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5. TECHNIQUES IN LOCAL PROGRAMME EXECUTION

5.1 Factors to be considered in selecting control methods

This section reviews the factors that must be considered before the decision is made to embark on a programme for rabies control in dogs.

5.1.1 Occurrence of human and canine rabies

The epidemiological picture and incidence of rabies both in the human and animal population in the area in question must first be established. The disease may be present in humans, dogs, cats, other domestic animals and wildlife. The techniques of human and animal rabies surveillance are described in Section 5.4.

5.1.2 Characteristics of the proposed control area

The size of the area and the population to be covered in a planned programme will be dictated by the epidemiological situation. It may contain cities, towns, villages, commercial and rural areas and should be large enough with, if possible, secure and identifiable borders, to enable control, once established, to be maintained.

5.1.3 Size, dynamics and mobility of the dog population

The next decision must be to carry out a dog census to ascertain the size and distribution of the dog population in the proposed control area, as the basis on which the vaccination and control campaigns can be planned.

Techniques of dog censuses are fully described in Sections 2.2 and 2.3. Dog habitat can be divided into urban and rural areas, these being sub-divided into residential and non-residential areas.

Experience has shown that residential areas can probably be divided into three types based on the human socio-economy - upper class, middle class and lower class. In each of these types of area, the pattern of dog ownership and the response to control procedures will vary. The dog census must consider these sub-divisions so that due account can be taken of them in planning control operations. Compare also Section 2.4.

5.1.4 Community attitudes

Success in rabies control is dependent on community participation and acceptance. Factors which will affect this will be:

Socio-economic class

Reaction to the control operation and the ease with which measures can be carried out will vary with the socio-economic class of the residents in different parts of the area. Due account must be taken of these factors when planning the operation (see Section 5.2).

Cultural background

Communities may adopt differing standpoints on control measures. In a number of countries or areas people may consider dogs as unclean and will have little or no contact with, or responsibility for, the dogs present in their communities. In other countries and areas, particularly in south Asia people
may object to the destruction of dogs and other animals. Communities in many other countries hold no strong views towards dogs and cooperation with dog vaccination and destruction is not impeded by religious and other factors. (see also Section 2.10).

5.1.5 Infrastructure of professional and other services

Due account must be taken ot the veterinary, medical, health and other social services in the area in the planning stage. The implication for, and involvement of, these services are described in Section 3 and Section 5.2.

5.1.6 Resources available for a dog vaccination programme

Account must be taken of the resources in the area, both human and material, and financial, before a decision is made to mount a dog vaccination programme. These factors, together with the supply of vaccine and various techniques of dog vaccination, are described in Sections 5.2.1 and 5.2.2.

5.1.7 Resources available for a dog control programme

The factors which have to be considered in deciding to initiate and maintain a dog control programme, and the differing techniques available, are described in Section 5.2.3. The choice of techniques will depend on the results of the dog census, the success of the dog vaccination campaign, the type of area as defined in Section 5.1.2 above, the size of the stray dog population and the protection and subsistence afforded to them by the environment. A decision will have to be made on the resources available for a clean-up of stray dog-supporting foci in the environment.

5.1.8 Availability and training of personnel

The trained personnel in post, or the potential and facilities for recruitment and training of personnel for a dog control campaign must be taken into account as factors in programme planning. These factors are discussed in Section 5.2.3.

5.1.9 Public relations and information

Effective community education is essential, both to inform the population of the hazards of rabies, how it is spread and controlled, etc., and also to give them precise information on forthcoming dog vaccination and control programmes. Good information will ensure good cooperation.

The availability and intended use of all information media must be assessed before the campaign begins, see Section 5.2.8.

5.1.10 Government support and finance

An essential factor is the full legislative, administrative and financial support of the government, at national level from the appropriate departmental minister downwards, through regional and local government levels. See Section 3.5.6.

No programme for the control of rabies in dogs can succeed without full government support at all levels.
5.3

5.2 Resource mobilization and application of field methods

Each country must plan, organize and implement its rabies control programme with consideration of the epidemiology of the disease, the magnitude of the problem, the structure of its urban and rural society and the resources available. The main factors to be considered are listed in Section 5.1 and possible methods of control are described in detail in this section. Each country must adapt these standard methods to its own national or regional situation - the programme adopted by one country may not be suitable for any other area.

Reliable information on the dog population and on the disease situation are important for the planning of dog vaccination and control programmes. Dog censuses are dealt with in Section 2 and rabies surveillance methods in Section 5.4.

5.2.1 Vaccination of dogs

Vaccination campaigns are directed toward breaking dog to dog endemic transmission cycles. In countries in which dogs are the sole maintaining reservoir hosts, the elimination of transmission in the dog has been shown repeatedly to eliminate the disease from cats and domesticated animals. Even in countries with endemic wildlife rabies, the dog and the wildlife transmission cycles are separate and disruption of endemic dog rabies transmission reduces the incidence of the disease in dogs to occasional sporadic cases from wildlife exposures. The necessary powers for dog vaccination can be obtained by law — see model legislation in Section 4.2.

Vaccination goals:

The minimum vaccination level for all areas of communities is 70% of all dogs at risk of entering rabies transmission cycles. In areas of unregulated breeding, where approximately 15% of dogs may be less than 3 months old and thus would not be vaccinated, one third of these puppies would be sufficiently mobile that they must be considered at risk of exposure to rabies. Thus about 5% of the dog population at risk would in any case be excluded from vaccination.

Methods of dog vaccination campaigns:

(a) Continual dog vaccination at private or government veterinary clinics to which dog owners take their dogs. It is the responsibility of dog owners to obtain first vaccination at 3-4 months old, a second dose one year later and booster doses every 2 to 3 years thereafter with live or inactivated and adjuvanted virus vaccines. The period between vaccinations will depend on the duration of immunity conferred by the vaccine in use. Owners may take their dogs to clinics of their choice, where vaccination may be provided free or at a fee set by the government or the veterinarian in charge. Standard certificates and identification of vaccinated dogs should be used nation-wide.

The advantages of this method are that little government effort or expense is needed where veterinary clinics already exist. It works well in conjunction with mandatory licensing and taxing of dogs with the requirement that valid certificates of vaccination be furnished by the owners. This method is most applicable where there are few unowned dogs and the population will voluntarily follow the regulations. When fully implemented, it continually maintains a high immune level in the dogs, ensuring against focal outbreaks of rabies.
The disadvantages of the method are that it does not reach unowned dogs and because of the owner efforts and expenses involved, it encourages people to declare their dogs unowned. However, the government requirement for dog vaccination may be much easier to proclaim than to enforce and although money may be saved in vaccination programmes, continual enforcement may be costly and difficult to achieve. Regulations are useful in this case which would lead, for instance, to saving the lives of rabies-exposed animals (if proved to be properly vaccinated and owned) after one booster injection. A high level of compliance by upper and middle class owners of pet dogs may mask a low level of coverage in the lower class neighbourhoods and non-residential urban areas where rabies is being maintained endemically.

(b) Dog vaccination campaigns through neighbourhood vaccination centres. Community-wide campaigns are usually conducted every 2 years or when focal outbreaks appear between regularly scheduled campaigns. Neighbourhood vaccination centres are usually set up for 1/2 to 1 day, to serve about 100 households up to 250 dogs within easy walking distance. The centres are set up in large open buildings or under natural or temporary shade, preferably during a dry season. If campaigns take place during school vacations (dogs can be brought by children) the percentage of vaccinations is usually higher. If cats are to be included, separate dog and cat vaccination lines must be organized and widely separated. Multiple dog vaccination lines should also be widely separated. Sufficient vaccine in cold storage and sufficient supplies must be obtained and at hand. Vaccination centres are usually under the supervision of veterinarians, often rabies control officers. The personnel are commonly government employees, trained for their work, pre-exposure immunized against rabies, and temporarily assigned to the rabies control programme. Local resident persons usually make the most effective vaccinators. Three person vaccination teams usually operate most efficiently. One person reconstitutes vaccine, fills syringes and injects the dogs. A second person instructs residents bringing their dogs and assists in restraining dogs during vaccination. The third person completes the certificates of vaccination and identifies the vaccinated dogs. A vaccination team can vaccinate and register 12 to 25 dogs per hour, depending on the level of mobilization of the neighbourhood. Usually dogs are vaccinated free or at nominal cost in vaccination centres. With a modified certificate (see Section 4.2) or with already identified dogs, time can be saved and up to 100 dogs per hour can be vaccinated.

The advantages of this method are that neighbourhood centres are more conveniently located for most dog owners than are veterinary clinics. They are relatively easy to publicize and frequently civic organizations and local mass media may effectively participate in community education and announcing schedules. Neighbourhood centres are relatively economical to operate and if neighbourhoods are well mobilized, can be very efficient in vaccination time. Maintenance of vaccine in cold storage is relatively easy and supplies may be rotated quickly for re-use. Vaccination centres may be the only feasible approach where household compounds are not open to vaccinators to enter and vaccinate dogs during times when the masters of the households are away.

The disadvantages are that vaccination clinics rely on owner initiative to bring their dogs. Unowned dogs, and owned dogs which may be difficult to manage off the owners' premises will not be brought. This method encourages people to disclaim ownership of dogs which may live on and even receive food on their premises. Dog owners may be hesitant to mingle their dogs at vaccination centres, recognizing hazards of animal fights, picking up of fleas or ticks, and transmission of infectious diseases. In mixed neighbourhoods, high compliance by some portions of the neighbourhood may mask low compliance of the most critical portions.
(c) One-day campaigns covering whole municipalities or states

In Brazil, programmes based on house-to-house visits have been proved to be very effective. However, the main difficulties encountered in implementing vaccination over a relatively long period of time (30-60 days) included: secondment of personnel from other institutions (e.g., military) to act as vaccinators, keeping up the interest in the campaign (community, personnel, mass media), obtaining motor vehicles, and decreasing other rabies control activities (e.g., surveillance, dog control), during the vaccination period.

Where experience has been gained in vaccination campaigns and trained personnel and infrastructure are adequate, a one-day campaign can be organized. Preparation requires a group of planners, since the organization of vaccine supply, vehicles, material, personnel, training, public information, etc., must begin months before the vaccination day. For example, the campaign in one state of Brazil was carried out through 10,000 fixed vaccination posts with the direct participation of 11,000 people, including vaccinators, supervisors and coordinators. Vaccine was administered by glass syringes and needles from 8 a.m. to 6 p.m. In the one-day campaign 1,053,914 dogs were vaccinated. This total represents 99 per cent of the figure for the previous year, when the house-to-house traditional vaccination method was used.

(d) House to house dog vaccination campaigns with complete coverage of residential areas and selected dog removal. Communities are usually systematically covered every 2 years or when local outbreaks appear between regularly scheduled campaigns. The campaigns are based on vaccination of all dogs, both owned and unowned, in residential neighbourhoods, and on vaccination or removal of dogs in non-residential areas. Usually dogs are vaccinated during campaigns without charge to residents. The operations are usually supervised by veterinarians but the vaccinators are usually local government employees assigned for this duty on a temporary basis. The vaccinators follow set house to house schedules. They must both convince the local residents of the importance of dog vaccination as well as vaccinate the dogs, so it is important that they be well acquainted with and preferably from the areas where they are working. They must be trained for both their educational and vaccination functions and must be provided with pre-exposure immunization. Usually 3 persons comprise each team. One person helps owners catch and restrain dogs as needed, or catches dogs which are recognized but not claimed by anyone in the community. A second person prepares and injects the vaccine. The third person completes the certificates of vaccination and identifies vaccinated dogs. They may vaccinate cats at owners' residences if this is desired in the programme. Vaccination teams are scheduled on a basis of 10-15 minutes per household or 8-12 dogs per hour per team. A sufficient number of teams should be assigned to completely cover a political jurisdiction like a city and adjacent densely populated area, or a province, within 2 months and a neighbourhood of not over 100 homes within 2 days. Vaccinators must be transported or take locally available transportation to work sites each day or arrangements may be made for them to obtain room and board within the communities in which they are working. Best hours for catching and vaccinating dogs are usually between 9.00 a.m. and 4.00 p.m. when dogs are least active. Residents are encouraged to vaccinate puppies which reach 3 months of age, and any dogs acquired between campaigns, at local veterinary clinics.
The advantages of this method are that a sufficient percentage - usually at least 90% - of all dogs in communities are vaccinated. Residents are encouraged to claim dogs as owned or at least recognized. Vaccinators become highly proficient in capturing and vaccinating dogs. Community acceptance is maximized by not killing dogs except on special request.

The disadvantages of the method are that training and pre-exposure immunization of the vaccinators are costly. House to house vaccination does not work well in large urban centres where neighbours do not know each other, and does not work well if owners are charged fees for dogs vaccinated.

(e) House to house dog vaccination campaigns with entire community coverage and no dog removal. This modification involves complete systematic coverage of entire communities by vaccinators going house to house in residential neighbourhoods. In addition the vaccinating teams capture, vaccinate and release all dogs at non-residential plazas, markets, abattoirs, fishing beaches, food processing centres, garbage piles and other areas providing food and harborage to dogs. Usually at least 90% of the dogs in all areas of control communities are vaccinated. Surveillance must be continuous, and prompt action taken upon any re-entry of rabies. Campaigns are usually repeated every 2 years or when rabies reappears.

The advantages of this method are that there is minimal disruption of normal community functions and community acceptance will be high. Only one agency, the government service vaccinating dogs is involved, compared with programmes in which animal patrols, police or other agencies must follow the vaccinating teams. Coordination of multiple agencies may be more complex than operation of a single agency. Continuous control actions, which are always difficult to fund and maintain, are not applied between campaigns.

The disadvantages are that this method is least effective where natural barriers to re-entry of rabies into control areas exist. Surveillance must be continuous and campaigns promptly reinstated upon re-entry of rabies. Moreover, unowned (stray) dogs, even if non-rabid, remain a threat for other health reasons, e.g. echinococcosis.

Age schedules for dog vaccination

In rabies control programmes based on owner vaccination of dogs, puppies should be vaccinated between 3 and 4 months of age, with a second dose 1 year later and booster doses every 2-3 years. Dogs first vaccinated as adults should be revaccinated every 2-3 years. In programmes based on community-wide campaigns every 2 years, all dogs 3 months of age and older are vaccinated in each campaign. Local campaigns should be held promptly in any communities in which cases of rabies appear between regularly scheduled campaigns. In such emergency campaigns, all dogs above 3 months of age may be vaccinated for greatest assurance of success, or dogs vaccinated as adults within the past year may be exempt from revaccination.

Continuing vaccination

Community residents should obtain new dogs only from within their or other rabies control communities. If they obtain puppies from non-control communities, they should keep them strictly confined until 1 month following vaccination. Adult dogs should either be vaccinated at least 1 month before being brought from non-control communities or should be strictly confined for 1 month following vaccination. Vaccination services must be provided in each community for residents bringing in unvaccinated dogs and people must be required to follow these rules.
5.7

Repeat vaccination campaigns

Dog vaccination campaigns are usually conducted every two years. In the event of appearance of rabies between regularly scheduled campaigns, an area within a 25km radius of the appearance should be quarantined and vaccination campaigns be promptly reinstated within the area.

5.2.2 Certification, identification and recording of dogs

In national and community operated rabies control programmes, the certifying and recording process should be as simple and uniform as possible.

Dog registration

Some countries may require registration of dogs along with other animals. If there is an associated fee, this may encourage people not to acknowledge ownership of dogs, and if registration is associated with rabies vaccination, not to cooperate actively with vaccination programmes. Serious consideration should be given to exclusion of dogs from registration, and if it is required, it should be completely separated from the rabies vaccination programme.

Dog licensing with assessing of a license fee is required by some countries. In other countries the certificates of vaccination serve as the record of dog ownership and copies may be filed in the appropriate government office. If separate licensing and fees are required, this must be completely separated from the rabies control programme and the records of dog vaccination must not be available for licence purposes. Presentation of certification of current vaccination may be required of dog owners for licensing, however.

The vaccination certificate

This is the essential record of vaccination. It should be in the local language, provide uniform information throughout the nation and be in plentiful supply, possibly by the company producing the vaccine. A model certificate is included in Section 4.2. The certificate should contain the following information:

(a) the owner's name and address
(b) the animal species, if cats or other animals may be officially vaccinated in addition to dogs.
(c) a description of the vaccinated animal, including predominant breed, colour, approximate size, sex and approximate age
(d) the manufacturer, serial number and type of vaccine used
(e) the dates of vaccination (and of required revaccination)
(f) the rabies tag, tattoo or other identification number if used
(g) the signature and stamp of the vaccinator.

Note:- Items (d) and (f) may be omitted in local mass campaigns of dog vaccination (see section 4.2), but are essential components of the International Certificate of Vaccination Against Rabies (Section 6.3).
The original copy of the certificate shall be given to the owner. One copy may be retained by the vaccinating agency if the vaccination was performed other than under the rabies control officer. One copy must be filed by the rabies control officer and be easy to locate for any medical inquiry as to the status of a dog which bites a person or another animal.

**Dog identification**

A visible form of identification of vaccinated dogs is essential particularly in campaigns during which dog removal phases are incorporated and it is highly desirable that in these cases it lasts through the period between campaigns. Paint or other indelible liquid marks are short lasting and can only be used if the removal phase follows immediately after vaccination; tattoos, though permanent, are of low visibility, time consuming, dangerous and difficult to apply. Leather collars with attached tags are prohibitively costly for most campaigns and can be lost or exchanged, but consideration should always be given to affixing metal engraved tags to the dog's own collar as proof of vaccination.

Collars made of plastic strips or plastic tubing are most practical for general use in campaigns. They can be colour coded for years of vaccination or by communities and have good durability. Plastic stripping approximately 1 x 10 mm in size may be purchased in rolls and cut to fit individual dogs. It is applied by placing 2 rivets through the overlapped cut ends. The plastic is economical but the riveting tools are expensive and the supply of rivets may be inconstant.

