Onderstepoort J. vet. Res., 54, 461–471 (1987)

REARING AND INFECTION TECHNIQUES FOR *AMBLYOMMA* SPECIES TO BE USED IN HEARTWATER TRANSMISSION EXPERIMENTS

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ABSTRACT

HEYNE, HELOISE, ELLIOTT, E. G. R. & BEZUIDENHOUT, J. D., 1987. Rearing and infection techniques for *Amblyomma* species to be used in heartwater transmission experiments. *Onderstepoort Journal of Veterinary Research*, 54, 461–471 (1987).

Techniques for the rearing of *Amblyomma* species to be used in heartwater *(Cowdria ruminantium)* transmission experiments are discussed. These involve the breeding and maintenance of infected and non-infected strains of ticks. They include the feeding of ticks on sheep, rabbits, mice, tortoises and guinea fowl.

INTRODUCTION

All proven vectors of heartwater *(Cowdria ruminantium)* are 3-host ticks belonging to the genus *Amblyomma* (Uilenberg, 1983a; Camus & Barré, 1982; Bezuidenhout, 1987). Except for 1 report on transovarial transmission (Bezuidenhout & Jacobsz, 1986), transmission is transstadial.

During heartwater research, infected as well as uninfected ticks are needed to study the mode of transmission, morphology, distribution and life-cycle of the organism in the tick. Infected ticks are also needed for the preparation of antigen for serological tests, tissue culture inoculations and for the production of a vaccine made from ticks infected with *C. ruminantium* (Bezuidenhout, 1981).

Rearing techniques are therefore aimed at supplying uninfected ticks as well as ticks with a high rate of infection. These techniques may also be used to determine the disease transmission status of laboratory and wild hosts such as mice, tortoises and birds, e.g. guinea fowl. In this paper the various methods and hosts used to feed, infect, maintain, collect, and record ticks, especially *A. hebraeum* during heartwater experiments, are briefly discussed. It is not intended to compare the suitability of the various published methods, but rather to present the standard and special techniques used at the Veterinary Research Institute, Onderstepoort.

Robinson (1926) gave detailed descriptions of the 10 *Amblyomma* life cycles that had been determined at that time, including information on the behaviour of these species in the laboratory. Subsequently Enigk & Grittner (1953) gave a detailed review which covers most aspects of tick rearing and the maintenance of hard as well as soft ticks. Gregson (1964) also reviewed problems pertaining to tick-rearing and maintenance.

ESTABLISHMENT OF A HEARTWATER-FREE TICK COLONY

To establish a colony, fully engorged *A. hebraeum* females are collected from animals at an abattoir or in the field and placed in glass vials for egg-laying as described below. The larvae and subsequent stages are fed on heartwater-susceptible sheep. During this period rectal temperatures, blood smears and the clinical signs of the sheep are monitored daily to establish the possible presence of any pathogenic organisms. At the same time the haemolymph of unfed adult ticks is also screened, according to the method of Burgdorfer (1970), for the presence of rickettsiae that may interfere with further studies.

After 1 or 2 generations, when enough ticks are available, heartwater experiments can commence. Care must be taken to save sufficient uninfected ticks to maintain the strain.

Received 14 May 1987—Editor

REARING OF UNINFECTED TICKS

Standard laboratory hosts

Backs of sheep

All stages of development of uninfected ticks may be fed on sheep that have never been in contact with heartwater-infective material or ticks. The wool on the back of the sheep is shaved with an electric hairclipper and then with a razor to ensure a smooth, hairless surface.

Three or more calico bags (Fig. 1), each 200 mm long with a 35 mm diameter suede ring attached around the 280 mm circumference of the bottom of the bag, are glued to this shaven area. A contact adhesive, e.g. "Genkem"[1], is applied to the bottom of the suede ring and to the sheep's back. When the glue is finger-dry the suede ring is pressed onto the sticky area on the sheep's back. It is then left to dry for at least 24 h before ticks are placed in the bag. This allows volatile solvents to dissipate and the glue to harden properly.

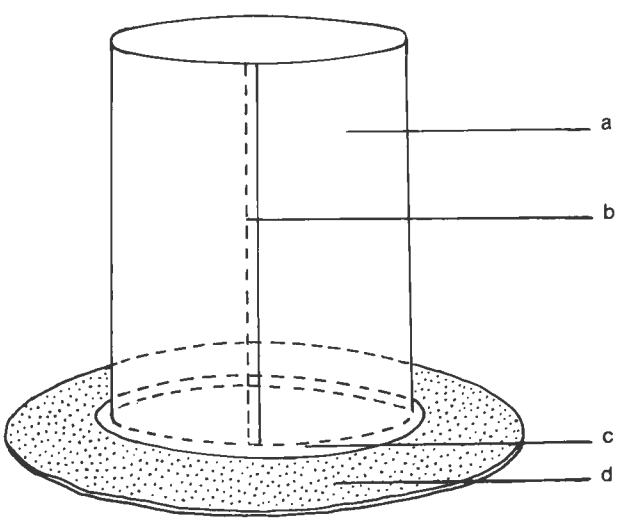

FIG. 1 Diagram of a calico bag for use on the backs of sheep:
(a) 200 mm × 280 mm calico cloth stitched to form a tube,
(b) stitching,
(c) stitching onto suede ring,
(d) 35 mm diameter suede ring

A minimum of 2 weeks should be allowed after ticks have hatched or moulted before they are fed. This is necessary to ensure proper hardening of the mouthparts. In the case of adults, equal numbers of males and females (100 ♂♂ + 100 ♀♀) are usually used to infest sheep. It is inadvisable to feed very large numbers of ticks in confined spaces as their close proximity may cause intense haemorrhagic conditions (Arthur, 1970). It

[1] Genkem: registered trade name of General Chemical Corporation.

is important to remember that most *Amblyomma* females will not attach and feed in the absence of males (Lounsbury, 1899). Females will only attach and commence feeding when pheromone-producing males are present. Pheromone secretion takes place 4–8 days after attachment of the males (Rechav, Parolis, Whitehead & Knight, 1977). Uilenberg (1983b), however, found that it was not necessary to feed *A. cajennense* males before females. Stoppered vials containing the ticks are placed in the bags, which are then sealed with rubber castration rings using the special ring applicator (Fig. 2). Without opening the bags the vial stoppers, consisting of cotton wool covered with fine gauze, are removed to liberate the ticks. Two days later, once the ticks have attached, the bags are opened and the stoppers and vials removed.

