the helminth breeding and research facilities for signs of disrepair that could result in compromised biosecurity requirements such as restricted access or risk of animal escape.

3. Monitoring records must be kept and stored for at least 5 years for auditing purposes.
4. In addition to the above, the following specifically applies to *Echinococcus* spp.: Logs must be kept detailing the number of intermediate hosts purchased, infected, necropsied and incinerated, so that all intermediate hosts used can be accounted for.

PART VI

OUTSOURCING OF HELMINTHS

1. No helminths may be outsourced to other research facilities in South Africa, except if such collaboration has been approved by DAFF in writing following successful application for Section 20 approval to conduct said collaborative research.
2. Helminths may be outsourced to other international research facilities, provided that all documents and approvals required by the importing country have been obtained, but DAFF must be informed of such shipments.

PART VII

FACILITY COMPLIANCE MONITORING

1. DAFF reserves the right to inspect the facility to ensure compliance with the above requirements at any time.
2. In the event of non-compliance, DAFF reserves the right to have the helminths kept on the facility destroyed and to not issue further veterinary import permits or Section 20 permits for the importation of or research with helminths, until all non-compliance issues have been rectified to the satisfaction of DAFF.

PART VIII

COMPLIANCE WITH LEGISLATION

- The relevant legislation applicable to this VPN, includes the following Acts:
  - The Animal Disease Act, No 35 of 1984 ("Animal Diseases Act);
  - The National Road Traffic Act, No 93 of 1996;
  - Medicines and Related Substances Control Act, No 101 of 1965;
  - Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, No 36 of 1947.
- This VPN relates to the requirements of the Animal Diseases Act, No 35 of 1984 and save to state that the abovementioned legislation or any other relevant legislation shall be adhered to in as far as it is applicable to each individual situation, only the details relating to the Animal Diseases Act will be set out and dealt with further.
- Reference to the Animal Diseases Act includes reference to the Animal Diseases Regulations published under the Animal Diseases Act.
- The following Sections of the Animal Diseases Act is of particular importance in this VPN:
  - Section 1 – Definitions;

- Section 6 – Importation of certain controlled animals or things;
- Section 8 – Removal and further detention of imported animals or things;
- Section 20 – Limitations on investigations, experiments and research with, and manufacture and evaluation of certain products

- Packaging of helminths for transportation during importation must be compliant with IATA requirements as well as the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996).

1. Section 1 of the Animal Diseases Act

- The following definitions given in this section are relevant:
  - ***'contaminated thing'*** *means any thing other than an animal which is capable of introducing into, or spreading in, the Republic, any controlled animal disease or parasite, or by means of which any such disease or any parasite can so be introduced or spread, including any prescribed thing contemplated in subsection (7) (a) of this section;*
  - ***'controlled animal disease'*** *means any animal disease in respect of which any general or particular control measure has been prescribed, and any animal disease which is not indigenous or native to the Republic;*
  - ***'controlled animal or thing'*** *means any animal, infectious thing, contaminated thing, animal product or parasite, and any progeny or product in respect thereof;*
  - ***'controlled purpose'*** *means the prevention of the bringing into the Republic, or the prevention or combating of or control over an outbreak or the spreading, or the eradication, of any animal disease or, where applicable, of any parasite;*

2. Section 6 of the Animal Diseases Act

- In terms of Section 6 of the Animal Diseases Act, 1984 (Act No 35 of 1984), the importation of helminths is subject to obtaining a Veterinary Import Permit prior to the importation thereof.
- Veterinary Import Permits will only be issued to research facilities that provide written proof from DAFF that their facility was inspected and they are compliant with the requirements stipulated within this VPN and may therefore work with helminths.
- Helminths must move from the port of entry to the research facility under cover of a Red Cross permit in addition to the import permit.
- For the sake of convenience Section 6 is quoted below:

6 (1)

b) A permit referred to in paragraph (a) –

(i) Shall be obtained by an importer before the relevant animal or thing is removed from or out of any place outside the Republic by means of any conveyance or by any other means for the purpose of importing it into or conveying it in transit through the Republic;

(ii) shall, in respect of any animal or animal product referred to in section 16 (1) of the Livestock Improvement Act, 1977 (Act No. 25 of 1977), only be issued if the written authority contemplated in that section has been granted in respect thereof; and

(iii) shall, where the director requires that the animal or thing be detained in a quarantine station, only be issued on proof being adduced to him that a confirmation of accommodation has been furnished and fees have been paid, as contemplated in paragraphs (a) and (b), respectively, of section 5 (4) of this Act.

c) *When any person imports into or conveys in transit through the Republic animals or things of the same class on a regular basis from the same country, the director may, if he is satisfied that it will not defeat a controlled purpose, issue to such a person a permit referred to in paragraph (a) to so import or convey during the period specified therein consecutive consignments of animals or things of the same class.*

(2)

Any animal or thing in respect of which a permit has been issued –

a) *shall only be imported into the Republic through or at a place of entry referred to in paragraph (a) of the definition of "place of entry" in section 1 (1), or, in the case of any animal, through or at any other place which the director has, subject to the provisions of the Customs and Excise Act, 1964 (Act No. 91 of 1964), determined for purposes of this paragraph;*

b) *shall be imported within the period specified in the permit;*

c) *shall be detained in the prescribed manner at the relevant place of entry, and shall be made available to the director for purposes of the performance of controlled veterinary acts; and*

d) *shall not without the written authority of the director, or contrary to any condition of such authority, referred to in section 8 (1) (a), be removed from such place.*

(3)

a) *The director may, if he knows or on reasonable grounds suspects, that any animal or thing is, contrary to any provision of this Act, or any condition of a permit –*

   (i) being removed, or has been removed, from any place outside the Republic for purposes of importing it into the Republic; or

   (ii) about to be imported by any person into the Republic; or

   (iii) present on or in any conveyance, or forms part of any consignment, which is being or has been brought or sent by any person to the Republic, direct that the animal, thing, consignment or portion thereof determined by him, shall not be imported into the Republic or unloaded or removed from the conveyance, as the case may be, except with his consent and, if he has determined conditions in connection therewith, in accordance with such conditions.

b) *The director may, if he deems it necessary, make such direction known by notice in the Gazette, and shall, irrespective of whether it has so been made known or not, make known the provisions of the direction as soon as may be practicable to all persons who, to his knowledge, are or will be involved in the importation, unloading or removal, as the case may be, or to any person in whose service any such persons are, or who exercises control over them, or in respect of such unloading or removal.*

c) *The provisions of subsection (2) (c) and (d) shall mutatis mutandis apply in respect of any animal or thing referred to in subsection (3) (a) which has been imported, unloaded or removed with the consent of the director as contemplated in the last mentioned subsection: Provided that in such application of the said sub-section (2) (d) a removal contemplated therein shall not be effected unless the importer concerned has paid the fees which are in terms of this Act payable in respect of the relevant required permit.*

3. Section 20 of the Animal Diseases Act

- In terms of Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984), approval to do research with Helminths must be obtained prior to conducting such research.

- Section 20 is quoted below for the sake of convenience:

20. Limitations on investigations, experiments and research with, and manufacture and evaluation of, certain products. –"No person shall, except under a permit and in compliance with the conditions which are prescribed or, in any particular case, determined by the director -

a) conduct any investigation, experiment or research with any vaccine, serum, toxin, anti-toxin, antigen or other biological product which consists or originates wholly or partially of, or from, any micro-organism, or of or from the glands, organs, fluids, or any other part, of an animal or parasite:

Provided that the foregoing provisions of this paragraph shall not apply to any substance in so far as it is controlled under the Medicines and Related Substances Control Act, 1965 (Act No. 101 of 1965);

b) for the manufacture or evaluation of a product or remedy used for or intended to be used at or for the testing, diagnosis, prevention, treatment or cure of any animal disease or parasite, or for the maintenance or improvement of the health, growth, production or working capacity of an animal, use any vaccine, serum, toxin, anti-toxin, antigen or other biological product referred to in paragraph (a); or

c) for the purposes of any investigation, experiment or research referred to in paragraph (a), or for the manufacture or evaluation of a product or remedy referred to in paragraph (b) –

i) infect or contaminate any animal or any other thing with any animal disease or parasite; or

ii) introduce into or collect in the Republic, or have in his possession, or remove or transport from the place where it is normally found or kept, any controlled animal or thing, or any protozoon, bacterium, virus, fungus, parasite, other organism or agent which is capable of spreading any animal disease or parasite."

ANNEX A

Application for permission under Section 20 of the Animal Diseases Act 1984 (Act no 35 of 84) to perform research/study

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

APPLICATION FOR PERMISSION UNDER SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) TO PERFORM RESEARCH / STUDY

IMPORTANT NOTICE

a) *Please complete this form fully, preferably typed in text and email to Mr Gololo at HerryG@daff.gov.za or fax to 012 319 7470 for Attention: Mr Herry Gololo.*
b) *Application must be submitted at least 3 months prior to the proposed starting date of the research.*
c) *Records relating to the information supplied in this section must be kept for auditing purposes for five years.*

I hereby apply for permission from the National Director of Animal Health, South Africa, to do research under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984):	
Date:	
Study/protocol/ethical approval reference number	
DAFF reference number (to be completed by DAFF)	
1. Researcher	
Full names and title of the researcher:	
Work address of the researcher:	
Contact details of relevant person for correspondence regarding application :	
Name:	
Tel:	
Fax:	
E-mail:	
2. Project	
Title of research project:	
Aim of research project:	
Proposed starting date:	
Proposed date of completion:	
3. Institutions (Details of all research institutions or laboratories where research will be done. Kindly amend table if more space is needed)	
Name:	
Physical address:	

Compiled by:
The Section 20 Team

Date:

Postal address:	
Laboratory/ sub-section:	
4. General	
4.1. Pathogen/disease/vector to which study relates:	
4.2. Micro-organism, parasite or animal material (including vaccine, serum, test kit, toxin, anti-toxin, antigen, biological product which consists or originates from a microorganism animal or parasite) to be used in study:	
4.3. Does the study involve the importation of the material mentioned in 4.2 above and/or unregistered pharmaceutical products? Please list these products and the exporting country	
4.4. Biological origin of the micro-organism, parasite and/or animal material:	
4.5. As part of the study, will field samples be collected from any animal or obtained from a biobank or laboratory? Please provide the details thereof and list all samples and species of origin	
4.6. Please attach a letter from the relevant state veterinarian of the research/ sampling area stating whether it is under any disease restrictions and/or a letter of permission from the biobank or laboratory concerned. (complete only if "Yes" to 4.5)	SV letter: Attached: Yes ☐ No ☐ Not applicable ☐ Biobank/laboratory letter Attached: Yes ☐ No ☐ Not applicable ☐
4.7. If samples are to be collected at an abattoir, please supply the registration and name of the abattoir, written permission from the abattoir owner and written permission from the relevant state veterinarian in the province.	Permission for abattoir owner: Attached: Yes ☐ No ☐ Not applicable ☐ Permission from relevant state veterinarian Attached: Yes ☐ No ☐ Not applicable ☐
4.8 Will samples be packaged and transported in accordance with International Air Transport Association (IATA) requirements and/or the National Road Traffic Act, 1996 (Act No. 93 of 1996);	Yes ☐ No ☐ Not applicable ☐ If no, please describe alternative method/SOP:
4.9. Does the study involve genetically modified organisms/material?	Yes ☐ No ☐
5. Facilities	
5.1. Indicate the Biosafety of all facilities involved in the handling of samples/animals for this study:	
5.2. Are these facilities DAFF approved /compliant? If yes, supply certificate number of BSL 3 or evaluation by DAFF for another BSL level, if available. If not DAFF approved for a specific BSL, provide a short description of BSL related precautions in place:	