Plastic tubing approximately 1 mm in thickness and 4 mm in diameter may be threaded with wire and used in a similar manner. It is secured around the dog's neck by tightly twisting the ends of the wire together. Pliers must be supplied to vaccinators for cutting the wire and twisting the ends; these are readily available in all countries. This identification, because it can be lost or cut off as puppies grow, does not constitute a definite proof of identity (as a tattoo does) but may be useful for associated vaccination and dog removal campaigns.

5.2.3 **Control of dog movements**

Control of dog movement, if fully enforced and accompanied by continual removal of dogs not in compliance, can be very effective in preventing rabies transmission. It is applicable principally to upper and middle class urban communities in most countries. The necessary powers can be obtained by enacting legislation declaring the area an infected area - see model legislation in Section 4.2.

(a) **Absolute control of movement**

Dogs are confined within walled or fully fenced compounds, tethered on the premises, kept in closed kennels with attached runs or restricted to inside the house. Whenever off the owners' premises, they must be on leashes not over 2 meters in length, and may be required to be muzzled. Such controls work only if there is strict and continuous enforcement and if dogs not in compliance are unable to reach those which are properly controlled. Absolute movement control is much easier to legislate for than to implement and much easier to enforce during emergency periods than continuously.
5.9

(b) Partial control of movement

During daylight when members of households are active outside their homes, dogs which accompany them may be restricted to voice command. The most important time for confinement of dogs is between 5.00 p.m. and 8.00 a.m. when most active socializing commonly takes place.

(c) Control of movement during specific times

During vaccination campaigns, confinement of dogs on owners' premises may be required to assist vaccinators in their work. During campaigns to remove unvaccinated dogs from a community, confinement of owned vaccinated dogs speeds the work of the animal patrols and reduces the need for owner redemption of vaccinated but inadequately marked dogs. During rabies outbreaks and emergency control campaigns, confinement of all dogs is very important, but is easier if owners are aware that unrestrained dogs could be killed. During such times, dogs off the owners' premises should be leashed and muzzled. Confinement of female dogs during heat periods greatly reduces community dog movement and helps to control dog population levels.

5.2.4 Dog removal

(a) Personnel

Animal wardens should be part of the local civil service, working full time in animal control. They should be trained and supervised by the rabies control officer. Their training should include humane capturing and handling of dogs. They should understand and be able to explain to community residents the rationale and importance of their work. They should have specific instruction in the handling of rabies suspect dogs and should be pre-exposure immunized against rabies. During dog vaccination campaigns, they may be assigned to temporary service as vaccinators; if so, they should be specially trained for this task. Animal wardens should be supplied with trousers made of sturdy cloth, with heavy leather gloves covering hands and wrists and with dog catching and restraining nooses. See Annex 5.1. Animal wardens usually work in teams of 2 persons plus a driver or with one of the wardens also driving. A team can capture up to 3-4 dogs per hour depending upon the number of dogs which are present.

(b) Vehicles

Animal patrol vehicles are usually jeeps or pickup trucks equipped with cages. Cages should be of welded angle iron and heavy chain link fence of 4-5 cm mesh construction with strong hinged doors and latches, so constructed that no sharp edges protrude which might injure dogs. Cage systems with 4-6 compartments opening to the sides and back of the vehicles are preferable to one single cage when handling dogs which may fight. One compartment may be a cage within a cage for handling rabies suspect dogs. There should be at least 5 cm space between the two sets of walls. Cage floors should be solid sheet steel for easy cleaning with a hose. Cage doors should be spring closing and open inward on larger cages for ease in putting dogs inside. All doors should have locks; these are essential for rabies suspect cages. Minimum cage sizes should be 0.4 m² floor space x 46 cm height for small dogs and cats, and 0.5 m² floor space x 75 cm height for larger dogs. Designs for cages for large pickup trucks, small pickup trucks and jeeps are shown as examples in Annex 5.2.
(c) **Dog pounds**

Animal pounds are costly to build and operate. They are practical in large urban centres in which unconfined and unowned dogs may wander through neighbourhoods and dog removal is needed as an adjunct to owner vaccination and control of dogs. The community must be committed to rapid, effective and continual removal of dogs not in compliance and to the placement or humane destruction of such dogs. The pound compound must be fully walled and isolated from escape of dogs or entrance of people. A practical design for an animal pound for handling up to 7500 dogs per year is shown as an example in Annex 5.3.

(d) **Euthanasia in pound dogs**

The carbon monoxide chamber included in the pound design in Annex 5.3 is a concrete structure 1.8 x 2.4 m x 1.2 m high with a door for dogs to enter from the corridor behind the communal cages, a window for observation of euthanization and a large door for allowing the carbon monoxide to dissipate and for removal of carcasses. It must be sealed to prevent escape of gas during operation. Carbon monoxide is provided through a flexible hose connecting the exhaust of a truck to a scrubber. A scrubber is a vertical tank 2.7 m high x .65 m diameter nearly filled with water. A tall inverted "U" pipe carries exhaust into the tank at the base without back siphonage of water. As the exhaust bubbles through the water it is cooled; water soluble components are removed and oily fractions form a layer on top of the water. The outlet carries carbon monoxide from the top of the tank down to the inlet near the floor of the chamber. If desired a box may be fitted into this line into which cloth gauze is placed to filter out remaining tars and carbon particles. In operation, the truck engine should have the carburetor set for a rich gas:air mixture and be operated at idle speed. The atmosphere in the chamber should quickly reach a carbon monoxide level of 8%. Dogs die most rapidly at carbon monoxide concentrations of 6% or higher. Water in the scrubber should be drained and replaced monthly.

Alternative methods for euthanizing dogs may require other specialized facilities and training of personnel. Special considerations must be made in the use of other inhalant poisons. Ether soaked cotton or gauze pads may be used for small dogs or cats in a small chamber. It is relatively inexpensive but is both flammable and explosive. Chloroform may be used in a similar manner. It is cheaper and nonflammable although in the presence of flame, phosgene gas is formed. Halothane, methoxyfluorane, and enflurane are nonflammable liquids which may be used in the same way but are much more expensive and not so readily available. Nitrogen gas in pressure cylinders is quite widely available as a by-product of the production of medical oxygen. It may be used in small chambers through which it is flushed to reach a final concentration of at least 98.5% for at least 5 minutes. Nitrous oxide gas is quite readily available in pressure tanks but is relatively expensive for animal euthanasia. Hydrogen cyanide gas is generated by placing pellets of sodium cyanide into sulphuric acid. It is economical and is rapid in action. Following use, dissipation of gas from the chamber is such a personnel hazard that it should probably be used only where pounds may be located in uninhabited open areas. Carbon dioxide gas generated by sublimation of dry ice out of contact with the animals or from a pressure cylinder is effective above 30% concentration. Because carbon dioxide is heavier than air, the charging of the chamber must be sufficient to prevent climbing dogs from avoiding exposure.
Two compounds given as intravenous injections are appropriate for euthanasia use if restraint facilities and trained personnel are available for administration. Pentobarbital, of intermediate action and relatively low cost, is the most used barbiturate. It must be stored in a locked cabinet and all use strictly accounted for to avoid possible abuse of the drug. Compound T-61, a combination of 3 drugs is effective for intravenous or intracardial euthanization.

Rapid decompression chambers produce hypoxia by evacuation of air to 68.8 mm of Hg or lower. The equipment is costly and must be properly maintained and operated.

Electro-lethality is also available in some countries.

(e) Destruction of dogs without initial capturing

All methods of killing dogs in residential areas present hazards and must be only used at night. In rural areas where owned dogs are turned loose by night to guard gardens and houses, stray dogs may preferably be killed during the daytime, after proper warning has been given to residents. Animal protection societies in general do not approve of destruction of dogs without initial capture. In any case, local residents must be given full information on the plan and schedule, which should be put into effect if possible on the same night and day of each week; they should be advised to keep their dogs confined and all members of the household indoors during the killing period. Anyone who must be out of doors in the area at the time must carry a plainly visible flashlight or torch.

(1) Shooting is the cheapest method, and can be applied at long distances, but requires special material, great caution and skilled marksmen.

In residential areas dogs can be killed at 30-50 metres either with a shot-gun using cartridges (no up to 4) or preferably with a.22 long rifle equipped with a silencer, a telescopic sight (5 x 40 or 4 x 32) and a device allowing burst fire. Hollow point, subsonic bullets are needed if a silent gun is used, the telescopic sight being slide or swing-over mounted. Dogs can be killed either during the daytime or by night. At night a strong light shone directly into the eyes of a target dog will briefly "freeze" it, permitting observation of an eventual identifying collar or tag, and firing of at least one shot. All shots must be low in front.

In rural areas dogs (and cats) can be killed when found far from houses, using a shot-gun (30-50 metres) or rifle type 22-250 "Remington" with telescopic sight using hollow point or soft point bullets only (type Federal or SAKO), preferably with a silencer. Long distance shooting usually concerns stray or wild dogs which can therefore be killed also by night using a powerful marine flashlight at up to 150 metres for a well trained shot. In all cases very strict precautions must be taken; only specially trained and authorized personnel (usually policemen) are allowed to handle and keep the guns. They must be aware of all possible dangers, firing only in safe positions and ensuring the identity of target animals. In every case injured dogs must be followed and killed and all carcasses must be promptly removed and properly disposed of (see Section 5.2.4(1)).
Capture guns use compressed carbon dioxide or percussion caps to propel syringes or darts to inject target dogs with immobilizing drugs. The drugs most commonly used are Etorphine, Fenanyl-Droperidol, Ketamine hydrochloride, Phenacyclidine hydrochloride, Xylanine. All of these are reasonably safe to bystanders and take effect in 3-25 minutes. The drugs are relatively inexpensive but may be subject to chemical abuse, and the guns are costly and supplies of carbon dioxide propellant cylinders and of the immobilizing drugs may be inconstant. Dogs are "frozen" by a strong light and following striking with the projectile are followed until anesthetized. Killing may be by further injection of a lethal drug or shooting with a captive bolt or bullet gun. All carcasses must be promptly removed and properly disposed of (see Section 5.2.4(ii)).

Oral poisoning must be done with great care to account for every bait set out and to remove every poisoned dog in different ways according to the type of area. In urban residential areas meat and fish baits or chicken heads are commonly used. A target dog is located and identified as unvaccinated by flashlight; then a single poisoned bait is tossed to the dog and the flashlight turned to provide only indirect light. As soon as the bait has been taken, the light is shone back to ascertain that it was eaten and not expelled from the mouth. The dog is then followed until it is unconscious. It may be most humane to kill a dog in convulsions or which is unconscious. The carcass must be promptly removed and properly disposed of (see Section 5.2.4(iii)) to prevent any possible secondary poisoning. Short to intermediate acting barbiturates are relatively cheap and take effect in 15-30 minutes. They must be kept in locked cases until used and every capsule must be accounted for to prevent possible drug abuse. Compound 1080 is too slow in action and the hazards of secondary and even tertiary poisoning are too great for consideration. Strychnine and sodium cyanide are frequently used and are cheap but very hazardous and inhumane.

Strychnine must be handled under strict safety conditions, and an example of one country's code of practice for the purpose is given in Annex 5.4.

The editorial group decided to include these instructions for the use of strychnine in these guidelines since this poison is still used in a number of countries, and therefore advice should be given on its safe storage and application. The editorial group by no means wishes to advocate the use of strychnine but it felt responsible for pointing out risks and precautions.

In non-residential areas, destruction of dogs must be done when no other operations are going on or when the area can be cleared of people. Shooting and capture guns are preferably used. In areas where foods are sold or processed, poisoning may be very hazardous. When oral poisons are to be used, initial baiting with non-poison baits is done to ensure that the right dogs take and actually eat the baits. On the following night, the poison baits are placed. Animal wardens patrolling the perimeter of the area try to turn back dogs leaving the area and follow any which escape after taking poison bait. When all dogs which have taken poison baits are dead, the carcasses and remaining baits must all be collected and deeply buried to prevent any scavenger animals or persons from accidental poisoning.
In rural areas where numerous unvaccinated stray dogs are living, oral poisoning can be easily accomplished on a large scale, specially around slaughter houses or community garbage dumps. Poison can be deposited under surveillance, at night, in sheltered places either in meat balls or in the carcass of a dead animal.

(f) Scheduling of animal patrol activities

Removal of dogs not in compliance is most important immediately following completion of vaccination campaigns when it is essential that chains of transmission of rabies be broken. Because, as dogs are removed from one neighbourhood, dogs from other areas quickly move into the vacant areas, dog removal campaigns should be conducted quickly. Patrol wardens should be scheduled to cover the area systematically, removing all dogs not in compliance which can be captured and destroyed on a neighbourhood by neighbourhood basis. They should cover the same neighbourhoods again one and two weeks later to remove dogs missed or entering since the principal removal campaign. In some areas the capturing of dogs is easiest during the hottest part of the day; destruction without initial capture must be carried out principally at night in urban residential areas.

In the maintenance of dog removal, animal patrols should spend the first part of each day responding to requests by residents to pick up unwanted dogs or dogs which have been observed not in compliance in their neighbourhoods. Then they should systematically patrol the area on a schedule that covers each residential neighbourhood and each non-residential area once each month.

(g) Rabies suspect and hazardous animals

Animal patrols should promptly respond to any notification of a suspected rabid animal in the area, apprehending it for confinement and observation, or if necessary, destroying it by an applicable means. At any time a rabid animal is recognized in a neighbourhood, local residents should be told to confine their dogs and an intensive dog removal effort should be immediately undertaken. Any vicious dogs which are uncontrolled and hazardous to community residents should be removed or destroyed by animal patrol wardens or by other public officials. Animal wardens, local officials or community residents should promptly remove or destroy any unidentified dogs which wander into a neighbourhood from outside.

(h) Animal patrol and pound records

Accurate records should be kept of all dogs captured by the animal patrols by time and location, of all dogs impounded and their disposition and of all dogs confined as rabies suspects and of the fate of these animals. Dogs which are redeemed or placed must be properly vaccinated and undergo a quarantine period of 3 months; dogs may be supplied to government recognized research or teaching institutions without vaccination.

(i) Safe disposal of animals killed or found dead. As a minimum measure, carcases should be buried at a depth of 1 metre and covered in quicklime, without contaminating sources of drinking water, before more elaborate or expensive methods, such as incineration or recycling, are instituted.

Carcases of dead animals suspected of rabies infection should, before disposal, only be handled by authorized persons for the collection of specimens for diagnosis. In no case - not even after cooking - is meat fit for human consumption if it originated from an animal showing symptoms suspicious of rabies.
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(j) Societies for animal protection. Members of societies for animal protection may operate animal care shelters, may receive unwanted dogs after proper quarantine and vaccination, or help owners in placement, may sponsor neutering clinics and may conduct public education programmes in responsible animal care. Their expertise and services should be integrated into rabies control programmes.

5.2.5 Reproduction control

The stray dog populations of many urban areas consist primarily of released and abandoned pets. In these areas it is important to limit the surplus offspring production of the owned dogs. It should be kept in mind that all the approaches mentioned in the following need the participation of the dog owners. Control of irresponsible dog ownership and education of dog owners is therefore an initial step of dog reproduction control.

- Sterilization (neutering) Community residents and particularly owners of numerous animals (e.g. hunters, farmers, police, etc.) should be encouraged to have both female and male dogs surgically sterilized at veterinary clinics. The sterilization of female dogs is especially important as the replenishment of the dog population is dependent on the number of female dogs of reproductive age which are present. Special neutering clinics may be operated in animal pounds, or in shelters organized by societies for animal protection. The percentage of dogs reached by neutering programmes is usually not large and this may be used as an adjunct to dog removal and not as a primary emphasis in rabies control programmes. Chemical sterilization is not sufficiently developed to be considered for use on any community basis.

- The simplest contraception is the prevention of mating by physically controlling the animals in heat. Chemical contraceptives should be safe, simple to deliver, inexpensive and accessible. Different commercially available products fulfill these requirements to different degrees. Some need administration during appropriate times in the heat cycle. Chemically induced interruption of pregnancy is occasionally practised in order to eliminate the consequences of uncontrolled mating. It is unlikely that such methods will ever become important on a larger scale.

- The elimination of puppies can be promoted by legally limiting the litter size. Such an approach is already taken by kennel clubs allowing not more than a certain number of puppies per litter for certain breeds. All legislation needs surveillance and enforcement. It might therefore be more efficient to pay a premium for each puppy brought for elimination to humane societies or animal control agencies.

- The imposition of higher taxes for fertile females than for neutered bitches or for male dogs should be a limiting factor to the number of puppy-producing individuals in a population.

5.2.6 Habitat control

Reduction of food and harborage supporting scavenging dogs in residential areas and control of specific habitats in non-residential areas are of great importance in dog population control. It is essential that community health and development departments play their part in habitat control as part of an overall rabies control plan. All public entities involved must participate; leadership may be provided by any of these entities, including rabies control officers.
(a) Community garbage collection on a frequent and regular basis can control the major food source for scavenging dogs. Residents must be encouraged to place garbage in covered dog-proof containers, and local authorities to provide such containers.

(b) Sanitary waste disposal facilities can remove this source of food for scavenging dogs in some communities.

(c) Removal of harborage in and under which dogs find shelter and protection and where female dogs whelp and successfully raise their litters should include unused and collapsed buildings, old vehicles, piles of lumber or brush and other junk. Low spaces under houses or porches should be securely sealed; dogs can enter through surprisingly small spaces.

(d) Clean-up of specific habitats within communities is most important. These include control of garbage dumps by thorough burning or burial; enclosing markets, abattoirs and food processing establishments to prevent dogs from entering these compounds; thorough clean-up after marketing, butchering or food processing operations; and thorough clean-up after fish cleaning operations in fishing villages rather than dumping such offal back into lakes, rivers, or the ocean.

(e) Education of the whole community must be developed to avoid multiplication of dogs by reducing sources of food. Particular attention must be paid to personnel in markets, abattoirs, food handling and processing establishments (hotels, restaurants, schools, military establishments) and fishing villages, who should be taught not to discard scraps, trimmings and other by-products where they might be eaten by stray dogs.