FIG. 2 Calico bags fitted to the back of a sheep being sealed by means of the ring applicator

Other body methods on sheep

The mass-rearing device for calves described by Samish (1982) can be adapted to suit sheep. In other methods that have been described ticks may be confined on the ears, legs, or dorsal surfaces of the trunk of sheep and goats under canvas (calico bags and covers) (Lounsbury, 1900, 1904; MacKenzie & Norval, 1980) or in ear bags (Irvin & Brocklesby, 1970).

Cattle

Ticks are confined in linen bags using the same method described for sheep. Whole body rearing of adults is impractical without specially designed cages to contain the released ticks. Cattle may be stanchioned using rearing cages similar to those described by Sutherst, Wharton & Utech (1978) and Samish (1982).

Ears of rabbits

Rabbits are often used as alternative hosts on which to feed the immature stages. A maximum of 1 000 larvae or 500 nymphae are fed on a medium-sized rabbit (3–3,5 kg).

Ticks may be confined to individual ears or both ears may be kept in a single ear-bag (Fig. 3). Linen bags are used and are fixed to the skin around the base of the ears with "Elastoplast" (Bailey, 1960), Unna's paste (Hadani, Cwilich, Rechav & Dinur, 1969) or "Genkem" contact adhesive. Details regarding the size and shape of bags, and methods to introduce and collect ticks, have been described by Neitz (1937) and Bailey (1960). To prevent the rabbits scratching off or damaging the earbags various collars have been designed (Bailey, 1960; Irvin & Brocklesby, 1970; Smith, Goulding & Priano, 1970; Watts, Pound & Oliver, 1972; Srivastava & Sharma, 1977).

FIG. 3 A rabbit fitted with a closed single linen ear bag enclosing both ears

Backs of rabbits

A circular plastic container with a lid can be modified to feed ticks on the backs of rabbits (Fig. 4). The bottom of the plastic container is cut off and the base of the container is then shaped to fit over the rabbit's back. A ring of suede is glued and stapled onto the shaped edge. For ventilation purposes pieces of the lid and container are cut out and replaced with pieces of linen glued on the outside.

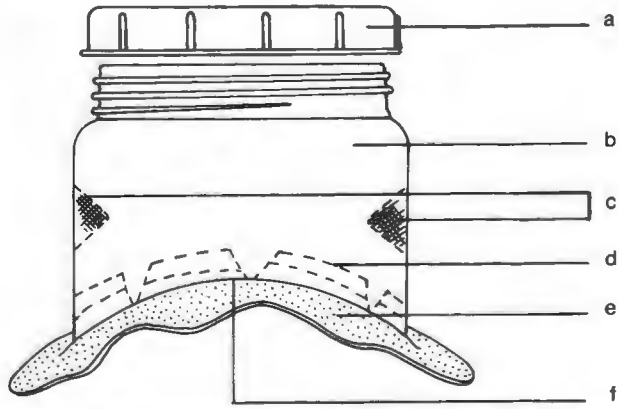

FIG. 4 The adaptation of round plastic containers for tick feeding on rabbits:
(a) lid with linen covered ventilation holes,
(b) 90 mm diameter screw top plastic container,
(c) linen covered ventilation holes,
(d) glued and stapled connection to suede,
(e) notched suede ring,
(f) shaped bottom edge of container

An area on the back of the rabbit, corresponding to the outside diameter of the suede ring, is shaved clean. The container is then glued onto the rabbit's back, using a contact adhesive such as "Genkem", as described above for fixing bags on the backs of sheep (Fig. 5). The glass vial containing the ticks is held inside the container, the stopper is removed and both are left inside. The lid of the container is screwed on and sealed with masking tape, particularly when larvae are involved. The vial and stopper are removed after 2 days.

Other types of containers for the confined rearing of ticks on rabbits have been described (Jellison & Philip, 1933; Sacktor, Hutchinson & Granett, 1948; Rau, 1965). A method described by Theis (1968a) using

FIG. 5 A rabbit fitted with a plastic container, ready for tick feeding

FIG. 6 A mouse fitted with a 'Velcro corset'

transparent chambers on the necks of dogs can also be used. Smith *et al.* (1970) sutured containers to the backs of rabbits.

Whole body methods on rabbits

Whole body and mass rearing techniques using rabbits are also in use in some laboratories (Morton, 1929; Patrick & Hair, 1975). The method described by Samish (1982) may prove to be suitable for the mass rearing of multi-host ticks on rabbits. Loomis (1961) used the Berlese funnel for mass rearing.

Non-standard hosts

Mice

A number of strains of *C. ruminantium* that are pathogenic for mice have recently been isolated. These are the Kümm strain (Du Plessis & Kümm, 1971), Kwanyanga strain (MacKenzie & Van Rooyen, 1981) and the Nonile strain (MacKenzie & McHardy, 1984). Mice provide an inexpensive system to study the transmission of these organisms, to determine the percentage of infected ticks and to serve as models for chemotherapeutic treatment. Suitable techniques which allow ticks, especially individual adults, to feed on mice would therefore greatly facilitate such studies.

Certain techniques have been developed for the feeding of ticks on mice and other small laboratory animals. Since mice groom and effectively rid themselves of any parasites, including ticks, it is necessary to restrain them in some way.

Eichenberger (1970) described a method using light aluminium haircurlers or cylinders made from a multi-perforated light metal sheet to restrict the animals. Aluminium haircurlers, however, are nowadays difficult to obtain and the authors have replaced them with Velcro[2] corsets.