Compiled by:
The Section 20 Team

Date:

5.3. Describe the containment of the pathogen/material at facilities in detail (includes handling of food, bedding, waste, access control, vector proof etc.) or provide/refer to relevant SOP:	
6. Live Animals	
6.1. Will live animals be used in study? If yes, list which species and approximate number:	
6.2. If live animals will be used specify origin of animals:	
6.3. Please attach a letter from the relevant state veterinarian of the sourcing area stating whether it is under any disease restrictions.	SV letter: Attached: Yes ☐ No ☐ Not applicable ☐
6.4. Describe containment of live animals in facility in detail:	
6.5. Fate of live animals after completion of the study: *(Refer to guidelines if to enter human food chain)*	
7. Disposal of materials and/or animals	
7.1. Describe the disposal of all biological/contaminated/potentially infectious waste at end of study:	
7.2. Method of disposal/ destruction used:	
7.3. If this function is outsourced provide name and registration certificate of accredited waste contractor used:	
7.4. If incinerated on the premises, supply calibration certificate and discuss disposal process from study site to incinerator:	
8. Storage and/or distribution	
8.1. Will any vaccine, serum, toxin, anti-toxin, antigen, biological product which consists or originates from any microorganism, animal or parasite be stored beyond the duration of the study? **If yes, specify in detail: which samples, how they will be stored and where they will be stored.**	
8.2. Will any vaccine, serum, toxin, anti-toxin, antigen, biological product which consists or originates from any microorganism, animal or parasite be distributed? If yes, specify where and for what purpose.	
9. Kindly provide a concept note, abstract or brief overview of the study in the space below:	
If insufficient space, please provide additional information as attachment/annex to the application form (maximum 2 pages) This information must be signed off as true and complete and representing complete disclosure.	
10. Details of person responsible for research	

Compiled by:
The Section 20 Team

Date:

Name:	
ID/Passport number:	
Physical address:	
Postal address:	

I hereby confirm that the summary and the information of the research/study as provided with this application, is true and correct and represent a complete disclosure. I further confirm that, where applicable, the following conditions will be adhered to:

1. No part of the study will commence until valid ethical approval has been obtained from the relevant South African authority as applicable;
2. Approval under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1947) and/or the Medicines and Related Substances Control Act, 1965 (Act No 101 of 1965) will be obtained prior to the commencement of the study if applicable;
3. Any suspicion of a controlled/notifiable disease in terms of the Animal Diseases Act, 1984 (Act No 35 of 84), will be reported immediately to the responsible State Veterinarian;
4. If a test for a controlled/notifiable disease was not performed in a DAFF approved laboratory for the specific test, the results will not distributed as a possible diagnostic test result to anyone other than the responsible State Veterinarian;
5. Consent from the owners of animals to be used in the study will be obtained in writing prior to the commencement of the study, if applicable;
6. Should there be any deviations to the descriptions, specifications or conditions described in this Section 20 application and/or Section 20 permit approved by the Director: Animal Health for the research/study; the Director: Animal Health will be informed immediately.

Researcher Name	Researcher signature	Date

11 Details of person(s) responsible for the institution(s):

Name:	
ID/Passport number:	
Physical address:	
Postal address:	
Designation:	

I am aware of the research referred to on this application form and take responsibility for this project to be done according to the research/study summary provided, at the above mentioned institution. Should there be any descriptions, specifications or conditions described in this Section 20 application and/or Section 20 permit approved by the Director: Animal Health for the research/study, the Director: Animal Health will be informed immediately;

Signature: _____ Date: _____

ANNEX B

Guidelines for Section 20 applicants