(f) Encourage development of backyard livestock and poultry raising to turn household garbage into an economic resource. Promote development of larger livestock or poultry operations to utilize garbage and food by-products on a community-wide basis.

5.2.7 Emergency and contingency plans

Surveillance of rabies in control areas is the basis on which decisions for further planning and action are made.

Continuing presence of rabid dogs following completion of rabies control campaigns

Within 6 weeks following completion of a comprehensive dog vaccination and/or dog removal campaign in sufficiently large and isolated areas, rabies should have virtually disappeared in dogs, though rarely cases may occur following incubation periods as long as 6 to even 8 months. Continual presence of rabies means that the dog census on which coverage was based recorded fewer dogs than were actually present, the vaccination and/or dog removal efforts did not reach 70% of the dogs at risk of exposure, or the vaccine lacked potency.

Immediate investigation must be conducted to assess the reason. Further vaccination, probably on a house to house basis, or further dog removal must immediately be initiated, starting with the problem neighbourhoods and progressing areawide. If the problem is use of vaccine of low potency, the entire campaign must be repeated with vaccine of demonstrated potency. Public opinion will not be favourable but the campaign must be totally repeated.
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**Declaration and enforcement of quarantine areas**

Any appearance of rabies in dogs in or adjacent to a rabies control area should, according to local conditions, lead to the placing of all communities under quarantine within a radius of at least 15 km. All dog owners within the quarantine area should confine or secure their dogs entirely within their premises. When off their premises, owners should have each dog under direct control on a leash or confined within a vehicle or cage.

If the last dog censuses in the quarantine area were conducted more than one year ago, new estimated or enumerated censuses should be conducted. Emergency dog vaccination campaigns should be promptly instituted to include all dogs 3 months of age and older except those which had been vaccinated at 12 months or older within the last year. Entire community coverage of all dogs or coverage only of owned dogs may be done. If unowned dogs are not vaccinated, they must be promptly removed or destroyed.

If rabies is present in adjoining countries or adjoining districts of a country, zones may be established in which comprehensive rabies control measures are enforced as long as there is danger of rabies entry. Continuous vaccination efforts with annual vaccination campaigns and continuous control of dog movement, removal of dogs not in compliance, and surveillance of rabies in dogs must be conducted in such zones.

Whenever a hazard of rabies recurrence appears in a control area between regularly scheduled campaigns, due to an influx of dogs with a human population shift, a breakdown in animal patrol coverage, an increase in incidence of rabies in adjacent areas or other factors, emergency programmes should be instituted. These may include comprehensive community-wide dog vaccination campaigns, restriction of dog movements, removal of dogs not in compliance or destruction of dogs not in compliance.

5.2.8  **Community education and public relations**

Strong community organization and participation are necessary for successful rabies control campaigns and continuing programmes. Public education and information on schedules must reach all sectors of communities. Educational programmes and materials must be applicable to local settings and be in local languages.

(s) National government

At the national level, national laws or proclamations form the bases for rabies control in the country. A model for legislation is shown in Section 4.2. National laws should be well publicized to the public.

Educational information on rabies, its transmission and control, and the national programme for rabies elimination should be publicized through newspapers, magazines, radio and television for as wide coverage as possible.

Brochures, posters and other informative material should be made available to community programmes to assist local promotion. Film strips, slide sets and movies on rabies may be prepared at national levels for local use if they are applicable to local settings.

Compulsory inclusion of rabies control in courses on hygiene in elementary and secondary schools and provision of appropriate material to schools for such use may be very effective.
Local government

Local ordinances or pronouncements should form the bases for local implementation of rabies control. They should be well publicized to community residents. Model legislation is shown in Section 4.2.

Educational and promotional materials received from the national government or prepared locally should be extensively used in all local mass media and public information channels. A brochure providing concise pertinent information on rabies, along with space for printing a local vaccination schedule is shown in Annex 5.5. A poster with space for announcing a vaccination schedule is shown in Annex 5.6. Suggestions for local radio announcements providing educational and promotional information are given in Annex 5.7. A radio interview in which the hazards of rabies are described and information provided on the forthcoming dog vaccination campaigns is shown in Annex 5.8. Another poster is shown in Annex 5.9 and one designed to warn travellers not to bring their pets into a country is shown in Annex 5.10.

An educational unit on rabies control in elementary and secondary schools can be effective in reaching children and their parents. An outline colouring booklet which children can read and take home is shown in Annex 5.11, and a school's educational wall chart is Annex 5.12.

Many community officials may function in community education on rabies. They need to be well and accurately informed. Local mayors and council members, physicians, veterinarians, rabies control officers, animal wardens and vaccinators and their drivers all have contact with the public and have important roles in educating community residents on the importance of rabies control.

(c) Community organizations

Informational letters to community organizations and talks or other presentations to such groups by administrators of the rabies control programme can reach a very influential group of community leaders, school teachers, religious and political leaders, etc.

Community organizations may be enlisted to assist in promoting dog vaccination and control campaigns and in operating neighbourhood vaccination centres.

The day before the campaign, the dates and hours of vaccination can be announced by posters, banners, radio, etc. One of the best ways to do it is by driving a car around the whole area to be vaccinated, with a megaphone mixing music and announcements of the next campaign.

5.2.9 Rabies control programme personnel

Personnel involved in rabies control must include the participation of both veterinary and human medicine as well as all necessary government departments. The leadership, both national and local may either rest with the Ministry (Department) of Agriculture or of Health but ultimate responsibility must be identified within a single ministry. The assignment and support of personnel from each ministry to the rabies control programme must be clearly defined. In the community education, community development and local enforcement aspects of the programme, the roles and support of
persons from these agencies must also be clearly delineated. Ambiguities which may lead to the failure of any agency in fulfilling its responsible role may lead to curtailment or collapse of the entire programme.

(a) National and regional rabies control personnel

If rabies control will be directed or coordinated at a national level, a director should be designated within the Ministry of Agriculture or Health. In many countries the director is a public health veterinarian within the Ministry of Health. He will have the following responsibilities:

He will direct and coordinate the national rabies control programme through community level actions, including the possible participation of private veterinary practitioners.

He will conduct and compile community dog censuses to assess quantities of vaccine needed and to establish programme allocations and goals.

He will ensure availability of equipment, vaccines and supplies for community programmes.

He will ensure adequate numbers of trained and properly immunized vaccinators for community campaigns. Vaccinators assigned from the national level to work in community campaigns must be paid transportation and living expenses. Local residents usually are more effective in local campaigns but both time and money are required for their training and pre-exposure immunization. Combinations of national and local personnel may compose vaccination teams.

He will ensure availability of national or regional rabies diagnostic laboratories and collect their results for epidemiological reports at national or international level.

He will conduct rabies control educational programmes in collaboration with the education authority and ensure availability of educational materials for community programmes.

He will ensure, with the appropriate authority, the training and plan of action of the personnel for dog destruction.

He will monitor the functioning and records of community programmes and take necessary actions to ensure their proper operation.

He will declare rabies quarantine areas where rabies emergencies exist and provide such direction and/or assistance as may be needed at community levels.

A national rabies control policy-forming council may be composed of representatives of all participating agencies with the national director. It will function in programme planning and its members will liaise with their agencies in allocating personnel and budget to the programme.

If regional coordinating centres with regional directors will be created, the regional directors will liaise between the national director and the community programmes in the effective implementation of rabies control in the communities of the region.
(b) Local rabies control officers

At provincial, city, district, or other political subdivision level, a rabies control officer will be employed or assigned to direct and coordinate local rabies control programming. He will be given certain police powers with respect to animal control. Usually this officer will be a veterinarian. His duties will usually include the following:

He will conduct and analyze dog censuses.

He will schedule, organize and conduct community dog vaccination campaigns.

He will monitor the vaccination coverage at the community level and will ensure that at least 70% of all dogs above 3 months of age are vaccinated.

He will investigate, report and take proper action in animal bite incidents.

He will assist in obtaining laboratory diagnosis of suspected rabid animals, and transmit positive results to national or international level.

He will provide or recommend through local medical services, pre-exposure immunization for persons at high risk of exposure to rabies.

If a community animal patrol is organized, it will be under his direction, or he will prepare its plan of action with police or army, and he will coordinate the work of the animal patrol wardens.

If a community pound or animal shelter(s) will be operated, he will direct or coordinate with the director(s) in maintaining, placing or destroying animals.

If animals may be destroyed without initial capture in the community, including dogs not in compliance with the rabies control programme or any animals which are a hazard to community residents, this will be under his direction, and he will prepare the plan of action with the police and local authority.

He will ensure that humane procedures are followed in all capturing, restraining, vaccinating, transporting, placing or destroying of dogs and other animals during the course of the rabies control programme.

He will direct clean-up of community areas harbouring or attracting unowned dogs and will work with community residents to try to prevent the casting out of unwanted dogs.

He will conduct and assist educational programmes in responsible pet ownership and rabies control.

He will follow directives and report to regional or national rabies control authorities as required.

Local rabies control councils may be formed representing local government boards or councils, local government health and veterinary services, local police or constabulary, local medical and veterinary societies and local animal protection societies. Such councils will function in local policy and scheduling and the members will liaise with their agencies in assigning personnel and allocating budget to the programme.
(c) Government agencies participating in rabies control programmes

Established agencies of government functioning in rabies control programmes will work with the rabies control officer. Agencies established as parts of rabies control programmes will work under the rabies control officer, as for example:

- finance department officers to pay bills and payrolls and receive funds in the operation of rabies control programmes
- community development officers to assist in community education and organization of clean-up of food and harborage supporting unowned dogs
- police to assist rabies control officers in the orderly performance of community rabies control programmes
- community animal patrols to capture and remove or destroy unowned, hazardous or rabies suspect dogs. Animal wardens are frequently assigned to short term campaign duty as vaccinators.

(d) Local medical and veterinary services and animal protection societies

Local health care services, whether government clinics or hospitals or private practitioners, have very important educational and promotional roles in rabies control programmes. They must immediately report incidents of animal bites in which rabies may be suspected, any other exposures to rabies and all cases of human rabies, to local rabies control officers. Frequently local government health care units assign personnel to temporary duty as vaccinators.

Local government veterinary units and clinics vaccinate, identify and complete official certificates on animals brought for vaccination. Personnel from these units commonly serve as vaccinators in local neighbourhood vaccination centres and often in house to house campaigns. They play important roles in rabies education and in promotion of its control.

Private veterinary practitioners perform official vaccinations. They frequently volunteer to serve in vaccination campaigns. They are important in the education of animal owners and in the promotion of rabies control, but the question of their role and remuneration (particularly if vaccination is free to dog owners) must be discussed in detail with their representatives before the start of the campaign.

Local animal protection societies frequently operate animal care shelters and function in placement of unowned animals. They play important roles in community education.

(e) Recruitment of personnel

In programmes conducted through national coordination, supervisory and some field personnel are commonly recruited, employed, trained, pre-exposure immunized, and assigned to field service by the national rabies control programme. In locally developed programmes, all of these functions are performed at community level. It is always best to use as many local persons as possible within the constraints of training and pre-exposure immunization costs. Local personnel know the communities, speak the local dialects and are usually better accepted by the public in vaccination campaigns. Drivers are usually employed by the agencies owning vehicles assigned to the programme.
(f) Training of personnel

All personnel involved in rabies control programmes, including supervisory personnel, field personnel, drivers and service personnel must be given training in their responsibilities, and education in the control of rabies. Following instruction in their duties, personnel must be given field practice, first assisting experienced personnel and then performing them alone.

Personnel associated with the programme are very important in public information and in answering queries from dog owners. National supervisory personnel or local rabies control officers usually conduct the training programmes. When vaccination teams are composed of personnel assigned by the national programme and locally recruited, part of the training of the local personnel may be by the team members assigned by the national government. The training should include the following 3 aspects:

(i) All personnel should be taught the epidemiology of rabies and the method of its control, including legislation and national or local regulations. It is most important that they fully understand the importance of rabies control. They must know their rights as well as their duties and be prepared to explain their duties to community residents and be motivated fully to perform their duties in dog vaccination and/or control.

(ii) Personnel must be taught the respective duties they are assigned to perform.

- the proper way to prepare and announce the campaign
- the proper handling, cleaning, sterilization and preparation of syringes and needles
- the care and handling of vaccines to preserve potency and prevent contamination
- safe and humane procedures for capturing and restraining dogs for vaccination
- the proper procedures for vaccination
- the identification of vaccinated dogs
- the proper completion of vaccination certificates
- the safe and humane release of vaccinated dogs.

(iii) If destruction of dogs without initial capture will be part of their duties, personnel must be carefully trained in the use of firearms to ensure effective and humane operation combined with safety for themselves and community residents. (See also Section 3.2.4 above)

(g) Pre-exposure vaccination of personnel

Programme personnel who will be involved in handling dogs should be pre-exposure immunized prior to assuming their duties using authorized human vaccines. Human diploid cell vaccine is commonly given in 3 doses 1 and 2-3 weeks apart with booster doses every 2 years or in the event of exposure. Other vaccines should be given according to schedules authorized for the particular types of vaccines.
The usual schedule of vaccination and training is to provide the first
dose in conjunction with one full day of study on rabies and its control.
The second dose is given in conjunction with the field practice day. The
third dose is given on the first day of the community campaign. Because of
the time needed in the pre-exposure immunization, personnel must be recruited
or assigned at least 3-4 weeks before starting work in a campaign. As far as
possible antibody estimation should be made one month after injection, any
person having less than 0.5 International Units/ml of serum being excluded
from rabies field work.

(h) Transportation, room and board for personnel

Vaccinators may travel to and from operational areas by commercial buses
if schedules are reasonably dependable. Vehicles assigned to the programme
may be more convenient but are much more costly. Provision of room and board
is a major consideration only for personnel assigned by a national
programme. In urban centres, room may sometimes be provided in health
centres or other public buildings, but often hotels and restaurants provide
the only, and unfortunately costly, provision for their needs. In provincial
towns and villages, vaccinators are usually accepted in local homes for which
payment should be made. Every effort should be made to avoid burdening local
residents. Personnel maintenance problems are greatly reduced when local
personnel are recruited or assigned to community campaigns.

5.2.10 Supplies for vaccinating teams

All necessary supplies must be in stock before the final scheduling of
community vaccination campaigns is made. Vaccines must be stored between 0
and 4°C continually to ensure retention of potency. During vaccination
campaigns, vaccine must be delivered to vaccinators as needed, and syringes
and needles must be continually recycled. The following provisions must be
made for effective operation of community campaigns:

(a) Information

Each team should have material (posters, leaflets, etc.) to distribute
before or during their work. It has proved very useful to have a portable
megaphone for summoning people or addressing groups.

(b) Vaccines

Vaccines must be of effective potency. This is of prime importance.
The quantity to be obtained for community campaigns is based on dog censuses,
adding a 15% overrun to ensure a sufficient supply if a dog census should have
been as much as 10% low and to allow for losses up to 5% in spillage or
vaccine remaining in vaccinators' kits at the end of days in the field.
Vaccines must be shipped, stored and transported to the field in a cold chain.
(See Section 3.4.3) In vaccination centres, vaccines must be kept in ice
chests or refrigerators. In house to house campaigns, vaccinators should
carry vaccines in ice chests which they may make themselves using polystyrene
sheets for insulation, or in commercial polystyrene ice chests or vacuum type
cold chests which are now quite widely available worldwide. Liquid type
vaccines should be kept in the cold chests until used. Lyophilized vaccines
should be used within one hour of reconstituting and should be kept cold
throughout the period of use. In accessible areas, vaccines for one day's
use should be delivered each morning before the vaccinators begin work. For
vaccination clinics, an estimate of doses per vaccinating team per day based
on experience of the previous campaign or first day's work may be made. For
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House to house campaigns, this initial estimate may be 100 doses per team per
day. At the end of each working day, unused vaccines should be discarded.
In less accessible and more distant areas, it may be impractical to deliver
vaccines more often than twice weekly. Under such circumstances, keep
vaccines as cool as possible. Vaccines should be administered according to
the manufacturers' instructions. Most dog vaccines in current use are
administered intramuscularly at a single site in the thigh muscle or
subcutaneously in the thoracic region (for inactivated vaccine only - see
Section 5.5.1).

(c) Syringes, needles and sterilization equipment

Sturdy metal and glass syringes and reusable needles are commonly used.
Pistol-type syringes are easier and safer to handle. Syringes should not be
used longer than one hour before washing and resterilizing, and needles should
be changed frequently. Washing may be done with household detergent followed
by a clear water rinse. Sterilization is usually done in a small covered
pan. Use enough water to cover needles and syringes completely and heat to a
rolling boil. In vaccination centres, kerosene or gas burners or electric
hot plates may be practical but in house to house campaigns, vaccinators
usually request use of stoves at local residences or make their own open
fires. Following boiling, the water is drained from the pan without removing
the cover and the syringes and needles are removed only as needed. Used
needles and syringes are placed in a box or other container until sterilized
and re-used.

Disposable syringes and needles are very convenient but also costly and
may appear quite extravagant to local residents. Vaccinators may find
themselves pressed with requests to buy these syringes and needles from
them. It is quite practical to sterilize and re-use disposable syringes and
needles. Many disposable syringes can be washed and boiled up to 10 times
and many such needles up to 100 times. Needles to be re-used should be
checked for sharpness and any with burrs or damaged points resharpenned.
Frequently syringes and needles are washed and resterilized by vaccinators
during noon breaks and evenings.

(d) Dog catching and restraining nooses

Dog catching and restraining devices and muzzles are useful in vaccination
centres and essential in house to house campaigns. A practical noose which
can be made by vaccinators themselves is shown in Annex 5.1. It consists of
an aluminum pipe, or bamboo pole with the nodes knocked out, allowing a rope
attached to the end to form a loop, the shank being threaded back through the
hollow handle. In use, the rope loop is dropped over a dog's head and drawn
firmly around the dog's neck. The dog's head is then pressed to the ground
with the handle and held during vaccination and identification. (See
photographs in Annex 5.1). Prior to release the dog is faced towards an
escape route; the head is then raised and the noose released. The dog will
move quickly to freedom without danger to the vaccinators.