Velcro adhesive material consists of 2 finely toothed nylon surfaces which adhere firmly when pressed together. Prior to the application of the corset, the mouse is lightly anaesthetized using ether. Two pieces of Velcro 25 mm wide are used to encase the mouse's body between its front and back legs. One piece 45 mm long with the soft under-surface passes over the animal's back. Another strip, 55 mm long and cut from the spiny counterpart of the material, fits underneath the mouse's belly and around most of its body (Fig. 6). The ends are firmly pressed together and stapled close to the body using a mini stapler. The spiny surface of the strip around the belly to a great extent prevents slipping, while the soft piece prevents unnecessary irritation of the spine. Skill and experience in anaesthetizing and corsetting the mice are necessary to ensure successful application.

[2] Velcro: registered trade name

Female mice, 6–8 weeks old, are more suitable than males because they are less aggressive, less active and do not smell so strongly.

Ticks in small groups are either released on the anaesthetized mouse or, in the case of adults, placed underneath the corset.

A small plastic collar may also be applied to restrain the animal even further from any effective grooming (Fig. 7).

FIG. 7 The small plastic collar around the neck of a mouse which to a large degree prevents grooming

Handling and care of the mice during the feeding of ticks is done according to the method of Eichenberger (1970).

A method of recovering ticks from small laboratory or field-caught rodents has been described (Kaiser & Hoogstraal, 1968). The method published by Hadani *et al.* (1969) may also prove to be applicable in the case of mice. Irvin & Brocklesby (1970) published a method of feeding *Rhipicephalus appendiculatus* on mice which could be used for feeding *Amblyomma* ticks.

Birds

Immatures of many *Amblyomma* species feed on birds (Hoogstraal & Aeschlimann, 1982; Horak & Williams, 1986). In order to study host susceptibility and transmission of *C. ruminantium* to and from birds it is important to have methods for feeding ticks on them.

The authors worked mainly with helmeted guinea fowl *(Numida meleagris)* but other birds could probably be used in a similar way. Only immatures of *A. hebraeum* have so far been fed on guinea fowl.

Birds are kept individually in cages fitted with a wire mesh floor. These cages are placed or suspended either over a tray of water or over a funnel of water in a specially designed tick collecting apparatus (Fig. 8 & 9).

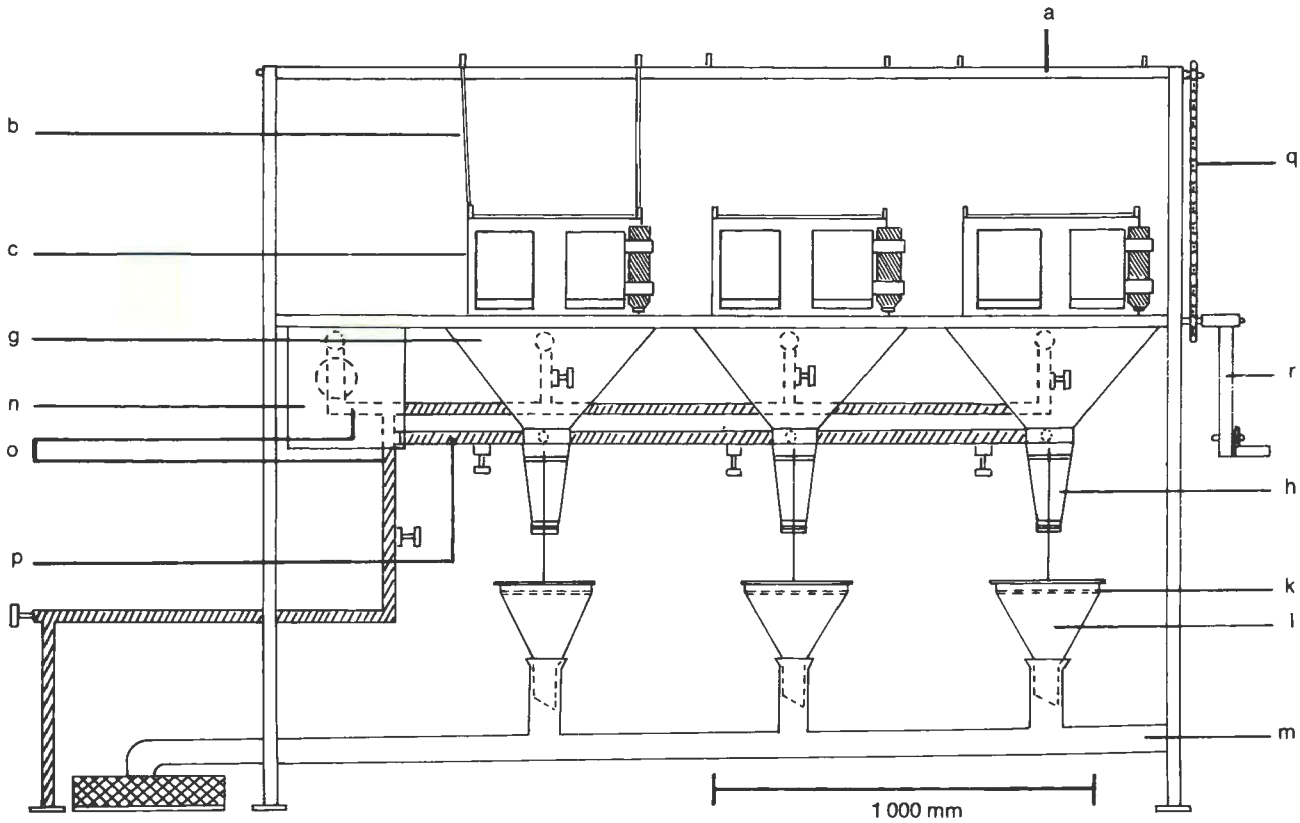

FIG. 8 A tick-feeding apparatus designed for the free infestation of ticks on hosts:
(a) rotating axle,
(b) nylon rope,
(c) animal holding cage,
(g) metal funnel,
(h) perspex funnel,
(k) fine nylon sieve,
(l) plastic funnel,
(m) drainage system,
(n) water reservoir,
(o) mains water supply,
(p) water supply to metal funnel (g),
(q) bicycle chain,
(r) handle to rotate axle (a)

A glass vial containing ticks is glued onto the bird's back. A period of approximately 3 h is allowed for the glue to dry, then the stopper is removed to release the ticks onto the host. Temporary immobilization of the birds may be necessary. This can be done by taping the wings cross-wise using broad linen tape (25 mm wide). The empty vial is removed a day later.

The water in the collecting tray is replaced once or twice a day. When the engorged ticks start to drop they are either removed by hand from the water tray, or, when the tick collecting apparatus is used, accumulated by allowing the water to run through a nylon sieve placed inside a large plastic funnel. The ticks concentrated on the sieve are then collected and thoroughly dried on filter paper.

Other methods for feeding ticks are also available (Hadani *et al.*, 1969). Loomis (1961) refers to a method described in 1953 for feeding Argasidae on chickens. Lancaster (1955) debeaked chickens prior to feeding ticks on them.

Tortoises

It has been established that certain tortoises may act as subclinical carriers of heartwater organisms under laboratory conditions (J. D. Bezuidenhout & J. A. Olivier, unpublished results, 1985). It has also been demonstrated that the tortoise tick, *A. marmoreum*, may act as a vector of the disease (J. D. Bezuidenhout, unpublished results, 1986). To establish a colony of *A. marmoreum* for these studies and to maintain the strain, the following method was used:

Leopard tortoises *(Geochelone pardalis)* were kept in an enclosure as specified by the Nature Conservation authorities. The enclosure is surrounded by a brick wall, 600 mm high, with a wire mesh fence, 900 mm high, on top of it. Lawn and Buffalo grass provide natural surroundings and shade.

Immature ticks: A vial containing 10 000 larvae or 100 nymphae was stuck to the top of the tortoise's shell with "Prestik"[3] and the stopper was removed to liberate the ticks. Ticks attached to the head, neck, tail and legs. Nine days later the tortoise was transferred to a cage with a grid floor, suspended over a collecting tray. The tray edges were taped with double-sided tape[4] to prevent the ticks escaping, and the ticks which detached were collected.

Adult ticks: Not more than 10 females plus 10 males were placed under the shell on the hind-part of the tortoise. Fully engorged females were pulled off before they detached of their own accord.

TICK COLLECTION TECHNIQUES

Immatures

A modified vacuum cleaner, normally used on the interior of motor vehicles, is used to aspirate the detached ticks from the inside of the calico bags. Great care should be taken that no ticks from a previous collection

[3] Prestik, registered trade name of Bostik

[4] Tesa: registered trade name

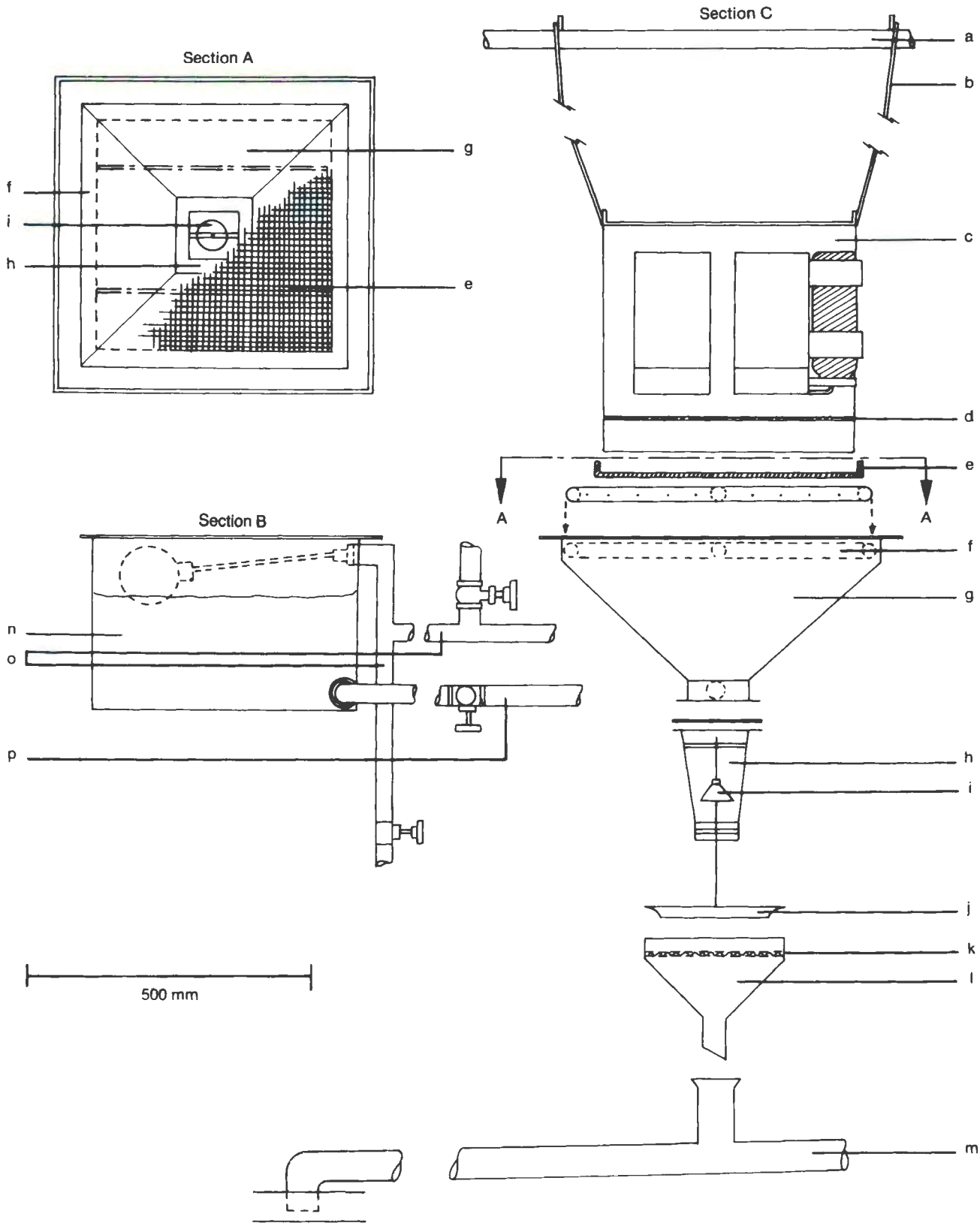

FIG. 9 Detailed aspects of Fig. 8 illustrating: A overhead view of grid for collecting faeces from the hosts plus the metal funnel under it; B the water reservoir; and C the cage and water drainage assembly:

(a) rotating axle to hoist animal holding cages,
(b) nylon rope,
(c) animal holding cage with food hopper and water bottle,
(d) grid floor of cage,
(e) faeces collecting grid,
(f) pipe with small holes fixed in upper part of metal funnel (g) and connected to mains water supply (o) to wash down sides of metal funnel during tick collection,
(g) metal funnel,
(h) perspex funnel where most ticks accumulate between collection periods,
(i) modified basin plug,
(j) metal handle to release water through (g) and (h),
(k) fine nylon sieve on which ticks collect when water flows through (l),
(l) plastic funnel,
(m) drainage system,
(n) water reservoir to maintain constant water level in (g),
(o) mains water supply,
(p) water supply to (g)

FIG. 10 Sealed glass aquarium tanks containing salt solutions and plastic holders for the storage of non-parasitic stages under optimum conditions

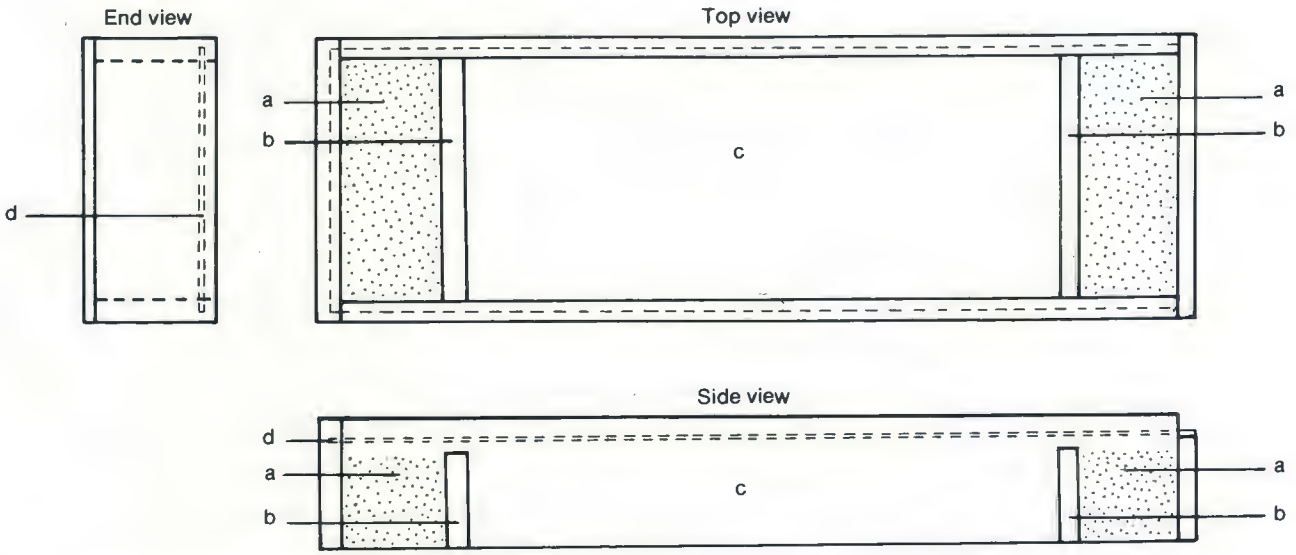

FIG. 11 Perspex containers designed for high humidity holding of non-parasitic stages;
(a) side compartments filled with moistened cotton wool,
(b) division slightly lower than container to allow humidity flow,
(c) compartment where tick vials are kept,
(d) sliding lid

remain in the collecting chamber or nozzle of the apparatus so as to prevent accidental contamination with other species or strains.

Adults

Adults are usually collected from the bags by hand or, if ticks are needed in the prefed stage, are carefully removed using fine curved forceps. The mouthparts of the tick are grasped firmly, as close to the animal's skin as possible, and gently pulled away (Theis, 1968b).

After detachment from the host, ticks sometimes die as a result of a condition similar to the "blackening" syndrome described by Hendry & Rechav (1981). Various bacteria are suspected of being pathogenic to ticks. The manner in which ticks are collected could possibly also be responsible for these deaths. Collection of detached ticks from the urine and faeces of an animal or the washing of ticks with a strong jet of water could be predisposing factors leading to this syndrome (Sutherst *et al.*, 1978).

REARING MANAGEMENT

Storage

After collecting the various developmental stages of ticks from animals they are separated from tick excreta and other debris by using a fine sieve. If ticks are contaminated with blood or other animal fluid exuding from feeding wounds they are carefully washed in running tap water and allowed to dry thoroughly on filter paper.

Depending on the developmental stage collected, a variety of containers are used to store ticks during oviposition and moulting. Larvae are kept in glass vials (20 mm inner diameter × 50 mm long). No more than a third of the vial must be filled with ticks. These vials are plugged with tight-fitting absorbent cotton wool stoppers covered with fine gauze. Nymphae can either be stored in a similar way to the larvae or in a Petri dish (150 mm diameter), the bottom half of which is layered with 2 discs of filter paper. In this case the upper and lower halves of the Petri dish are sealed together with masking

TICK BREEDING

V.R.I. Onderstepoort — Entomology

Species ..... **Origin** ..... **Ref.** ..... **Group no.** .....