5.2.11 Continuous community functions in rabies control programmes

Following achievement of rabies elimination on a local or national basis,
continual vigilance must be maintained to prevent re-entry or to detect
re-occurrence. When rabies is re-introduced into a susceptible dog population,
it will spread rapidly and quickly re-establish its former endemic status
unless prompt control measures are instituted. On one Philippine island from
which rabies disappeared following a single vaccination campaign which reached 85% of the dogs, rabies remained absent until reintroduced 11 years later. Within 3 months, the former endemic status was re-established throughout the island. When rabies reappears in a control area, very rapid action must be taken to halt its spread and to eliminate it again.

(a) Continual surveillance

All reports of suspected and confirmed rabies must promptly reach the medical and veterinary officials and the rabies control officers involved. Continual surveillance must include the following:

- Prompt reporting and investigation of animal bites. When rabies is suspected, the animals involved must be apprehended and detained for observation.

- All cases of clinically diagnosed rabies in animals must be promptly reported by government or private veterinary clinics.

- Diagnostic laboratory reports on all specimens received, both positive and negative, must be rendered promptly.

- Surveillance of border areas between rabies control and infected areas within the country and at international boundaries must be continuous.

- Human exposure and cases of human rabies must be reported promptly. If the probable source of exposure was within a rabies control area, it must be investigated at once.

(b) Continual control procedures

Community education on rabies control and encouragement of community participation must be continuous. The following procedures must be maintained:

- Community residents should have their puppies vaccinated when they reach three months of age, followed by a second dose one year later and a booster dose every two to three years. Adult dogs brought into the area should be vaccinated, followed by booster doses every two to three years.

- If dog removal and/or destruction are part of a local rabies control programme, they must be continually carried out. All too often these measures falter or become sporadic following initial campaigns, or vivid memories of aggressive dog control campaigns mitigate against continuation of such action.

- Dogs entering the country across an international border or entering a rabies control area from an endemic area must be vaccinated at least 30 days before entry or held in quarantine for 30 days following entry and vaccination. (see Section 6.5).

- Community residents must be continually exhorted not to cast out unwanted or surplus puppies or adult dogs.

- Habitat control must be continuous.
5.3. Diagnostic procedures

5.3.1 The need for diagnostic laboratory services

As dogs are the most important transmitters of rabies to man, even where the disease is prevalent in wildlife, it is of paramount importance in the prevention of the spread of rabies to man and other animals to be able to detect the disease as early as possible in a rabid dog. This is especially so in a country where rabies is not endemic but which is under constant threat from the disease being introduced from neighbouring or other areas.

Since post-exposure anti-rabies vaccination in humans is not recommended in every case of dog-bite because of possible serious reactions to the vaccine, it is necessary to determine, wherever possible, whether the biting dog was indeed rabid or not before administering the vaccine. If the dog shows typical clinical signs of rabies it will be possible in an endemic area to presume with some degree of accuracy that the dog is rabid and institute immediate treatment of the exposed person with hyperimmune serum and vaccine. However, if the dog has silent rabies or the dog-bite incident occurs in a hitherto rabies-free area, laboratory testing is the only way whereby rabies can be confirmed.

The role of the diagnostic laboratory in the control of dog rabies is, however, not only a guide to the proper management and treatment of the bite victim, be he human or animal, but also a necessary element for anti-rabies control measures in the country even when no one has been bitten.

Not all countries or parts of countries, however, have access to a diagnostic laboratory and in many developing countries, lack of communication and transportation seriously hampers surveillance by laboratory methods. For foci of infection in such areas there is no other alternative for the control of dog rabies than to set up local teams of workers, recruited from the locality itself, to destroy stray or unlicensed dogs, and vaccinate and license owned dogs. To eliminate dog rabies in such areas, these tasks must be carried out relentlessly and continuously.

All members of the team should be given pre-exposure vaccination and their immunity tested and maintained by booster injections. Newer and better vaccines have been developed for both dog and man and these should be used instead of the out-dated ones, wherever possible.

5.3.2 Clinical diagnosis

All warm blooded animals are susceptible to rabies though not equally so. Dogs are only moderately susceptible but are the species most likely to spread the infection to man. It is therefore of the utmost importance, particularly in a rabies-endemic area, to be able to detect signs of rabies as early as possible in an infected dog.

The epidemiological history of a dog can be highly suggestive when coupled with early subtle behavioural signs. Such history might include the absence of immunization, the prevalence of rabies in the immediate region, whether the animal is allowed to roam freely or is confined, thus changing its level of exposure risk, and any history of an incident such as a fight with another dog or some other wild animal. If the possibility of rabies does not enter into the differential diagnosis, laboratory examination will not be necessary.
The clinical picture in dogs

Incubation period. The incubation period is very much variable. In the majority of cases when rabid dogs bite other dogs or animals the bite goes unnoticed and the incubation period can only be estimated. Where a history of a bite is available the incubation period in dogs appears to be about 10-12 weeks. Periods shorter and longer than this average are, however, not uncommon. Since dogs tend to bite one another in the head and neck, and much less frequently in the hind quarters, the incubation period may on occasions be as short as 2-3 weeks.

Prodromal stage. In dogs the first sign is, without question, a sudden change in disposition. It is a signal very often unrecognized by owners. A dog normally affectionate suddenly becomes snappy and uncertain, and one normally reserved or uncertain in temperament suddenly becomes affectionate and seeks human companionship. In the absence of a rabies outbreak, owners can be excused for disregarding this cardinal symptom but its recognition during an outbreak is of importance if the owner and his household are to be spared much subsequent personal stress.

The prodromal state lasts 2-3 days and is followed by either the "furious" or the "dumb" form of the disease.

The "furious" form. The symptoms in this stage follow no definite sequence. The dog becomes unusually restless, seldom lying down or sitting in one spot for more than a short time; if confined or restrained it moves about ceaselessly. The eyes assume a watchful, puzzled or apprehensive look - a most important sign - and it may snap at imaginary objects. There is a dilation of the pupils and sometimes a squint. The characteristic rabies "howl", not easy to describe, may or may not develop; it is neither a bark nor a howl but possesses elements of each. At certain periods of the "furious" stage a dog seems possessed of more than normal strength and to be insensitive to pain. Bars of cages, expanded metal netting, steel chains, corners of concrete, furniture, etc. are frequently attacked to the point where the animal's teeth are reduced to stumps and the mouth and gums grossly lacerated. In a dog not under restraint this excitable energy is manifested by furious, aimless running and by snapping indiscriminately at objects, animate or inanimate, in its path. It is this syndrome that is responsible for the rapid spread of the disease, as, within a comparatively short time, a furiously rabid dog can create many new potential foci of infection. The appetite during this stage is frequently perverted, and affected dogs swallow stones, sticks, earth, etc.

The "furious" stage lasts 1-4 days and gives way eventually to ataxia, paralysis and death.

The "dumb" form. There is little of a spectacular nature to note in this form and for this reason many cases go unnoticed, particularly in communities in which dogs are not held in much esteem. The dog usually remains quiet and bites only when provoked. The watchful, apprehensive look in the eyes, noted in the "furious" form, is also present in the "dumb" form. Paralysis may affect the hind quarters. In the vast majority of "dumb" cases paralysis of the jaw ("dropped jaw") supervenes with a concomitant paralysis of the tongue which becomes flaccid and protrudes from the side of the mouth. Saliva drools from the mouth, a symptom not infrequently associated in the owner's mind with the presence of a bone stuck in the dog's throat. In endeavouring to relieve this supposed condition, many owners have risked infecting themselves gravely through getting bitten or getting their hands and fingers contaminated with the dog's saliva.
The dog is unable to take food and becomes more and more emaciated. Paralysis of the tongue makes it impossible for the animal to lap water; many try very hard to do so but cannot.

Death in either form, occurs 3-7 days after the prodromal stage. Since, however, the saliva can be infective as early as 3 days before these initial symptoms are manifested, suspected dogs are usually quarantined for a period of 10 days from the date of their having bitten another animal or man.

Diagnostic features: the clinical symptoms of canine rabies are so characteristic that little difficulty should be experienced in making a clinical diagnosis with some assurance in all but a few cases. A combination of several of the following may justify the diagnosis:

1. Sudden change of disposition
2. Watchful, apprehensive expression of the eyes
3. Characteristic "howl"
4. Drooling of saliva
5. Posterior paralysis
6. Marked excitability and restlessness
7. If restrained, attacks objects within reach
8. If at large, runs aimlessly, biting anything in its way
9. Depraved appetite
10. Lies quiescent, biting only when provoked
11. Snaps at imaginary objects
12. Paralysis of lower jaw and tongue
13. Inability to drink

Differential diagnosis: in its early stages the rabies syndrome in dogs resembles the extensive group of conditions known as "canine hysteria". The veterinary surgeon should be able to eliminate a number of potential causes at the first examination; those with experience of rabies acquire a very acute and reliable diagnostic "sense" for the disease. In all cases of doubt, however, it should be the invariable routine to place the suspected animal in a strong kennel for 10 days' detailed observation. If the animal is still alive at the end of this period it can be assumed that the case is not one of rabies. If death occurs within the 10-day period the brain should be examined for Negri bodies by histology, for rabies antigen by immunofluorescence and for virus isolation by animal inoculation.

The clinical picture in other animals

In cattle, horses, goats, domesticated monkeys and cats the disease conforms in general to the clinical picture for the dog. Both "furious" and "dumb" forms are recognized. Where the "furious" form occurs in cattle and goats, aggression is much less marked than in dogs. They will frequently, however, butt trees, posts, etc., until the forehead is badly lacerated. Cats are highly susceptible to rabies and most of them tend to show the "furious" form. As they usually attack their victim on the head and face they are particularly dangerous. Moreover, because they can hide easily, they could be responsible for the introduction of rabies into controlled areas.
The clinical picture in man

Incubation period. Although it may vary from ten days to several months, the incubation period is usually between two weeks and two months. Bites with large and deep lacerations, bites in children, and bites of the head, neck and upper limbs, are all associated with a short incubation period.

Premonitory stage. This lasts for 1-4 days. There is usually low pyrexia, malaise, anorexia, nausea and often headache. Respiration is often so shallow that the patient has to stop speaking and take a long sighing breath. Changes in personality and nervousness are common and there may be episodes of acute anxiety. In more than three quarters of cases there is some form of abnormal sensation around the causative wound, which will have healed by then. It may be itching, stabbing pains, numbness, tingling ("pins and needles"), coldness, burning or aching with trembling or weakness of the affected limb. The patient may be hypersensitive to stimuli such as bright lights, cold and draughts. A positive "fan test" has been described as diagnostic of rabies in which a current of air directed across the face causes violent spasms of the pharyngeal and neck muscles (aerophobia).

Stage of excitement. The majority of patients show increasing excitement often continuing until death, which usually occurs within one to three days. Aimless wandering about the room is common and speech is often incoherent. Periods of acute mania may alternate with depression and acute morbid anxiety. Convulsions which may be fatal often occur during these maniacal attacks. Pyrexia is usual and may reach 39°C. The classical, although not constant, symptom of hydrophobia is very characteristic when the patient tries to drink, or even sometimes thinks of drinking; the muscles of the pharynx go into sudden acute spasm so that any fluid taken in is violently ejected and the head is thrown back. Cases with typical hydrophobia, yet able to swallow solids, have been described. Hydrophobia is not seen in other brain infections and never in rabid animals.

The respiratory muscles may be affected causing apnoea followed by cyanosis and severe dyspnoea. In the late stages Cheyne-Stokes breathing is not uncommon. The patient is unable to swallow his saliva and becomes dehydrated due to lack of fluid intake. The saliva which is infective, becomes veryropy and tenacious. Tremors and weakness especially of the ocular and facial muscles may be observed. The reflexes may be increased in the early stages but are usually absent later. There may be some stiffness of the neck but Kernig's sign cannot be elicited. Babinski's sign may be positive.

Stage of paralysis. Patients seldom live longer than three days after the onset of acute excitement and may die during this stage. Those who survive the acute excitement usually pass into a stage of increasing apathy and paralysis leading to coma and death, often within a few hours. Some patients may, however, live in this stage of apathy and paresis for as long as ten days. Consciousness is usually retained until shortly before death. Rare cases are predominantly paralytic from the onset and may present as (i) Landry's ascending paralysis (ii) transverse myelitis, or (iii) a paralysis indistinguishable from poliomyelitis or a neuroparalytic accident of vaccine treatment.
Diagnostic features: the definitive diagnosis of rabies during life depends on the detection of the viral antigens in skin or brain material, or the isolation of the virus from the saliva. However, a negative result could not exclude the disease. Rabies diagnosis is evident if the following are present:

1. A history of a bite
2. Sensory symptoms at the site of the bite
3. Excitement or convulsion
4. Hydrophobia
5. Paralysis

Differential diagnosis:
- Follow-up is very close to rule out encephalitis.

There is, however, a history of an accidental sensory symptoms do not occur if there is one.

- The viral encephalitis must be considered in the differential.

- Tetanus may follow bites, but the incubation period is generally shorter (usually 6-14 days). Tetanus symptoms do not appear actively, between attacks and are not followed by tetany. There is general spasticity as compared with the flaccidity of rabies between spasms. The typical tics and tremors are not found in rabies.

- Hysteria precipitated by the fear of rabies after a bite, or to be borne in mind, the "incubation period" is short.

- Neuraparalytic accidents of various treatment may be very difficult and, if fatal, somewhat impossible to differentiate from rabies.

Only by demonstration in virus xenograft, no conditions other than ruled out.

5.3.3 Quarantine and observation

The terms quarantine, confinement, and observation are often used imprecisely in the field of rabies surveillance and control. Quarantine is often used to designate the act of confinement of animals under quarantine conditions; the purpose of this quarantine or confinement is observation. Quarantine may also refer to the isolation of movement of animals or persons (usually accompanied by intensified immunization, control of stray animals or other efforts) within a circumscribed area which is experiencing epidemic rabies. The terms quarantine and confinement are used interchangeably when referring to a single or small number of individuals, but not when relating to an area.

a) Confinement of individual animals

Apparently healthy animals may be confined for observation for the development of symptoms of rabies. In most cases, the animal has bitten a person or another animal and the observation period determined if the biting animal could have been infectious at the time of the bite. Existing data show that dogs and cats can be sick in the rabies of the dog or cat for 1 to 5 days before the onset of symptoms. A fact widely applied and used is identified by the 10 day observation period following a bite as recommended by WHO. Comparable data are few for other species (in fact, few wildlife species have considerably longer periods of exposure) and so the 10 day observation is appropriate for dogs and cats only.
An animal showing early and poorly defined behavioural, paralytic or neurological deficits may be confined for observation for the development of more clearcut symptoms, for the acquisition of specimens (skin, corneal impression smears, cerebro-spinal fluid) for laboratory study, or for the application of more sophisticated clinical examination. In areas where laboratories use Negri body examination rather than the fluorescent antibody test (FAT), additional time is often felt (but not really proved) necessary to increase the numbers of Negri bodies, and hence elevate the percentage of cases diagnosable by this method.

Confinement is also indicated in the case of an animal which has been exposed to rabies. In this case the observation is to allow the maximal limit of an incubation period to pass before the animal is considered risk free. Six months is considered to encompass the incubation period of the vast majority of rabies cases.

The properly immunized animal in case of exposure should be immediately given 1 or 2 booster injections of rabies vaccine, and confined for 30-60 days. As the average incubation period for rabies is 3 to 6 weeks, a confinement of 45-60 days seems most logical; the antigenic stimulation from the revaccination should provide adequate protection beyond this point.

In all cases, the optimal state-of-the-art diagnosis is accomplished by examination of the brain. However, the proportion of biting non-rabid animals to those biting and actually infected with rabies is extremely high. This fact, coupled with the common resistance to killing animals simply for obtaining a diagnosis, make in vivo techniques a requirement. In the academic sense, we will never learn the true character of rabies by relying upon brain examination to identify the cases.

b) **Quarantine as a national protective measure**

The purpose of quarantine is to prevent rabies from entering a rabies-free or rabies controlled area through infected animals. Imported animals are generally subject to quarantine and/or measures of observation in order to ensure diagnosis (see Section 6.6).

c) **Area quarantine**

Quarantine is imposed upon a circumscribed area rather than an individual for the purpose of intensification of control efforts. As quarantine constitutes a control measure, with various provisions and ramifications, diagnostic services play a major role in reaching the initial decision, monitoring the progression of the epidemic and the success of control measures, and in reaching a decision as to when the quarantine can be removed.

5.3.4 **Location of laboratories**

The number of laboratories required for the diagnosis of rabies in a country depends on the size of the country and how well-developed its communication and transportation system is. If the system of communication and transportation is good and the country is small, only one central laboratory is needed to cope with all the specimens from the country.

Of course, the larger the country, the more laboratories are needed, and these should be sited where they can best serve different areas.
Local laboratories, serving smaller geographical areas, however, may suffer from inadequate access to positive material, thus reliability of
techniques and have more problems in maintaining equipment and obtaining
equipments. Where trained techniques are used, with a mix out of 90% positive
specimens per week, the reliability of results will be a subject of concern.

LABORATORY TECHNIQUES

Collection. In the collection of specimens from imported cases of cattle,
beef or animal, it is of utmost importance to bear in mind that highly
contagious material is being handled and therefore all precautionary measures
in present infection are to be strictly followed. As this applies not only to
the time of collection of the parents but throughout the journey to the
laboratory, every effort must be taken to the packing of the specimens for
transit.

Specimens may consist of live intact animal, dead animal, clinical
expressions, fluid from organs, blood and alimentary fluid. Although by
the time the sample arrives at the laboratory, the causative material may
have been killed or decomposed, post mortem processing by the pathologist
will ascertain the nature of the disease as either a viral or invertebrate factor
in the alimentary fluid.

Specimens are prepared as follows:

Brain or skin samples - refrigerate or freeze at -20°C or -70°C

For definitive periodic protein detection, for virus isolation only, 
preservation is 10% formalin acceptable. Preliminary observations
indicate that buffered formalin is preferable. Preservation for
systemic antemortem testing (SAM) after specific treatment of the brain
specimen, but the technique has not yet reached practical application in the
field.

Corneal samples for EM - specimens are best prepared on slides by an experienced
person, fixed in acetone, and sent to the laboratory refrigerated.

Saliva - refrigerated, freeze, or preserved in 10% formalin for virus isolation
only. Saliva sheets should be dried and folded in a buffered transport
medium.

Blood serum or convoluted tissues - can be held or shipped with no additives
for 1-4 days if specimens is secured and handled acceptably. Add 30%
glucose (sucrose reducing carbohydrates to compensate), refrigerate, or
freeze (for long term storage).

NMS or nerve fibres for electron microscopy - Contact NH laboratory to secure
proper advice, equipment and procedures.

There are specific research needs to be pursued in the near future to
expedite transit of specimens, and hence diagnosis and surveillance.

Emulsion fluid transfer, is needed for the technique of preserving tissues
for EM in buffered formalin, which has proved to have great promise for
both fresh or postmortem-embalmed tissues - etc.
Research is needed to determine the adequacy of examination of cranial nerves or cervical spinal cord as compared with standard examination of the brain. In some cases, the brain is missing and therefore no test is done. Also, small pieces of easily accessible tissue (e.g., optic nerve or cervical spinal cord) are much easier to acquire and ship to a laboratory.

Post-mortem specimens

Brain. The animal suspected of being rabid should be observed for at least 10 days and the disease allowed to progress until the fatal outcome. The premature killing of such animals may reduce the possibility of detecting Negri bodies in the brain as these are believed to develop in direct relationship to the length of clinical illness. Under certain circumstances the immediate killing of a dog may however be desirable or even inevitable.

When in agreement with the health authorities and the dog owner this is justified provided there is a reliable rabies laboratory with FAT facilities which will examine in addition to Ammon's horn also segments of the brain stem and the cerebellum. If it is necessary to destroy the animal it should be shot through the heart and not the head which will damage the brain. The use of chemical poisons should be avoided as they may interfere with the results of subsequent animal inoculation tests.

In the decapitation of the animal and opening of the skull and spinal column, all precautions must be taken including careful operative techniques and the wearing of protective clothing, including rubber autopsy gloves, rubber aprons, sleeved gowns, surgical masks and goggles. If the decapitation is done in the field and the brain cannot be removed immediately, the head should be cooled down promptly and kept cool until it reaches the laboratory.

Care should be taken to damage the brain as little as possible during its removal. The method sometimes described of fracturing the top and sides of the skull with sharp hammer blows is not recommended for obvious reasons. The skull should be sawn vertically through both parietal bones at the level of the supraorbital process and the cuts joined by a horizontal saw-cut on each side just above the level of the zygomatic arch. By the use of the saw (preferably a hacksaw) the whole of the roof of the cranium can be lifted off in one piece. A fresh saw should be used for each head, or at least sterilized between heads.

Having exposed the brain the membranes are incised and the brain divided antero-posteriorly into halves using a long, sharp knife. Each half is removed separately and with care, one being placed in Zenker's fixative (Annex 5.1) and the other in 50% glycerol-saline (Annex 5.14), the former for histological examination and the latter for virus isolation and detection of viral antigen. Even brain which is too mangled for histological examination should be sent in glycerol-saline as virus isolation may be possible.

The brain of a human victim should be removed as soon as possible after death. The method is similar to that for the animal brain.

Salivary glands. A dog's saliva may contain rabies virus several days before Negri bodies are demonstrable in the brain although the brain is infected first. If virus is present in the salivary glands, the submaxillary glands will contain most virus. Preferably, both submaxillary glands packed in glycerol-saline should accompany the brain when suspected rabies material
is submitted for laboratory examination. If Negri bodies are not found in
the brain, the inoculation of preparations from the salivary glands into mice
can demonstrate the presence of virus.

It is suggested that both submaxillary salivary glands accompany the brain
on all occasions where a clinically suspicious case arises in an area after a
long period of freedom from the disease or in other cases of special interest.

Shipment: It is imperative that all specimens for rabies diagnosis be
adequately packed to prevent breakage or leakage and labelled, e.g., "CAUTION.
SPECIMEN FOR RABIES EXAMINATION. DO NOT DELAY". The laboratory must be
notified by telephone or telegram of each shipment and the specimens must be
despatched promptly.

The entire animal head or post-mortem specimen should be placed in a dry,
watertight unbreakable container and closed tightly. This in turn should be
placed in a larger watertight container and the space between both containers
packed with cracked ice or freezer packs. The specimen must be delivered to
the laboratory within the shortest possible time after the death of the human
or animal.

Specimens in fixative or glyceroinsaline should be handled with care to
ensure that they are not damaged or exposed to excessive temperatures.

5.3.6 Personnel

Personnel should be reliable, calm and deliberate, and with the highest
possible educational and experience level. It must be assumed that persons
who are more nervous and impulsive in their activities are not at particularly
good risk in working in the laboratory with a dangerous virus which can be
transmitted by accidental exposure. Not only should new employees be
selected with these criteria in mind, but experienced employees should receive
periodic reminders of the risks and other dangers (e.g., written courses) to
make sure their safety attitudes and procedures are uppermost in their mind.

While very few potential employees have experience with rabies, it is not
so unusual to acquire persons who have had training and experience in other
laboratories and in the handling of high risk microbiological agents. New
employees must therefore gain experience in rabies safety and laboratory
procedures by direct training and supervision. It is also advisable for new
employees to spend brief training periods in other laboratories and often this
will represent a cost effective way of securing certain types of training
experience.

All persons in the laboratory or field who are at risk of rabies exposure
should be pre-exposure immunized. Proper immunization means either having
received a minimum series of acceptable rabies vaccine injections or by
demonstrating a neutralization titre of 0.5 International Units or
greater. Booster doses at 2 year intervals are recommended. After
successful immunization, all appropriate safety precautions should be
exercised in the laboratory to prevent the possibility of accidental exposure,
so that the immunization merely serves as a back-up in case accidents do occur.
5.3.7 Diagnostic laboratory techniques

The available laboratory techniques are capable of detecting:

- negri bodies in brain tissues;
- viral antigen in the cells of various tissues (e.g. brain, salivary glands, cornea, skin and mucosal scrapings);
- infectious virus in organs such as brain, salivary glands including saliva; and
- specific antibodies in blood-serum and CSF.

Detailed descriptions of the laboratory methods are available in the WHO Monograph Series No.23 (1973).

Laboratory handling of specimens: It is imperative that specimens be processed promptly on arrival at the laboratory. Care must be taken in the recording and labelling to avoid confusion. Fresh and unfixed specimens in preservative must be handled aseptically to prevent contamination of specimens, and infection of workers. All laboratory instruments, solutions, containers and facilities must be sterilized or disinfected and every precaution taken to prevent transfer of rabies virus from any one specimen to another.

Unfixed or fresh specimens, whether dry or in glycerol saline should be kept at 4°C in the laboratory until processed for examination to detect Negri bodies by the Seller's method, viral antigen by the FAT and infective virus by mouse inoculation.

Fixed specimens for histology must be submitted to the pathologist with the request to prepare and stain at least 2 sections each of the hippocampus, cerebrum and cerebellum for the detection of Negri bodies and for histopathological examination.

(a) Detection of Negri bodies

Although these acidophilic inclusion bodies may be absent in about 15% of cases, when detected they are pathognomonic of rabies. They should, therefore, be looked for, especially in laboratories which do not possess a fluorescent microscope.

Negri bodies may be detected by the microscopical examination of smears or impressions of fresh material stained by the Seller's stain (Annex 5.15) or by the examination of fixed material sectioned and stained for histopathological examination (Annex 5.16).

By Seller's staining, Negri bodies appear magenta-red in colour and are characteristically formed having dark blue/black basophilic granules (Innenkörperchen). They vary in shape and size and their matrix is heterogeneous in appearance. Their typical intracytoplasmic position is not retained in smear preparations as in fixed sections. All parts of the nerve cell are stained blue. Interstitial tissue stains pink and erythrocytes a coppery red colour.
Negri bodies may be confused with the acidophilic non-rabies inclusion bodies of canine distemper and canine infectious hepatitis or with those of cats, mice, deer and other animals, all of which have the same staining characteristics for Sailer's stain. However, the non-rabies inclusion bodies are found more frequently in other parts of the brain than the hippocampus, have no internal structure but a homogeneous matrix and tend to stain plainer.

The longer a biting and suspect animal is held in quarantine during life, the better chance there is of detecting more abundant and better developed Negri bodies with good internal structure.

Many methods of staining have been described and advocated for fixed sectioned materials. One method of choice is the Fast-Green-Acid Fuchsin stain (Smes 3,10).

In brain sections, the Negri bodies are electron lucent inclusions. Electronically, they are found between the nucleus and one corner of the neuron. Higher power examination reveals characteristically arranged inclusion. The inclusion reaction can also be clearly seen.

(b) Immunoperoxidase technique

The rabies antigen in the cells of an infected host may be detected by the FAT or the immunoperoxidase reaction, both of which are based on the principle of an antigen-antibody reaction.

In the FAT, the antibody which is tagged to a fluorescent dye (fluorescein isothiocyanate) reacts with specific antigen, if positive, allowing direct observation of the reaction product under the fluorescence microscope. In the immunoperoxidase reaction, the antibody is attached to an enzyme (peroxidase) and reacts with the specific antigen causing it to appear as yellow brown particles under the light microscope.

The chief merits of these tests are speed and accuracy. However, reliable results depend very much on the availability of satisfactory reagents and equipment, and on the experience of the person operating the fluorescence microscope. The Immunoperoxidase test, though needing simpler equipment, requires greater experience for reading than the FAT.

The fluorescent antibody test

The FAT has been widely accepted as a reliable and specific method in rabies diagnosis. In laboratories not yet thoroughly familiar with its use, it is recommended that it be employed in conjunction with the mouse inoculation test and with Negri body detection for about one year in order to gain experience and confidence.

This test is described in detail in the WHO Monograph Series No.23 (1973). Materials for examination by the FAT include brain, salivary glands, corneal cells, eardrum scrapings and skin biopsies. If specimens are received in glycerol saline, the smears or impressions must be prepared from tissue taken below the surface of the specimen. The tissue must be washed several times in saline since glycerol may mask the fluorescence by combining with acetone used during the fixation step.
Four impression smears are prepared from the hippocampus and two from the cerebellum and brain stem. Some laboratories prefer to have the two additional slides prepared from a paste by dry grinding in a mortar of equal portions of material from hippocampus, cerebellum and brain stem. The remaining ground tissue may be diluted with appropriate diluent for the mouse inoculation test. Staining procedures include the direct, indirect and complement staining method. The direct method will be described here.

Conjugate is a Fluorescein-labelled antiserum which may be obtained commercially. Conjugates from reputable firms have been pre-titrated and the recommended working titre can be used with reproducible and reliable results. It may be more advisable, however, to predetermine the optimal working dilution for one's own laboratory and microscope by preparing serial two-fold dilutions of the conjugate and test these against a known positive specimen. The one before last dilution giving 3-4+ fluorescence is regarded as the standard working dilution. Freeze-dried preparations can be kept for 3-4 years at 4°C, but once reconstituted the solution will keep for only a limited time (about 1 to 2 months) at the same temperature.

Mouse brain suspensions, normal and infected

The brains of normal adult mice and of mice infected with rabies virus and harvested in a moribund state are aseptically removed, weighed and homogenized in 9 volumes of sterile normal saline. The suspensions are centrifuged lightly and the supernatant is stored at 4°C before use.

Acetone. This is for fixing the smear and should be reagent grade, freshly poured from stock and pre-chilled in a Coplin jar to -20°C. It should be discarded after every use. Acetone-fixed slides should be regarded as infectious, since non-inactivated rabies virus may still be present.

Washing solutions. Phosphate buffered saline (PBS) pH 7.4-7.6 is used in all washings. The final rinse is in distilled water. These solutions must be clear but not necessarily sterile.


**Staining technique**

Fig. 5.1: Procedure of modified direct method of detecting rabies antigen

1. Prepare fluorescein-conjugated antiserum
2. Dilute the specimen
3. Incubate at 37°C
4. Rinse
5. Add fluorescein-conjugated antiserum
6. Incubate
7. Rinse
8. Add fluorescein-conjugated antiserum
9. Incubate
10. Rinse
11. Add fluorescein-conjugated antiserum
12. Incubate
13. Rinse
14. Examine under a fluorescence microscope

Results:
- Negative: No fluorescence
- Positive: Fluorescence present

Slide with test specimen
5.38

- Dilution of the conjugate. Part of a freshly dissolved conjugate is diluted to its standard working dilution by mixing it thoroughly with appropriate volumes of normal (suspension A) and infected mouse brain suspension (suspension B). The mixtures are incubated at 4°C for 8-12 hours or overnight. Following centrifugation for 10 minutes at 2500 rpm the supernatant fluid is ready for use and retains its full staining capacity for a 1 to 2 week period thereafter when stored at +4°C.

- Preparation of the smears. The glass slides used for impression smears must be freed from grease by immersing them in an ether-alcohol mixture (96% ether - 4% alcohol). Two impression smears should be made as thinly as possible from the suspected tissue (one smear for the test and the other as control for specificity), placing them about 3cm apart on each slide. As an additional control it is recommended that parallel tests be run with known positive and negative preparations, which may be mouse brain or field material. When the smears are dry, they should be fixed in water-free acetone at -20°C for a minimum of 10 minutes. Just before staining, each of the smears is ringed with fingernail varnish and marked "A" and "B" respectively.

- Staining. Two of the 4 hippocampus smears from the test specimen are stained. The two others are stored at -20°C in case a re-examination is required. The remaining two which include other parts of the brain are stained and examined if the first examination is negative or equivocal. The smear marked "A" on the slide should be covered with suspension A (Fig. 5.1) and the other with suspension B, making sure the two suspensions do not mix. The control slides are stained at the same time. The slides are incubated for 20 minutes at 37°C in a humid chamber (a plastic box with wet filter paper fixed to the inner surface of the lid) to allow the antigen-antibody reaction to take place. Using a pipette, the conjugates are washed off from the slide with PBS, taking care they do not mix with each other during the process. The specimens are then washed twice for 10 minutes each in PBS (a magnetic stirrer may be used with the slides arranged on the periphery in a Coplin jar filled to the brim with PBS), immersed briefly in distilled water and dried in air. Examinations are made, with or without a cover glass, depending on the correction of the microscope's objective. If a cover glass is used, it should be mounted using buffered glycerol pH 7.2-7.4. If these stained specimens cannot be examined immediately they should be stored at 4°C until examination. The positive and negative control smears must always be examined prior to examining the test smears.

Interpretation of the test

- Smear stained with suspension A. Since only normal mouse brain is contained in the suspension, the labelled anti-rabies antibody is not absorbed and is therefore free to stain any rabies antigen that is present in the smear. This will be seen as Negri bodies, which fluoresce brightest at the margin, and/or as fine, dust-like fluorescent green particles. If these stained specimens cannot be examined immediately they should be stored at 4°C until examination. If a cover glass is used, it should be mounted using buffered glycerol pH 7.2-7.4. If these stained specimens cannot be examined immediately they should be stored at 4°C until examination. The positive and negative control smears must always be examined prior to examining the test smears.

If the hippocampus smear is positive, no further tests need be done, but if negative or equivocal, the smears of the other parts of the brain should be examined. If no fluorescence is observed, the smear is negative.
b. Smear stained with suspension B. Since rabies antigen is present in this suspension, the labelled anti-rabies antibody should be absorbed and is therefore not available for the staining of rabies antigen in the smear. No fluorescent green staining should therefore be seen in this smear. If bright fluorescence is present, it indicates non-specific staining. An additional check for non-specific staining is by examining the smear under an incandescent light source, which causes specific fluorescence to fade away rapidly while non-specific staining remains.

Fig. 5.2: Modified direct method of detecting rabies antigen

If non-specific staining or a less than satisfactory fluorescence is observed with the positive control smear, the entire procedure should be repeated with freshly prepared brain smears.
All smears, whether test or control, may be stored dry and kept in an airtight closed container with dry calcium chloride or silica gel at \(-20^\circ\text{C}\). In this way, the smears can be kept for 2-3 weeks without appreciable loss of conjugate binding activity. Material from which the positive control smears are prepared is best stored in small portions in dry vials at \(-70^\circ\text{C}\).

The use of infectious virus in the specificity tests presents a possible hazard to the laboratory personnel. This can be avoided by inactivating the brain suspension containing rabies virus (suspension B) with UV light. The suspension is poured onto sterile petri dishes, so that the thickness of the virus suspension does not exceed 1 mm, and is then exposed for 30 minutes to UV irradiation at a distance of 30-50 cm.

The use of monoclonal antibodies for the routine laboratory diagnosis of rabies is not yet recommended. Monoclonal antibodies have been found extremely helpful for the differentiation of certain virus strains (see below) but the test at present can be handled only by certain laboratories. The ease and rapidity by which a laboratory diagnosis is achieved can mainly be attributed to the diagnostic procedure itself. The time for instance required for processing a specimen by Seller's or FA staining is comparable. If a re-examination is not required, a single specimen should have been processed and read within 2-3 hours time after receipt. In most instances, the FAT report is final and no other tests need to be done. If Seller's staining is negative, however, other tests are required.

This already indicates the weakness of histochemical methods in general and of the Seller's technique in particular. This method is economic only where the majority of laboratory specimens are rabies positive and where Negri bodies are regularly encountered. If the submitted specimens are derived from animals other than the dog, and if the rabies-negative cases outnumber the positive ones, the FA-technique is the method of choice. Every effort should then be directed to convince authorities to have this technique introduced into the rabies laboratory. The high initial costs encountered for the purchase of equipment and conjugate and for the training of personnel are well justified and will turn out beneficial in the long run.