No.		Larvae				Nymphae				Adults					Remarks
	F No.	Host No.	Feeding Start	Feeding End	Date Moult	Host No.	Feeding Start	Feeding End	Date Moult	Host No.	Feeding Start	Feeding End	Date Ovip.	Date Hatch	

FIG. 12 The standard record form used in tick breeding at the Veterinary Research Institute

tape. Moulted adults are sorted into equal numbers of males and females, usually in batches of 25 each, and put into separate glass vials to facilitate the infestation of animals with males and females separately. Sorting of these ticks may be done when they are confined either in a Petri dish, or on a flat surface delimited by means of double-sided tape or, according to the method of Wilkinson (1964), using blackboard chalk and applicator sticks. Ticks can be chilled in a refrigerator for a short time and are then easier to handle. Engorged females kept for egg production are either stored in groups in Petri dishes as described above or separately in glass vials. In the case of the engorged females kept in Petri dishes, eggs are collected after oviposition, mixed to ensure heterogenous populations, and put into glass vials. Three to 4 glass vials, containing either eggs, larvae, nymphae or adults, are placed in a larger glass test tube (30 mm inner diameter × 205 mm long) which is plugged with a cotton wool stopper as an insurance against the ticks escaping. These large test tubes are kept in plastic holders[5]. These holders are available in a variety of colours, which greatly facilitates coding. The importance of labelling all vials, test-tubes and containers cannot be over-emphasized.

Temperature and humidity requirements

The non-parasitic stages of ticks are kept in either a temperature-controlled room or an incubator at 26 °C (± 1 °C). Breeding rooms should preferably be used only for tick rearing and storage, be free from draughts, and away from direct sunlight or extremes of temperature. The use of insecticides must be avoided (Bailey, 1960). A relative humidity of 75 % (± 5 %) is created either by means of an atomizer-type humidifier or through saturated solutions of sodium chloride (Peterson, 1949). We prefer the system of holding ticks in sealed aquarium tanks containing sodium chloride (Fig. 10) in an airconditioned room, because it almost completely eliminates the problem of too high or low humidity and run-away temperatures. We also prefer to use sodium chloride because it is readily available and relatively inexpensive, and the RH is stable over a wide range of temperatures (Winston & Bates, 1960; Young, 1967). Other authors use calcium phosphate (Patrick & Hair, 1975); sodium carbonate (Smith, 1975); and potassium hydroxide (Norval, 1977). Various other methods for providing humidity have been used: damp sand in metal containers (Bailey, 1960; Loomis, 1961; Walker & Parsons, 1964) and Plaster of Paris (Lancaster, 1952, 1953, cited in Lancaster, 1955). Malan & Heyne (unpublished data, 1974) used special Perspex holding chambers, (230 mm × 75 mm × 35 mm high) with a compartment slightly lower than the container at each end to allow for the free movement of humidity (Fig. 11). Absorbent cotton wool swabs are placed in these compartments and moistened with sterile water. The cotton wool is replaced weekly with fresh damp cotton wool swabs. The whole length of the container is sealed off with a sliding lid.

Fungus growth in storage containers is a problem under certain conditions, especially when the humidity is high and ventilation poor. This condition may be improved by keeping fewer ticks per container or using glass tubes stoppered at both ends. Ticks should always be dry before they are stored and it is advisable to use clean filter paper to absorb any body fluids that they excrete during egg laying and hatching. Ticks can also be washed in a solution containing an antifungal preparation, such as amphotericin B (5 $\mu g/m\ell$), to prevent fungal growth.

Record-keeping

Animals: In any study of heartwater it is of the utmost importance to know the immune status of the experimental animals. Records of their origin, use in previous experiments, and treatments should therefore be available before commencing any heartwater studies.

After inoculating infective material, or during the feeding of ticks, daily monitoring and recording of rectal temperatures is necessary. Any abnormal clinical signs as well as treatments, collection of blood, etc., are recorded.

Necropsies are done on all animals that may die and necropsy records are kept.

Ticks: Where possible, or necessary, the number of ticks that attached and the number that dropped are recorded.

Detailed records of ticks during and after feeding, moulting and oviposition are also kept using a standard tick record form (Fig. 12).

A knowledge of the life-cycles of the various tick species under experimentation assists in the planning and execution of experiments related to tick-feeding. Table 1 summarizes the life-cycles of some of the *Amblyomma* species known to be carriers of *C. ruminantium*.

The use of computers in the maintenance of breeding records is rapidly gaining acceptance and will no doubt play a major role in future laboratory administration.

HEARTWATER EXPERIMENTATION WITH TICKS

Infection of ticks with C. ruminantium *on sheep*

A susceptible Merino or Dorper sheep is injected intravenously with 5 $m\ell$ of heartwater blood vaccine of

[5] Addis cutlery drainer: Registered trade mark.

TABLE 1 A summary of the life cycles of various *Amblyomma* species according to the authors indicated. Periods are given in days; m = mean