Antigen variation among rabies viruses has recently been shown to exist by the use of monoclonal antibodies (see also Section 1.1). These highly specific antibodies will become an extremely helpful tool for future virus strain differentiation. Already, true variants have been identified from the classical rabies virus in regard to their protective activity such as Mokola, Lagos bat, Duvenhage viruses as well as other bat-origin viruses. All have so far been shown to originate exclusively from within the African continent. By the use of ordinary high quality rabies conjugates these viruses are as easily identifiable as are common rabies virus strains. Claims of the existence of atypical strains not being recognized by FAT are unrealistic and can from experience be reduced to the question of inadequate conjugates.

(c) Isolation of infectious virus

Virus isolation is occasionally important in human or animal exposure situations and should assume greater importance as epidemiological studies now seem feasible on virus isolates using batteries of monoclonal antibodies against different rabies antigens.
Intracerebral mouse inoculation, in conjunction with the fluorescent antibody test or microscopic examination of brain tissues for Negri bodies, is still one of the most useful tests in the laboratory diagnosis of rabies and should be used whenever human beings have been exposed to suspect animals. Suckling mice are generally more sensitive for virus isolation and should be used whenever possible. The observation period may be shortened by fluorescent antibody examination of brains of inoculated mice killed 4 days or more after inoculation.

Recent studies on the value of cell cultures for the isolation of rabies virus indicate that the method could be as sensitive as mouse inoculation provided that sensitive cells (e.g. neuroblastoma) are used.

Preparation of tissue

Brain. Pieces from the hippocampus major, cerebral cortex or cerebellum are weighed and ground in a mortar. 10% heat-inactivated sterile horse or rabbit serum in normal saline is slowly added to make a 10% emulsion. An equal volume of a solution containing 100 units of penicillin and 4 mg of streptomycin per ml is added and the mixture centrifuged at 1,000 rpm for 5 minutes. The supernatant is kept at 4°C for 30 minutes before inoculating the mice. Bacterial contamination should be checked by plating the inoculum on blood agar.

Salivary gland. The procedure is the same as above except that it is advisable to mince the tissue with sterile scissors first and then to use sterile sand in the mortar to facilitate grinding.

Inoculation

In routine practice at least 6 weanling mice should be evaluated per specimen. Under special conditions (e.g., a rabies-free country) the following procedure may be adopted: two litters of 10 suckling mice each and 6 adult mice should be inoculated, the latter being first anaesthetized with ether in a glass jar. The amount of inoculum for suckling mice is 0.01 ml each and for adult mice 0.03 ml each.

Observation and recording

Observation and daily recording of clinical signs and deaths should start from the very next day after inoculation. Weanling and adult mice are observed daily for 28 days. Deaths during the first 5 days are regarded as non-specific and probably due to trauma or bacterial infection. A check of the blood agar culture may assist in determining the cause of such deaths.

If additional suckling mice were inoculated, one suckling mouse from each litter is killed on days 3, 4 and 7 post inoculation, whether showing symptoms or not, and the brain examined for viral antigen by the FAT. Four smears are prepared from one half of each brain and two of them, stained by FAT. The other two smears and the remaining half of the brain tissue are stored at -20°C temporarily in case a re-examination is required. After unequivocal results are obtained, the stored materials may be discarded, taking the usual strict precautions for the elimination of infectious materials. The remaining suckling mice are left for the full observation period of 30 days before being declared negative (Fig. 5.3).
Fig. 5.3: Flow chart for the examination of specimens for rabies diagnosis

SPECIMEN

FA test/Seller's test and/or Histology
(if no FAT)

+ve -ve
FINAL REPORT TENTATIVE REPORT
(pending mouse inoculation results)

1/2 brain
4 smears
2 smears stained & examined by FAT
FINAL REPORT

Mouse inoculation

Suckling mice
(2 litters)

6 adult mice

+ve -ve
NEGATIVE REPORT

1/2 brain
2 smears stored at -20°C (temporarily)

One per litter sacrified on D3, 4 & 7

Alive & well for 30 days

Sick Alive & well for 30 days

Examine for Negri bodies & antigen

FINAL REPORT NEGATIVE REPORT

2 smears stored at -20°C (temporarily)

FINAL REPORT

Discard when final report obtained

FA by FT

NEGATIVE REPORT

FINAL REPORT
The signs denoting rabies are roughening of the fur, tremors when lifted by the tail, lack of co-ordination of the hind legs, paralysis, prostration and death. Positive results must be confirmed by examining the brain for viral antigen by the FAT or Negri bodies by the Seller's staining method if the FAT is not available. It will be noted that without the fluorescent microscope, the early detection of viral antigen in suckling mice would not be possible as the Negri bodies are unlikely to have been developed at such an early stage.

(d) Demonstration of rabies antibody

A number of serological procedures have been described for measuring rabies antibody (Kaplan & Koprowski, 1973). These include the complement fixation (CFT) test, the haemagglutination inhibition (HAI) test, the passive haemagglutination (PHA) test, the plaque reduction (PR) test, the gel-diffusion techniques, the mouse neutralization test (MNT) and more recently, the radio-immune assay (RIA), the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973), and the enzyme immunoassay (ELISA) (Atanasiu et al., 1975).

Among the antibody assay techniques, the conventional MNT is still the most commonly used procedure despite having the disadvantage of being time-consuming and costly. The test can be recommended for the routine evaluation of seroconversion if fast results are not required. Regional laboratories, however, offering serologic services to the public should consider the use of tests being more reproducible, faster and more sensitive. Among those the RFFIT has found general acceptance and is widely used in many laboratories. Results are available within 24 hours. The test can be highly recommended to those employing routine cell culture work. The ELISA test is another much promising procedure, especially since standard reagent sets for the sandwich method are commercially available.

5.3.8 Equipment for laboratories

The amount and type of equipment needed depends on the techniques involved, on the volume of work that can be handled, on safety requirements, and limited by the funds being available. A list of basic equipment and supplies required for a rabies laboratory is given in Annex 5-17. Categories of equipment are as follows:

Brain examination by conventional methods  Histochemical staining of brain smears for the presence of Negri bodies requires a good light microscope capable of 1000x magnification. Also included are routine laboratory items of low cost.

Safety items and instruments are needed for removal of brain specimens. Disposal of infective materials is accomplished using autoclaving or incineration.

Brain examination by immunofluorescence microscopy  Immunofluorescence microscopy requires an ultraviolet light source, appropriate filters, spare illuminators and a microscope suitable for ultraviolet illumination.

The FA microscope used must be of high quality, well-aligned and adjusted and the illuminating unit properly focussed. The mercury vapour lamp must produce a high intensity light. A record of the time the microscope is in use should be kept and the mercury vapour lamp changed after 200 hours of use if it has not burned out or diminished in intensity earlier. A heat dissipator on the bulb will increase its life-span.
5.3.10  Joint rabies diagnostic services for both human and animal specimens

Routine examinations such as screening tests for Negri bodies, antigen detection by immunofluorescence, virus isolation and detection of antibody are identical procedures regardless of whether the tissue is of human or animal origin. In a specialized diagnostic situation such as rabies, small laboratories have difficulty in maintaining adequate case numbers and volume to maintain a high degree of proficiency in carrying out tests. It is difficult to justify separate rabies diagnostic laboratories because the numbers are often small. There are likely to be many areas devoid of an animal rabies diagnostic laboratory service where human laboratories are operating; the reverse is no doubt true as well.

The problem encountered here is territorial or political rather than technical. Artificial dividing lines based on the species being examined make no biological or economic sense at all. Great savings could be accomplished by combining these activities in one rabies diagnostic laboratory and multiple benefits would accrue. Personalities often get in the way of this possible development. As the problems are behavioural, territorial, and perhaps cultural rather than technological and since the need is great, a concerted effort must be made to solve the problems blocking joint diagnostic laboratories to expedite an efficient service to the areas and segments of the population in need.

5.3.11  Reporting of laboratory findings

The importance of prompt reporting of laboratory results for rabies is obvious and cannot be over-emphasized. Preliminary results from the microscopy of the brain should be available within 24-36 hours in most cases so that the results can be used as guidance for antirabies treatment.

"Prompt" reporting however actually depends on: transportation to the laboratory, processing of the specimens within 24-48 hours followed by the reporting of the results. It can easily be seen that transportation often is the weakest link in the entire chain of circumstances producing delay. Laboratory results should be transmitted:

a. immediately to exposed patients:

Patients with bite exposures and their physicians need answers as rapidly as possible as the efficacy of anti-rabies post-exposure treatment decreases daily from the day of the bite or presumed exposure. Also, there is extensive economic drain of continuing treatment if the diagnosis is not conducted in a reasonably short period of time.

b. within one day to local health authorities:

Local health personnel need to be alerted to the presence of rabies because of the implications for their own communities. Also, educational campaigns can be planned the better with prompt diagnostic results.

c. bi-weekly or monthly to national and international agencies as required:

National agencies need summaries of reported cases or rabies for surveillance, determination of the adequacy of legislation directed at rabies control, the progression of epidemics, modification of control programmes,
dissemination of knowledge to the public about the situation, and for the establishment of policy regarding importation, exportation, port of entry regulations and quarantine.

d. Where conditions warrant (for instance, a rabies case in a rabies-free area), immediate notification of authorities at all levels is indicated.

5.3.12 WHO assistance

a. Training of personnel

WHO offers training in laboratory methods for rabies on regional and inter-regional bases. In the past, either seminars and workshops were held in developing countries for groups of workers or visits arranged to WHO-recognized rabies laboratories for individuals awarded research training grants or fellowships.

Two categories of workers should be trained in this way:

(i) the veterinary and medical graduate and

(ii) the medical technician. More training opportunities should be offered to the technicians than hitherto, as the health services in developing countries tend to depend more on this group of personnel than on the usually scarce number of graduate officers whom many countries can ill afford.

The graduate officer, interested in the latest advances in rabies, would profit most from seminars and conferences at which new knowledge in rabies could be shared. Laboratory techniques could be taught to both graduates and technicians in workshops conducted by WHO consultants. The technician, however, should be familiarized more with field control operations, e.g., the administration of vaccine to animals, performing post mortems on the suspected animal, removing the specimens and despatching them to the laboratory for diagnosis, and the health education of the public through films, video cassettes, posters and talks.

The subjects to be dealt with in comprehensive training programmes should include (i) epidemiology of rabies, (ii) recognition of clinical rabies, (iii) up-to-date guidelines in the control and treatment of rabies, (iv) laboratory techniques, (v) vaccines and vaccination, (vi) methods of quarantine, (vii) methods of dog control, (viii) methods of monitoring wildlife rabies, (ix) health education of the public, and (x) the importance of multi-disciplinary approaches.

b. Provision of materials

(i) For laboratory diagnosis. Anti-rabies conjugate of good quality and of high titre is very expensive and occasionally beyond the means of the laboratory in a developing country where rabies is endemic. As the FAT is recommended by WHO as the best single method available in rabies diagnosis, WHO should also collaborate in evaluating commercial or laboratory produced conjugate for availability and reliability. It seems feasible that in certain areas WHO should stimulate the production of conjugate so that areas with chronic shortages can have assistance in securing adequate reagents and supplies. This could be arranged through a network of WHO collaborating centres. Such centres could provide advice and resources as well as quality control, servicing of equipment and on the spot training.
(iii) For health education. Posters, booklets, films, video cassettes, etc. are needed to impress on the community the importance of their active participation in the control of rabies, especially in the area of dog control and vaccination.

(iii) For public health programmes. In collaboration with national authorities, WHO has recently begun to develop time-phased programmes aimed at the elimination of canine rabies at local and national levels. The guidelines contained in this manual are intended to form the technical basis for such programmes. Aid in programme development will be a priority function of WHO for the coming years. It is believed also that technical and financial support may be obtained once sound programmes have been elaborated by national and WHO authorities.

c. Provision of services

Zoonoses centres and collaborating centres of WHO (see Section 7.1) should offer their services in the following ways:

(i) Typing of virus isolates. Monoclonal antibodies capable of revealing antigenic differences among virus strains are produced and virus isolates are being tested at the WHO rabies laboratories at Philadelphia, U.S.A., Paris, France and Tübingen, Germany. The workload involved in this type of work restricts mass typing of strains to those showing biological or serological peculiarities on isolation.

More laboratories are needed to perform this testing.

(ii) Control of laboratory accuracy (quality control)

WHO should encourage visits at irregular intervals to rabies laboratories of participating countries – possibly within the framework of regular consultancies – to ensure that the accuracy of the laboratory tests is maintained. The submission of unknown test samples at regular intervals would further serve this purpose.

(iii) Equipment servicing

Another function of WHO would be in the servicing of equipment. In this context virtually all laboratories have items of equipment out of service as the cost is too great, parts are inaccessible or trained service personnel are not available. Sometimes established laboratories can become non-functional because some simple necessary repairs, adjustments or parts are unavailable. A WHO reference centre could help to solve these problems.

d. Disseminating information on rabies

Rabies workers need to be kept in touch with the latest developments in rabies, particularly in the areas of (i) properties of the virus, (ii) treatment and prevention of rabies by vaccine and/or human immunoglobulin, (iii) pathogenicity and immunity, (iv) new diagnostic procedures (aimed at diagnosing the infection before the development of the clinical signs), (vi) control of wildlife rabies, and (vii) the role of interferon in the treatment of rabies. This information could be in the form of newsletters and/or bibliographies.
REFERENCES


5.4 Case reporting and surveillance

5.4.1 Needs for surveillance of animal and human rabies; role in rabies control; rabies control in the absence of surveillance programmes

In accordance with the definition by the FAO/WHO Expert Committee on Zoonoses, surveillance in the field of rabies means the continued watchfulness over the distribution and trends of incidence through systematic collection, consolidation and evaluation of data on all factors which characterize or influence the epidemic process. Besides distribution and frequency of cases in animals and man, rabies surveillance has thus to consider the ecology of the causative agent, its reservoir, and of animals at risk outside existing reservoirs. Modern methods permit the evaluation of data on the density and dynamics of animal populations, topography, infrastructure, measures of rabies defence and control, specific treatment of man and animals before and after exposure, complications due to specific treatment, etc.

More than in any other zoonosis, surveillance has become essential in rabies not only for the application of control measures in animals but also for prevention in man. Rabies is the only virus disease where the movement of human exposure can accurately be recorded and appropriate post-exposure treatment be initiated. Accordingly, rabies surveillance must ensure
adequate public awareness of the risk of exposure, and rapid assessment of the risk of infection is necessary in view of increased travel and the international transfer of animals.

In many countries the technical services for collection and shipment of specimens of suspected cases in animals and man to the laboratory are still inadequate (see Section 5.3.5) and particular attention must be paid to the requirements of effective surveillance systems, making use of existing services and laboratory facilities.

It is thought to be of advantage to have one national centre for diagnosis and surveillance. Should it prove to be necessary to establish more than one centre, those sub-centres should be able to report their data quickly to the main centre.

Due to modern international traffic of humans and animals, however, national surveillance alone will not suffice. For the protection of travellers (tourists and others) as well as for the indirect protection of humans by the control of animal border traffic, authorities should be informed quickly about the rabies situation in countries other than their own. This can be made possible by founding international centres for certain regions of the world, such as the WHO Centre for Rabies Surveillance and Research in Tübingen, Federal Republic of Germany. Most important also is the direct reporting of cases across borders between local veterinary and medical services of adjacent countries.

It is recognized that canine rabies can constitute a constant problem in countries with limited economic and professional resources for the implementation of large-scale surveillance programmes. Under such conditions, dispersion of efforts, resources and manpower should be avoided, and the total effort directed towards strategically important areas. To rid an area of endemic or epidemic canine rabies requires a well organized programme for the registration of owned dogs, their swift mass immunization, the elimination of stray dogs and health education.

Before these measures can be put into effect, the situation has to be properly appraised by disease surveillance in order to avoid overaction.

In national programmes of canine rabies eradication, priority should be given to areas that are known to be principal foci of the disease. Such foci are often in the major metropolitan areas (e.g., in South America), or in rural areas (e.g., in East Africa), where emphasis should be given to carrying out the mass vaccination of dogs in the shortest possible time. All dogs should receive their primary vaccination at the age of 3-4 months and a booster approximately one year later. Since rabid cats may present a serious problem, cat owners should be encouraged to have these pets immunized as well and cats should be included, if possible, in mass immunization programmes.

5.4.2 Reporting of animal cases

A reporting system should make use of existing administrative units and governmental institutions. In this section the terms "community", "district", "province", "central government" and "Ministry" are used, as being applicable to most national administrative structures.

Every person should be bound legally to report to the local authority any animal or carcass suspected of being affected with rabies (see legislation - Section 4.2). Possible reasons for suspecting an animal of having rabies should be made public through the mass media and by printed matter.
Local authorities in this context are:
- the district authorities, which should include a veterinarian and a medical officer. Both should be empowered to deputize legally any local practitioner of their profession.
- the communal authorities, e.g. the mayor's office. Those should be equipped with standardized containers for the shipment of samples or carcasses to the diagnostic centre. They should also be furnished with sufficient supply of standard forms to accompany the samples in the containers (see Annex 5.18).
- The local posts of constabulary or police; they should immediately relay reports received to "local authorities".
- Hunters, foresters, rangers, game-keepers and wardens, who should be employed in the same way as local police and constabulary.

Every report of a suspected case of rabies in animals should be registered at the district veterinarian's office. He has to keep records for the district and should map the location of cases on large-scale district maps. In cases of human contact, he should immediately notify the medical officer (this section does not deal with cases where direct medical assistance is required).

Killed animals or animals found dead and being suspected of having been affected with rabies should be shipped to the diagnostic centre. There, another record should be kept according to the data given by the accompanying report forms.

In cases of living suspects, the decision to kill or to detain and observe the animal rests with the district veterinarian. Any killed animals sent in for diagnosis must be accompanied by a report form.