Species	Host	LL hatch	LL feed	LL moult	NN feed	NN moult	♀♀ feed	Pre-ovip.	Oviposition	Reference
A. cajennense American str.	—	37–154	2–7	10–(?)	3–13	12–105	7–12	9–22	19,7 m	Diamant & Strickland, 1965
A. cajennense Trinidad str.	Various	32–43	3–6	8–19	4–7	14–18	12–14	7–13		Smith, 1975
A. gemma	Various	69	5	17	6	28	12	12		Theiler, Walker & Wiley, 1956
A. hebraeum	Cattle	77–180	4–9	30–90	4–8	18–77	6–10	14–77	21–63	Lounsbury, 1899
A. hebraeum	—	70–180	4–20	25–120	4–20	18–160	7–20	14–90		Diamant & Strickland, 1965
A. hebraeum	Various	54–61	4–15	14–25	6–9	20–29	6–12	10–14	38	Norval, 1974
A. maculatum	—	21–142	2–10	7–121	4–11	17–71	5–18	3–9	13–75	Diamant & Strickland, 1965
A. marmoreum	Sheep	37	6–12	2–3	8–20	21–28	?	12–15	30	Norval, 1975
A. marmoreum	Tortoise	37	30	2–3	51	21–28	60	12–15	30	Norval, 1975
A. sparsum	Sheep	48–54	5–10	13–14	6–11	26–30	23–36	19–22		Walker & Parsons, 1964
A. sparsum	Tortoise	47–57	10	11–20	9	33	62	22–47		Walker & Parsons, 1964
A. tholloni	Sheep	50–59	6,10m	16–28	5,42m	23–28	8–18	14–29	24–44	Norval, Colborne, Tannock & MacKenzie, 1980
A. tholloni	Rabbit		6,69m	16–28	6,29m	23–28				Norval *et al.*, 1980
A. variegatum	Various	38–45	6–8	12–30	3–8	15–30	6–12	5–14		Ilemobade & Mohammed, 1978

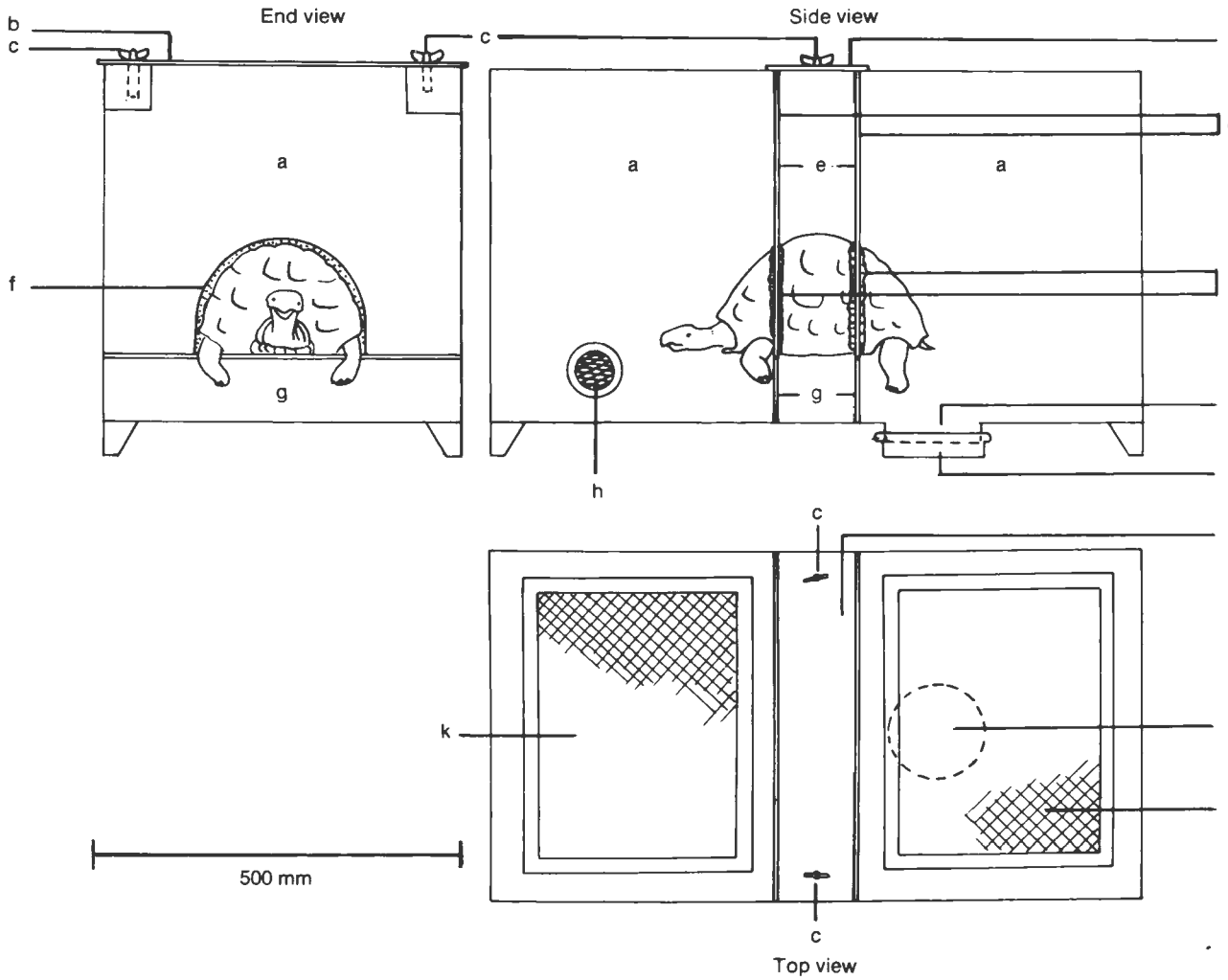

FIG. 13 A specially designed perspex cage for the isolated feeding of ticks on tortoises,

(a) cage,
(b) perspex portion to hold down perspex central sections,
(c) wingnut to secure (b),
(d) perspex central sections to separate hind and fore portions of the tortoise creating a "no man's land",
(e) "no man's land",
(f) plasticine seals around tortoise ensuring that no ticks migrate,
(g) platform,
(h) ventilation hole covered with nylon mesh,
(i) hole in cage floor for faeces,
(j) container for faeces,
(k) removeable mesh covered lids for access

known infectivity. Four days later the wool on the back of the sheep is shaved and bags applied using the technique previously described. Vials containing approximately 15 000 *A. hebraeum* larvae or 2 000 nymphae are confined in separate bags on the 5th, 6th, and 7th days after the sheep has been inoculated with infected blood.

Infestation of heartwater-infected animals with uninfected ticks must be timed so that most ticks detach during the height of the febrile reaction in the sheep. Only those ticks that detach when the febrile reaction is above 40 °C are regarded as highly infective and are retained for later use.