In this way all suspected cases are recorded at the national centre. Of course, an unknown number of unreported cases will remain. This number must be kept as low as possible by means of public information, education and instruction.

The national centre should be part of the ministry responsible for the control of animal infectious diseases and also coordinate the surveillance activities of the medical services. The ministry should have direct control of all infectious diseases of animals, e.g., foot and mouth disease, rabies, anthrax, etc. The district veterinarian should report periodically to his provincial veterinary service headquarters. The national centre should report back diagnostic results to the province, the district, the community and in many cases to the owner or sender. A report should at the same time be sent to the veterinary and the medical department of the Ministry.

5.4.3 Reporting of human rabies cases

In the interest of the validity of a reporting system one should make sure that the following rules are followed when reporting:
- human bites by non-rabid animals should be recorded by the clinic but should not be reported to the rabies unit,
- human bites by rabid animals (highly suspected or proven by the veterinary laboratory) should be reported as exposures,
human rabies (with clinical symptoms and verification through identification of intracytoplasmic virus or fluorescent antibody staining of certain tissues) are reported as cases.

(a) Every human case of rabies, and bites by rabid or rabies-suspected animals, should be reported to the health authorities of the district where the incident occurred. Within 24 hours the name, age and address and all relevant details of the victim should be reported to the health and veterinary authorities (see Annex 5.19).

(b) The following persons should be legally bound to report:

- every physician
- nursing personnel
- heads of institutions, house holders
- headmasters of schools or kindergartens, teachers and other school employees
- tenants of flats
- publicans, hotel-managers, etc.
- veterinarians, police and constabulary, game-keepers and foresters, such cases coming to their knowledge while working in their professions or trades
- coroners
- landlords or their caretakers.

(c) When authorities have been advised of cases as in (a), they should send physicians to make the necessary examinations and inquiries.

(d) Rabies-infected, rabies-suspected persons and contacts should be bound to:

- give all information required
- permit themselves to be examined medically
- allow the taking of samples.

(e) For the diagnosis of rabies in humans, the patient should be hospitalized and specimens sent to a rabies diagnostic laboratory.

(f) On confirmation of a human case of rabies, communal authorities should put control measures into effect.

(g) In human lethal cases, which are suspected to have been caused by rabies, health authorities have to issue an order for a post-mortem. The samples taken at autopsy should be brought to the diagnostic centre, preferably by one of its experts, assisting at the autopsy.

(h) Humans bitten by rabies or rabies-suspected animals should be brought for treatment immediately.

(i) Physicians carrying out post-exposure vaccinations must complete a report form as in Annex 5.19 and distribute it as follows:

- keep one copy for filing,
- one to the provincial government, and
- one to the Ministry.
Completed report forms serve also to establish vaccination statistics, and to complete WHO Rabies Questionnaire no.19.

(j) A vaccination certificate (as in Annex 5.20) should be issued to the vaccinated person.

(k) Communities, police and constabulary, vaccination posts and physicians must report all cases of dog bites to the district authorities; who advise their provincial government, who on their part send a monthly report together with the report on contagious diseases, sending copies to:

- the provincial government
- the district authorities.

5.4.4 Reporting by rabies diagnostic laboratories

Three priorities of diagnostic specimens exist:

Category A: cases of human exposure

These may be divided into major and minor bites or exposures.

Category B: cases in domestic animals and susceptible pets

In these cases it is advisable to assume human exposure even when reports do not say so.

Feral dogs and cats should be included here, too. Difficulties may arise in countries where the terms "wildlife" and "pet" overlap (e.g. skunks, racoons, monkeys).

Nevertheless, the principle idea is to be on the safe side where human health could be involved.

Category C: wildlife cases without human exposure

As these are purely of interest for surveillance and control they have the lowest priority.

The means of communication in reporting should be:

- telegram
- telephone or radio
- report forms.

If the possibility exists to use teletype networks of the police on 24 hour duty, the opportunity should be taken, especially after duty hours or during weekends: the local constables know where to find doctors or veterinarians.

Carcasses and samples arriving at the laboratory in the morning mail or rail delivery should be grouped and dealt with according to priorities.

Diagnostic work relating to category A and B specimens should start immediately, so that the results of FA-tests can be reported back to the district veterinarian on the same day. Reports on the results of the mouse-inoculation-test are necessarily delayed; it takes approximately 21 days for this confirmatory test to be completed.
It is essential to set a deadline for the daily results to be ready for transmission. Transmission by telegram or telex is to be preferred, with confirmatory written reports sent later.

In exceptionally urgent cases, district veterinarians should be contacted directly by telephone in order to enable them to act quickly. Again confirmatory written reports should follow.

Diagnostic work on specimens of category C may follow as soon as work on A and B specimens is finished. Generally, the results should be ready for transmission on the day following arrival, at the latest.

In wildlife epidemics large numbers of cases will have to be diagnosed and about 85 to 85 per cent may prove to be negative. It is understood that positive cases will be reported back to the district veterinarian within 72 hours after submission of the specimen, the negative reports could be issued in weekly or bi-weekly lists.

Reports on diagnoses of categories A and B as well as C should go to the district veterinarian directly.

In category A cases he will report to the district medical officer. In category B cases the district veterinarian should receive the report.

Category C cases are also dealt with by the district veterinarian, who will decide on the course of action.

At the diagnostic centre each case must be registered in a ledger or case book in a prescribed form.

Report forms should be copied to the following:
- the district veterinarian
- the medical rabies institute
- the veterinary officer of the provincial government
- the veterinary department of the ministry
- the medical department of the ministry
- to the owner or sender of the sample or carcass, if the address is known.

The diagnostic centre prepares quarterly maps of positive cases and quarterly reports. These are sent to:
- the international centre of the region
- the veterinary department of the ministry
- the medical rabies institute.

Copies of the quarterly report of international diagnostic centres are sent to national diagnostic centres and to national governments.

5.4.5 Data to be reported

In order to give quick and precise information, case reports should meet minimal requirements and be submitted together with the specimen. The data required for national rabies surveillance is as follows:
- number of animal rabies cases
- number of human rabies cases
- number of post-exposure treatments in man
- number of pre-exposure treatments in man
- post-vaccinal complications
- control measures applied.

**Animal rabies cases** reports should include the name of owner and sender, address, date, animal species, location and other information as to whether the submitted animal was killed or found dead, and on possible exposures of humans and other animals.

The location will preferably be the smallest administrative unit within the country (generally the community), or part thereof, where the case occurred.

Any other additional relevant information available to the reporting officer should be added, especially if it is possible or intended to have the data stored, processed and evaluated by means of computerized techniques through a regional or international WHO reference centre. An animal rabies case reporting form as being used in the European Rabies Surveillance System is given in Annex 5.21.

**Human rabies cases** should be well documented. Reports on human rabies should be divided into actual rabies cases, post- and pre-exposure vaccinations and post-vaccinal complications, giving information for each as follows:

(i) **Rabies cases**
- person exposed (name, age, sex, address)
- details of exposure (date, place, type, site)
- source of exposure (animal species, disease status at time of exposure, clinical and laboratory diagnosis)
- circumstances of exposure (provoked attack, other people exposed)
- post-exposure treatment (local and systemic, type and batch number of vaccine and serum, course of treatment, vaccine reactions)
- clinical course (incubation period, period of illness, treatment, final outcome, diagnosis).

(ii) **Post-exposure treatments**
- the person exposed (age, sex, address)
- treatments (local wound, vaccine, serum)
- post-vaccinal complications
- source of exposure (animal species, disease status at time of exposure, clinical and laboratory diagnosis)

A suggested case recording form is given in Annex 5.19.

(iii) **Pre-exposure immunizations**
- the person (name, age, sex, occupation, address)
- the vaccination (type and batch number of vaccine, immunization schedule, seroconversion, booster doses)
- vaccine reactions and complications.

(iv) **Post-vaccinal complications.** General allergic reactions and CNS-disorders (e.g. meningitis serosa, myelitis, encephalomyelitis, neuritis)
related to the anti-rabies treatment should be reported on separate forms containing information on
- the person (name, age, sex, occupation, address)
- details of exposure
- post-exposure treatment
- clinical course and diagnosis
- final outcome.

5.4.6 Processing of surveillance data at receiving centres

In order to present the accumulating rabies data in a given format, the data have to be processed and presented in a certain manner. The procedures involved may be divided into 5 categories, irrespective of whether the data are processed by hand or through an electronic data processing unit. The steps are as follows:

(a) Data preparation
- preliminary data screening to check for obvious errors or non-intelligible characters on the reporting forms
- transformation of data into computer-compatible form (coding).

(b) Entry of data into the computer or into main-files.

(c) Data editing
- detection of errors
- resolution of errors (deletion of records, adding records, or changing characters of information).

(d) Data analysis
- simple analysis (averages, standard deviations, frequency counts)
- complex analyses (mapping, multivariate statistics, time-series resolutions)
- retrieval of data for various purposes from the master or subsidiary files.

(e) Display of output

Data may be presented in various forms (tables, maps) for use in routine reports, epidemiological analyses, and statistics. By use of varying computer programmes the data may be presented in many ways, e.g., (i) the capability of displaying information on geographical distribution; (ii) period that is needed for epidemiological analysis (from months up to years); (iii) infected areas; (iv) densities of cases; (v) number of cases per location, number of cases per given species, or a combination of species. The computer can produce all these types of information output quickly if it is correctly programmed.
5.4.7 Reporting of data from surveillance centres

The principle function of surveillance centres should be to exchange information on rabies and to provide veterinary and other public health authorities with the essential data for the most effective application of control measures in animals.

The information distributed from surveillance centres should include the following categories of data on animal and human rabies:

i. Data on epidemiological conditions including mapping of animal rabies cases for immediate action in rabies control, prevention, and post-exposure treatment of humans.

ii. Information on human exposure, vaccination, post-vaccinal accidents, and human cases of rabies.

iii. Retrospective analyses of the spread of the disease, control operations, and human treatment.

iv. Information on current outbreaks or enzootics.

The information distributed from surveillance centres should be designed to assist local officials in their decisions on animal rabies control operations and prevention of rabies in man, and to give information on long-term trends of the disease.

Countries with inadequate surveillance services should be encouraged to collect data systematically. Appropriate evaluation and reporting of such information would encourage better cooperation between countries and should be disseminated through the WHO Collaborating Centres. There should be close cooperation with the International Office of Epizootics (OIE) and the Food and Agriculture Organization of the United Nations (FAO). WHO rabies surveillance helps to develop and improve national systems by bringing all countries up to a common acceptable standard. National and international systems could be coordinated so that the same forms and reporting systems are used. There should be quick exchange of information from country to country through a common data pool.

5.5 Vaccine procurement and delivery

5.5.1 Selection of type of vaccine

The widespread use of modified live virus (MLV) and inactivated nervous tissue vaccines prepared from fixed virus has significantly reduced the incidence of rabies in many countries.

Annex 5-22 summarizes the main animal rabies vaccines as used in 1980. The same types of vaccines are still in use today without any major modifications.

A world-wide, up-to-date (April 1982) inventory of available animal rabies vaccines as compiled by the WHO Mediterranean Zoonoses Control Centre is given in Annex 5.23. The detailed list specifies necessary information such as
producing laboratory, type of vaccine, virus strain, immunity, duration of
immunity, required potency tests, etc. The list is not complete and is still
lacking some important vaccines from different countries.

In planning and implementing a control rabies control program, the
following factors need consideration in selecting the type of vaccine to be
used: (1) modified live virus (MLV) vaccine; (2) killed vaccine
to be vaccinated in addition to dogs; (3) whether vaccination will be done
predominantly by mass vaccination or by practitioners' surprises;
(4) provisions of local dog licensing authorities and control procedures;
(5) adequacy of storage and distribution systems.

(a) Established types of vaccines for dogs

For dogs two main types of vaccines are commonly used throughout the
world: modified live virus and inactivated virus vaccines.

(1) Modified live virus (MLV) vaccines

Most of the vaccines currently used for dogs contain the Flury strain
either as live or killed vaccine. The Eastern Equine Encephalitis (EEE)
strain and its derivatives EEA and VEEK or the Pasteur strain
derivatives such as the Challenge Virus (AVS) or Pasteur Mouse (PM)
strains. It must be borne in mind that if a virus is not entirely and
definitely inactivated by physical or chemical means it must be considered as
a live virus vaccine. Without doubt most of the so-called "phoshokilled" or
"Killing-type" vaccines still used in many countries are of this type. They
usually contain from 10^3 to 10^7 units of viable live virus.

- Flury MLV vaccines

The LEP strain is no longer grown in the MLV vaccine production,
although it is still in use for research.

The HEP strain can be produced from chick embryos inoculated at the 7th
day of egg incubation, harvested 14 days later and made into a 1% tissue
suspension. The average yield is 1-2 dog doses per harvested embryo. It
can also be produced from primary chick embryo fibroblasts or cell lines of
dog or human kidney origin. Monolayer cells are inoculated after 24-28
hours of culture and harvested 5-6 days later, the average yield being 1-4 dog
doses per 10ml of infected cell culture fluid.

* Detailed procedures of production of rabies vaccine can be found in
KAPLAN, M. M. and KOFROWSKI, K., ed. Laboratory techniques in rabies.
Series No. 23).
Production of lamb or kid brain vaccine is detailed in Annex 5.24
Research requirements in this field are summarized in Annex 5.27.
- SAD derived MLV vaccines

Two SAD derived strains are commonly used. The ERA strain virus cultivated in porcine, canine, bovine or hamster cells and used in many countries. The Vnukovo virus cultivated in hamster kidney cells is mainly used in the USSR.

These vaccines are usually produced in monolayer cell-cultures

(ii) Inactivated virus vaccines

These vaccines are obtained by complete inactivation of rabies virus grown either "in vivo" (nerve tissue) or "in vitro" (cell culture).

- Nerve tissue vaccines

Fixed strains are recommended for use in the production of vaccines, but also strains isolated from local epidemics may be considered.

Animals inoculated can be of various species: laboratory mice, guinea-pigs and rabbits as well as farm animals such as sheep, goats, cattle or even horses and asses. Very young animals, preferably new-born, should be preferably used: the neuro-allergenic factor of brain tissue is much lower in the newborn and the titres obtained from such brains are 10 to 1000 times higher than from adult animals. Average yield, in these cases, is for instance about 3000 dog doses per lamb brain and 5-10 dog doses per suckling mouse brain.

Inactivation of vaccine virus can theoretically be achieved by physical inactivation (heat, ultra-violet radiation) or more safely by chemical agents. For practical purposes, only chemical treatment by either betapropiolactone or the immines is reliable and results in complete inactivation of the virus with negligible degradation of the virus antigen.

- Cell culture vaccines

The virus strains used are either the Pasteur derived strains, PV, CVS, Pitman-Moore or the Flury strain viruses. From a theoretical point of view any other rabies strain of well documented passage history and defined antigen properties (see below) can be used provided the virus yields from cell culture are sufficient.

Most of the cells used for rabies virus propagation originated from hamster cell lines, but sometimes primary explants of chick embryo fibroblasts, porcine, bovine or canine kidney cells are used. Cultivation of rabies virus on micro-carriers (sephadex beads) is under development and could increase the virus yield from cell culture.

Inactivation is generally achieved by chemical agents (see Annex 5.24).

Rabies vaccines are often combined with other bacterial or viral antigens (e.g. distemper, panleucopenia, hepatitis, leptospirosis, etc.). Such polyvalent vaccines are acceptable provided each of their components, and particularly the rabies antigen, has been properly tested for potency.

Adjuvants (e.g. aluminium hydroxide) added to rabies vaccines proved very useful and should be recommended in order to increase the duration of immunity in dogs.
Advantages and disadvantages of different types of vaccines

Any vaccine that has been properly tested for safety, purity and potency and has been shipped, stored and administered according to instructions is capable of providing adequate immunity in the recommended species.

There are, however, factors that may favor the selection of one type of vaccine over another according to local conditions such as high temperature, lack of appropriate cold chain, storage, transportation facilities, etc.

(i) MLV vaccines

- Advantages

When properly produced and administered, MLV vaccines usually provide a long lasting immunity lasting without adjuvants and after a single injection.

MLV vaccines have proven superior in reducing the incidence of canine rabies to a low level in the United States (10 cases reported in 1973 versus 266 cases in 1953) and other countries. Several MLV products currently licensed have been shown to protect at least 60 percent of vaccinated dogs for at least 3 years after vaccination at 66% when challenged with sufficient virus and 100% of control dogs.

Many countries are still accustomed to the use of such vaccines and can produce MLV to satisfactory standards at a low cost, with local equipment and already trained personnel.

- Disadvantages

MLV vaccines share the general disadvantages of any live virus vaccine. They must be stored properly. Stabilizers added by various manufacturers differ; some vaccines in past years were shown to have had a significant loss of virus titer when stored in improperly functioning refrigerators. Recently however, revised stabilizer formulas have improved vaccine storage characteristics.

The MLV vaccines are lyophilized; reconstituting diluent must be added before use. Adventitious contaminants more readily survive with lyophilization than with inactivating procedures.

It is necessary that MLV vaccines be given only intramuscularly at one site in the thigh. Other intramuscular sites of administration are potentially unsafe and may result in clinical rabies. Subcutaneous vaccination has been shown to be less efficacious. Reported cases of suspected vaccine-induced rabies are to be investigated and monoclonal antibodies have been beneficial in differentiating vaccine-induced rabies from vaccine failures. The MLV vaccines are prone to the deleterious effects of improperly cleansed syringes and needles, or disinfectants applied to the site of injection.

In a very limited number of dogs and cats there is circumstantial evidence that severely immuno-suppressed animals may react to modified live virus vaccination in an aberrant manner. Consider postponing vaccination of dogs when high levels of steroids have been administered or vaccinating with an inactivated vaccine when leukemia is present.
(ii) **Inactivated virus vaccines**

- **Advantages**

Inactivated vaccines have advantages that are attractive. Vaccine-induced rabies is not a problem with properly inactivated products. Subcutaneous administration appears feasible when intramuscular injection is painful or difficult, such as in toy breeds. Intramuscular inoculation, however, remains the route of choice. Combining rabies antigens with other antigens is feasible after proof of non-interference has been established. Inactivated products for use in dogs do not present safety problems when administered to exotic species. Although accidental inoculation of MLV vaccines has not been shown to cause rabies in humans, less anxiety is produced if inadvertent injection with inactivated products occurs. Multi-dose vials of inactivated products can be stored after initial use and do not rapidly deteriorate if all doses are not administered within a short time.