Infection of ticks on tortoises

Two days before the tortoises were injected with *C. ruminantium* infected material, uninfected *A. hebraeum* nymphae were placed on the ventral sides of the tortoises. This was done after the tortoises were immobilised by placing them on their backs in shallow dishes in suitably sized containers. They were kept in this position for 1 h. Vials containing the nymphae were attached to the shell using Prestik and the stoppers were removed. To prevent the ticks escaping from the containers, the rims were taped using double-sided sticky tape.

The tortoises were injected intracardially with either 2 mℓ of infective sheep blood or 2 mℓ of infective ground-up suspensions prepared from *A. hebraeum* nymphae (J. D. Bezuidenhout, unpublished results, 1985).

In the case of *A. marmoreum* only larvae were used. They were placed on the tortoise 2 days before injection, on the same day (Day 0), and on the 3rd and 6th days after injection. *A. hebraeum* larvae applied to tortoises did not attach.

After infestation the tortoises were kept in a metal cage with a wire-grid floor suspended over a tray of water. They were fed *ad lib.* with lettuce. The water in the tray was replaced daily and the engorged ticks that were collected were dried thoroughly before they were stored as described previously. The infectivity of ticks was tested by feeding subsequent developmental stages on susceptible sheep (J. D. Bezuidenhout & J. A. Olivier, unpublished results, 1985). Ticks which were to serve as uninfected controls were maintained on non-infected tortoises in a similar way.

Transmission of C. ruminantium *to tortoises and ticks*

The principal aim of this experiment was to transmit *C. ruminantium* to a tortoise by means of infected ticks.

At the same time uninfected ticks were allowed to feed on the tortoise in such a way that they had a chance of acquiring the infection. It was therefore of the utmost importance to prevent infected and uninfected ticks from mixing.

A special perspex cage was constructed for this purpose (Fig. 13). The tortoise was effectively immobilized in 1 position by securing it firmly by means of the 2 adjustable central sections of the cage onto the fixed platform at the bottom of the cage. This allowed for free movements of the legs and head. These central sections also divided the surface of the tortoise into 3 regions completely separated from each other. All possible openings between the shaped central sections of the apparatus and the shell of the tortoise were carefully sealed off with plasticine.

Infected *A. hebraeum* nymphae were placed on the hind-portion of the tortoise and 3–4 days later uninfected nymphae were released on the fore-portion.

Engorged ticks were collected separately from the front and rear sections of the cage. The infectivity of the ticks collected from the front section was determined by feeding on susceptible sheep. Alexander (1931) was unsuccessful in his attempts to feed *A. marmoreum* adults on sheep. Immatures, however, fed easily on sheep, goats and cattle. The authors found that if *A. hebraeum* males were placed on sheep prior to their infestation with *A. marmoreum* adults, the ticks would attach and feed.

Animal Care

The feeding of ticks on experimental animals is essential for various studies on the transmission of tick-borne diseases. If it is not done compassionately, and with great care, however, it could cause much discomfort to the host animal. One should therefore always strive to be humane, and house and restrain the animals in such a way that unnecessary stress is avoided. Animal cages must be kept clean and dry to avoid bacterial and fungal growth which create favourable conditions for blowfly attacks.

Skin sensitivity, abcesses, anaemia and toxicoses are all problems associated with tick feeding and not all animals react similarly to the bites of the various tick species. For example, guinea pigs are more sensitive to tick bites and to certain contact adhesives, especially those with an acetone solvent, than are other laboratory hosts. Certain areas on an animal's body may be more suitable for tick feeding than others. The widely used ear-bags were found to be unsuitable, especially in rabbits, for some ticks, e.g. *Hyalomma* and *Rhipicephalus* species. Excessive wax in the ears and inflammation interfere with tick feeding. Ear mites and their control may complicate the procedure even further.

After the cessation of an experiment, any remaining attached ticks are destroyed by handspraying the hosts with an acaricide. If these animals are going to be used in further tick feeding experiments, it is advisable to use a short-acting pesticide. In our laboratory natural pyrethrin[6] is used. Three to 4 days later the animal is washed with water and a liquid detergent to prevent any further residual activity of the product. Great care should be taken to keep sprayed animals away from others harbouring ticks for at least 1 week. Some acaricides, especially the synthetic pyrethroids, are very persistent in materials, on hands, etc., and due care should be taken to avoid contamination of clean hosts, ticks and materials.

[6] AVI C & B Concentrate Special, Avima (Pty) Ltd.

Discussion

Laboratories tend to continue to use the methods with which they are most familiar. A combination of the best techniques gleaned from published methods could greatly improve tick rearing.

Light and temperature influence the attachment, feeding, detachment and oviposition of ticks. Barnard, Morrison & Popham (1985) found that the highest proportion of *Amblyomma americanum* nymphae and females fed in a photoperiod of light:dark (LD) of 12:12 h. Feeding time was shortest for nymphae in LD 16:8 at 25 °C and females in LD 12:12 at 20 °C.

Artificial feeding of hard ticks has not been a successful alternative to the rearing of ticks on hosts. Various people have published techniques for artificial feeding (Burgdorfer, 1957; Tarshis, 1958; Gregson, 1964; Purnell & Joyner, 1967; Kemp, Koudstaal, Roberts & Kerr, 1975; Howarth & Hokama, 1983). If successful it could be very useful for infecting ticks with heartwater organisms. Intracoloemic injections of engorged *A. hebraeum* nymphae with *C. ruminantium* from infected tick suspensions have lately proved to be successful. The resulting adults are capable of transmitting heartwater to susceptible sheep (J. D. Bezuidenhout & J. V. Badenhorst, unpublished results, 1985).

It is obvious that there is still scope for the improvement of tick rearing techniques to be used in heartwater research.

Acknowledgements

The authors wish to thank Dr E. M. Nevill, Prof. I. G. Horak and Mr A. M. Spickett for their assistance in the preparation of this article. Mrs Susan Brett is sincerely thanked for drawing the figures.

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