- **Disadvantages**

Inactivated products also have disadvantages. Some products, particularly vaccines prepared from adult animal brain tissue, are of marginal potency. Inactivated products are not all free of stability problems when improperly stored. The production of potent inactivated vaccines is somewhat different from that of MLV-vaccines, requiring more sophisticated equipment and training.

Neuro-allergenic sequelae are more common in dogs when given inactivated products with a high proportion of neural tissue. This defect has been substantially eliminated in recent years with the introduction of products of cell culture or neonatal neural tissue origin.

(c) **Facilities needed for use of different types of vaccines**

Production facilities vary depending on whether the vaccine is of tissue culture origin or neurogenic origin. The first requires increased incubator space, glassware preparation area, and tissue culture production facilities. The second requires increased animal space.

Facility requirements will also vary for modified live and inactivated vaccines. Production of a live virus product will require a lyophilizer and increased freezer space for storage prior to lyophilization. Inactivated vaccines can be stored at 4°C prior to bottling.

**Handling of vaccines**

To be efficient, MLV vaccines (including "Fermi-type") have to be inoculated at a minimum titre specific for each strain used, for instance $10^2$ but not exceeding $10^{2.7}$ MICLD$_{50}$/$0.03$ ml for Fermi-type, $10^{3.3}$ for Flury and $10^4$ for ERA vaccines.

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*MICLD$_{50}$ = Mouse intra-cerebral lethal dose killing 50% of test animals.*
Vaccines below the required titre are unlikely to be efficient.
Therefore, great care must be taken to avoid decrease of this titre
particularly by thermal inactivation. Cold chain conditions must be ensured
gall along from release and control of each batch by the manufacturer until the
day of inoculation. Lyophilised vaccine must be used without delay (within
15 minutes) after dilution, and not kept at outside temperature or exposed to
sunlight.

IVM must be injected by the intramuscular route and never be mixed with
other products.

Inactivated virus vaccines are much more resistant to thermal
inactivation. Short accidental exposure to temperatures above 40°C does not
appear to destroy but can reduce their protective activity. They must,
therefore, be kept at low temperature (especially for liquid vaccines) but not
frozen.

Inactivated vaccines can usually be mixed with other inactivated (or even
dilute) vaccines and can be used either subcutaneously or intramuscularly. The
best route is recommended for vaccines without adjuvants.

(a) Cost of production at different volumes

The evaluation of the cost of vaccine production is difficult, in any
case, due to the variation of a number of parameters depending on:

the local conditions (price of basic supplies, laboratory material,
personnel expenditures, etc.). The production background (number of vaccine
batches produced, number of existing laboratories, depreciation of furniture and
equipment, etc.). Variations of these parameters can increase the cost of
production from 1 to 10. The cost of production, including overheads, of a
vaccine origin inactivated ovine vaccine in social list of 75 units is
the fourth has been estimated at 7.2 and 9.3 NS in 1970. The higher
cost for vaccine vials is a single use container. Although production
costs for other types of vaccine are not available, the market price of the
different vaccines are comparable, about 0.6 to 0.95 dollars/dose. This would
indicate that actual production costs are very similar.

In any case it appears advisable to ask national and international
companies for price estimates prior to the purchase of vaccine. The
corresponding offers will usually reflect the same type of vaccine, and
the lower prices usually offered will be in the range of 0.2 to 0.9 NS
per dose for inactivated vaccines, and from 0.2 to 0.5 NS dollars for MIV
vaccines.

As batch numbers increase, the production cost decreases. This is due
mainly to a decrease in the costs of quality control testing as it is spread
over more doses. A point to reach, however, when because of size, the
loss of a batch of vaccine due to an unsatisfactory test result becomes a
severe economic impact. If a high unsatisfactory test rate is anticipated,
then purchase of vaccines that has already been found satisfactory may be cost
effective.
(e) Stability

Stability is an important consideration in the selection of a vaccine to be used in a rabies control programme. Vaccines should be monitored for loss of potency during the storage period. The time between release and expiry date of a vaccine, sometimes referred to as its shelf-life, is usually not more than 6 months for liquid inactivated vaccines, but much longer, at least 18 to 24 months, for lyophilized inactivated or MLV vaccines.

It should be noted that MLV vaccines in particular, may be adversely affected by improper treatment during storage and shipment. The cold chain must be ensured from the moment the vaccine is released until it is used. This calls for special attention from and briefing of officials in various services concerned (i.e. airline and customs officials, freight agents, field agents).

5.5.2 Quality control

Quality control of rabies vaccines is of utmost importance in any programme of rabies control: vaccinations using uncontrolled vaccines should be avoided under all circumstances, because of the severe consequences of any false confidence in an animal's immunity.

No producer can assume that his product is safe and potent: every batch of rabies vaccine has to be controlled before release. This control comprises identification, safety, potency and stability of the vaccine.

(a) Control authorities

Quality control of rabies vaccines should be conducted at two levels: by the manufacturer and by a national control authority, e.g. the National Rabies Laboratory or National Veterinary Services Laboratory.

Tests required of the manufacturer (Annexes 5.25 and 5.26) comprise those on the master seed virus and serial lots of vaccine as well as those for viral and bacterial contaminants on all ingredients used in the vaccine or during production such as cells, nutrient serum, trypsin, etc.

It is considered beneficial for the quality control department of the manufacturer to be under different management from the production department.

Quality control conducted at the national laboratory should consist of tests on the master seed virus and selected tests on some batches of vaccine. The depth of testing conducted by the national laboratory is influenced by the degree of correlation present in results obtained from the manufacturer and the national laboratory. As the incidence of batches being determined satisfactory by the manufacturer (but unsatisfactory by the national laboratory) increases, the testing rate also increases.

Besides testing batches of vaccine at the time of manufacture, it is also the responsibility of the national laboratory to test batches of vaccine at the expiration date. This is accomplished by maintaining a repository of samples from each batch of vaccine produced. When a vaccine demonstrates a below-minimum result at the expiration date, the manufacturer is required to
shorten the period during which the vaccine can be marketed. This is accomplished by monitoring the potency at 6-month intervals and re-establishing the duration of acceptable potency. An alternative to shortening the dating is to increase the minimum required potency level at the time of manufacture.

Another function of the national laboratory is to supply the reagents necessary to conduct the various tests required of the manufacturers. Of primary importance is the reference vaccine used in the VN test. The reference for veterinary vaccines (as for example a CVS vaccine produced on Vero cells or suckling mouse brain) should be prepared, standardized and distributed by the national laboratory (see Section 5.6.2). The vaccine should be identified, and potency from batch to batch is controlled by the national lab to reconstitute the vaccine and by additional dilution if necessary.

(h) General procedures for quality control

Although many new methods of testing rabies vaccines have been proposed during the last decade, the main types of tests remained unaltered:

(i) Identification. This test is the same whatever the type of vaccine. It verifies that a vaccine protects animals (usually mice or guinea pigs) against challenge infection with a known rabies virus strain. In addition, vaccine virus strains should be identified by monoclonal antibodies.

(ii) Safety. Specific tests must be performed for different types of vaccine (see below). It is recommended that, particularly for VN, purity testing encompasses not only the major rabies virus but all the ingredients used in vaccine manufacture. Each new rabies vaccine must be tested for safety by direct inoculation of dogs at different dilutions.

(iii) Antigen testing. Many different tests are proposed for assessing the antigenic value of inactivated vaccines (see below). Apart from the routine test compulsory for each commercial batch, the immunogenic value, i.e. the ability to induce neutralizing antibodies in the target species, should be regularly assessed in dogs. This assessment will confirm the antigenic content of the vaccine. This analysis will be performed for each batch and for the production run to demonstrate the duration of immunity at least one year in the dog. The protective value, i.e. the ability to induce a resistance to challenge with a rabies virus, should be tested at least once for each new rabies vaccine. This challenge should be performed with a street rabies strain isolated from the country where the vaccine is to be used.

(iv) Vaccine virus strains and antigenic variants

The strains of virus presently used in veterinary rabies vaccines are derived from Challenge Virus Standard (CVS), Vinegar Voree (PV) Nogat-Alabama-Buffalo (SAD) and Glory Low and High Egg Passage (LEP and HEP) and Yellow viruses. These strains have proved effective in protecting against street virus exposures in dogs when potent vaccines have been properly administered. There is no proof of the existence of aberrant street virus strains against which these vaccine strains will not protect in most parts of the world.
The question of antigenic variation among rabies viruses from different parts of the world has been studied by monoclonal antibodies (see also Sections 1.1 and 5.3.7(b)) and is of two categories:

- **Virus strain variation.** The use of monoclonal anti-nucleocapsid and/or anti-glycoprotein antibodies makes it possible to show minor though significant differences between any two or more given virus strains under test. No differences in protection by established vaccine strains are noted.

- **Serotype variation.** This group of viruses has been shown by both types of monoclonal antibodies and by cross-protection tests to represent major antigenic variants of rabies virus being distinct serotypes.

The viruses of the latter group have been found so far to originate exclusively from the African continent from bats, cats and a dog. For Africa it may therefore be necessary to include local viruses in their vaccines, in order to protect dogs, cats, and possibly humans from exposure to aberrant field strains.

There are indications that viruses exist also in other parts of the world showing a low degree of serotypic variation. When assayed by cross-protection tests in mice, these animals proved to be not fully protected. The importance of these findings is not clear as yet. Do we need in such cases to include local virus strains in certain types of vaccines? Are failures seen in the post-exposure treatment of man due to antigenic variants or are they a result of late or inadequate vaccination? Carefully constructed experiments employing dogs and other animals, as well as variant strains for immunization and challenge should partially answer these questions.

(c) **Quality control for MLV vaccines**

Modified live virus vaccines are usually tested in respect of their identification, safety, and potency (see Annex 5.25).

(i) **Safety tests:** Several types of safety tests have been prescribed by national authorities for the various vaccines. Specific tests are described in the third edition of 'Laboratory Techniques in Rabies'. Contaminating agents (e.g. parvovirus) can be a problem in this type of vaccine, probably due to sera or trypsin used in vaccine manufacture, and must therefore be carefully controlled.

(ii) **Potency tests:** Standard potency tests for MLV vaccines and the general principles underlying them are described in Annex 5.25. It is recommended that samples from the field that are approaching their expiry date be tested again.

(d) **Quality control for inactivated vaccines**

Recommended control procedures dealing with identification, safety and potency test are described in Annex 5.26.

(i) **Safety tests:** Claims made that a vaccine contains only inactivated virus should be substantiated by adequate tests for residual live virus: each vaccine batch must be shown to be free of living agents including residual rabies virus, regardless of whether other safety requirements are followed.
(ii) Potency tests: A variety of potency tests are now available (see Annex 5.26) among which the Habel and NIH test are still the most widely used tests. Among other tests proposed, only the antibody-binding test (measuring the capacity of a vaccine to bind a constant amount of virus-neutralizing antibody) and the antibody assay in immunized mice (measuring the antibody levels in mice as induced by serial dilutions of the vaccine) have proved to be of practical value for the assay of the antigenic value of inactivated vaccines. When carried out in cell culture the antibody-binding test is a fast (overnight) and reproducible procedure and is highly recommended for use by the manufacturer as a during-process control method, and for quick re-evaluations of vaccines before expiration.

5.5.3 Distribution system

Rabies vaccines for animal use, either live or inactivated, should never be freely available for sale, but should be distributed by and to veterinarians with possible control at a national level. This system would allow general surveillance of quantities and quality of the vaccine distributed all over the country.

The vaccines, either manufactured in the country or imported, must be stored according to general recommendations, particularly by maintaining the cold chain during storage and further shipment. The stock must be regularly monitored in order to avoid delivery of batches after or near the date of expiry. This can be easily achieved if a national 'Drug Store' exists or if manufacturers or importers take this responsibility.

The circumstances of vaccination (date, type of vaccine, batch number, etc.) need to be filed in standard registers irrespective of whether the vaccine is applied to individual animals or used during mass campaigns. This may facilitate a retrospective evaluation of possible vaccine failures.

5.5.4 Responsibility for supply

Supply of vaccine is often one of the thorny points during mass vaccination campaigns. Roughly, two main sources of vaccine supply exist:

- vaccines manufactured in the country
- vaccines imported from foreign countries.

In both instances the national government should always have a direct control upon the quality and quantities of rabies vaccine, in order to organize rabies control at a national level.

As far as animal vaccine is concerned, the responsibility should be with the Ministry of Agriculture having

- legislation to control the production or importation of vaccines used throughout the country
- practical facilities for the implementation of quality control of these vaccines.
5.5.5 Training of personnel making vaccine

Senior staff personnel having the responsibility for producing rabies vaccine should be qualified by education and experience, preferably at university level, with major fields of study in veterinary medicine, microbiology and immunology. All personnel employed in the preparation of biological products should be competent in good laboratory techniques through education or training so as to consistently prepare high quality products.

One major difficulty in manufacturing rabies vaccines is the training of personnel. Actually most of the producers are located in developed countries, where this responsibility lies upon private companies. These are often reluctant to train people or to teach them technical procedures (except if a specific financial contract is foreseen), particularly for licensed vaccines. Therefore, in most of these cases, the training of personnel must be planned via bi- or multilateral cooperation contracts between governments where state laboratories produce rabies vaccines. This training can be achieved either by visits of people in charge of production, or by consultation with experts in their country, or by both.

The training and transfer of technical assistants to work on seed viruses, cell lines, formulas for stabilizers, etc. should also be accomplished in collaboration with rabies research laboratories or WHO Collaborating Centres. Researchers from these laboratories are prepared to work closely with institutions intended for vaccine production by giving guidelines, arranging for training courses and, most efficiently, by on-the-spot training. Once started, these researchers should be contracted to control vaccine production during the next years and to serve as consultants when problems arise.

5.5.6 Sources of supply for local vaccine production

The decision to produce a certain type of vaccine locally is much influenced by the necessary laboratory space, equipment and personnel as well as by the availability of supplies in the country. In general, inactivated brain tissue vaccines are easier to produce with local supplies than are high quality cell culture products.

(a) Brain tissue vaccines

The main source of virus is nerve tissue collected from young infected animals: mice, rats, rabbit, sheep, goats or even cattle or horses.

Any of these sources are convenient provided that a sufficiently high titre of virus is obtained, i.e., at least \(10^7\) mouse LD50 per gram of neural tissues. Lower titres are acceptable for "Fermi type" vaccines since the minimum required residual virulence (\(10^2\) but not exceeding \(10^2.7\) MICLD50) is far below the expected titres from brain tissue whatever the age of the animals.

The choice of animal species to be used depends upon their availability in the country in sufficient number and quality, and at the required times for vaccine production. For instance suckling mice can be obtained throughout the year whereas lamb brains are available only in the spring. About 300 to 600 suckling mouse brains are necessary to equate to the yield of one lamb's brain.
In addition, maintaining a mouse colony of pregnant females that will provide suckling mice in large numbers on a regular basis for suckling mouse brain vaccine is a complex undertaking that requires highly trained and skilled personnel and strict adherence to rigorous isolation procedures.

Stock colonies should be free at least from LCM and ectromelia, however, other endogenous viruses such as Sendai, mouse hepatitis and others may impose occasional but serious problems.

(b) Vaccines from cell cultures

An easy method of obtaining tissues for cell culture use is to arrange with a local abattoir to supply the desired organs, pack them in ice, and deliver them to the laboratory by the most expeditious means. This approach, however, can lead to difficulties. Porcine kidney has probably been the source of porcine parvovirus and rotavirus contamination. Bovine kidney is known to be the source of BVD, Pi-3, reovirus, and bovine herpesvirus (e.g. 18K) contamination.

If feasible, the use of cell lines that have been tested for the presence of extraneous agents (and found clean) is much preferable to using primary tissues. Research laboratories, WHO Collaborating Centres and the American Type Culture Collection are able to supply starter cultures of baby hamster kidney, porcine kidney, bovine kidney, and similar cell lines that have proved useful for vaccine production.

**Culture media, nutrient serum and trypsin for cell culture**

Cell culture media and other ingredients may either be imported (autoclavable stock media are available as dry powder) or prepared from biological or chemical products manufactured in the country.

For MIV vaccines care must be taken that contaminants or even pathogens are not introduced via biological sources.

Bovine viral diarrhea (BVD) virus and mycoplasma sp. are two contaminants that are frequently encountered in volume vaccine production using cell cultures. Strict attention must be paid to how these vaccine ingredients are harvested and pooled. It is suggested that trypsin of porcine origin be examined using the procedure of Craigie, et al., *Applied Microbiology* 26: 431-433, Sept. 1973) to detect any extraneous paroviruses. A dependable continuing source of BVD-free foetal bovine serum is a very difficult thing to locate.

5.5.7 Assistance by WHO Collaborating Centres

The necessity of support through WHO, and WHO Collaborating Centres in the field of training, advice and technical assistance has repeatedly been emphasized in this chapter.

For the control of inactivated vaccines (locally produced or imported) WHO does provide every country with the "3rd International Reference Preparation to be used in the NIH test". This will allow the expression of test results in "International Units" which is useful for the biological standardization of rabies vaccines throughout the world. In order to comply with the amount of reference vaccine required for daily routine use, each country is advised to prepare its own reference stock and to standardize it against the international reference preparation.
Similarly, an "International Reference Serum" for the titration of sera of vaccinated animals is available on request.

Seed virus stock for vaccine production (fixed strains) can be obtained through WHO Collaborating Centres which can also assist in the identification either of already used strains or of candidate strains isolated from the country since this strain may be more immunogenic against infection by indigenous street viruses.

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