Different methods to collect soft ticks of the genus *Ornithodoros* transmitting African Swine Fever virus (ASFV) in the field

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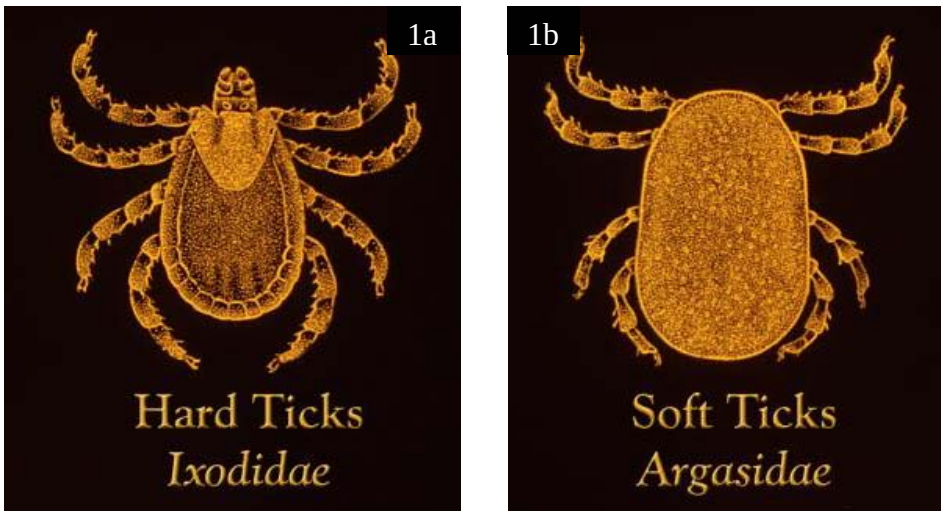
Systematic of soft ticks and their role as vectors of AFSV

There are two major tick families: the Ixodidae or "hard ticks", so called because their sclerotized dorsal plate, and the Argasidae or "soft ticks", so called because of their flexible cuticle (Sonenshine, 1991) (Figure 1).

Figure 1: Schemes of Ixodid ticks (1a) and Argasid ticks (1b).



In different countries in Africa and Europe, soft ticks of the genus *Ornithodoros* (*O. erraticus* and *O. porcinus*) have been described by several authors as biological vectors and natural reservoirs of ASFV; they transmit the virus during their blood feed on domestic pigs (Boinas, 1995, Boinas *et al.*, 2001, Boinas *et al.*, 2004, Oleaga-Perez *et al.*, 1990, Perez-Sanchez *et al.*, 1994, Plowright *et al.*, 1969, Roger F, 2001, Sanchez-Botija, 1982, Thomson *et al.*, 1983, Vigario & Caiado, 1989, Walton, 1979).

Reasons to adapt collection methods to soft tick ecology

Ixodid and Argasid ticks differ by their morphology, ecology and life cycle (Table 1).

Table 1: Morphological, ecological and life cycle characteristics of Ixodid and Argasid ticks.

Characteristic	Ixodidae (hard ticks)	Argasidae (soft ticks)
Morphological features		
Capitulum	Visible from dorsal aspect[a]	Not visible dorsal aspect[b]
Scutum	Present[a]	Absent[b]
Coxal pores	Absent	Present in adults and nymphs
Sexual dimorphism	Well-marked[a]	Slight
Ecology		
Habitats	Open environments (except in certain nidicolous *Ixodes* species, particularly immatures)	Sheltered environments (nest, burrow, cave, man-made primitive shelter)
Seasonal activity	Yes	No
Host-seeking behavior	Mostly non-nidicolous parasiting free-ranging hosts (except certain *Ixodes* species, particularly immatures)	Mostly nidicolous in the habitat of their hosts
Host	Usually 3 hosts (1 per stage), often different species	Usually multi-host feeding pattern
Life span	Range from several months to 3 years; less resistant to starvation and desiccation	Long-lived (up to 10 years); highly resistant to starvation
Biological features		
Nymphal instars	1	Many
Feeding	Each stage feeds slowly (several days), firmly attached and once only	Nymphs and adults feed briefly (minutes to a few hours) and several times
Weight gain while feeding	High (≤100 times unfed weight)[c]	Low (≤12 times unfed weight)
Osmoregulation	Salivary glands	Coxal fluid

Soft ticks are commonly endophilic at all stages of development (living in the nidiculous underground habitats of their hosts) and remain attached to their hosts for a few hours or less, so as to blood feed (Morel, 1969); these ticks usually feed during the night, fall off unnoticed and return quickly to their habitat which they occupy between feeds. In Europe and in East Africa, soft ticks transmitting ASFV were found in crevices and holes of walls, as well as wooden roofs, of pigsties used to shelter domestic pigs (their principal hosts). They were also found in burrows of warthogs which constitute a wild host for these ticks and a natural reservoir of ASFV.

As a result, it is almost impossible to find soft ticks on their hosts or outside; the only solution is to collect them directly in their underground habitat. Because the design and establishment of an epidemiological study (to assess the role of soft ticks in the maintenance and spread of ASF) lead to the collection of large numbers of ticks, collection methods have to be adapted to the ecology of these soft ticks in all field conditions.

Description of different collection methods of soft ticks

Manual collection

Basically, it is possible to manually remove the dust of crevices and holes of pigsties, as well as the content of wooden or tiled roofs, and to manually dig out the pavement of pigsties. Manual method can also be used in the field to examine warthog habitats.

However this method proved to be laborious, time consuming (30 min – 3 h for each manual collection session) and largely unsuccessful because of minute size of larval and nymphal stages and their dark coloration which makes them difficult to see . This method is unusable in large-scale studies.

Carbon dioxide trapping

Carbon dioxide gas has been found to be a good stimulant and attractant to certain species of ticks (Hokama & Howart, 1977, Nevill, 1964). Dry ice which is available at ice cream and beverage stores vaporizes carbon dioxide and can be used for trapping.

Several types of carbon dioxide traps have been tested. The most convenient and effective device was found to be a stainless-steel tray (30x45x8 cm) carrying a polystyrene plastic cup of about 500 ml capacity which was filled with solid dry ice pellets (Caiado *et al.*, 1990) (Figure 2). Traps have to be placed on the ground, close to potential underground habitats of soft ticks, with soil or other material covering them to their top edges and left. If possible, traps are set out overnight; this duration depends on the severity of local infestation, season, ambient temperature (the activity of soft ticks decreases at temperatures <15°C) and the eventual presence of vertebrate hosts utilizing the examined holding or wild habitat.

Advantages of the method:

- Traps can be left unattended.
- Traps are very easy handled and assembled.
- The material is inexpensive.
- The method greatly reduces the exposure of the operator to tick bites.
- A large number of ticks can be collected (even larval stages).
- By this method it is possible to simulate vertebrate hosts (domestic pigs) and to study the movements of soft ticks attracted by this host.

Disadvantages of the method:

- Dry ice may be difficult to find in some countries.
- Dry ice can not be stored more than 3 days at room temperature (gas production ended). It is difficult to use it during long field missions.
- The method is not easy to use when examining deep burrows of warthogs.
- By this method, it is impossible to know where collected soft ticks come from.

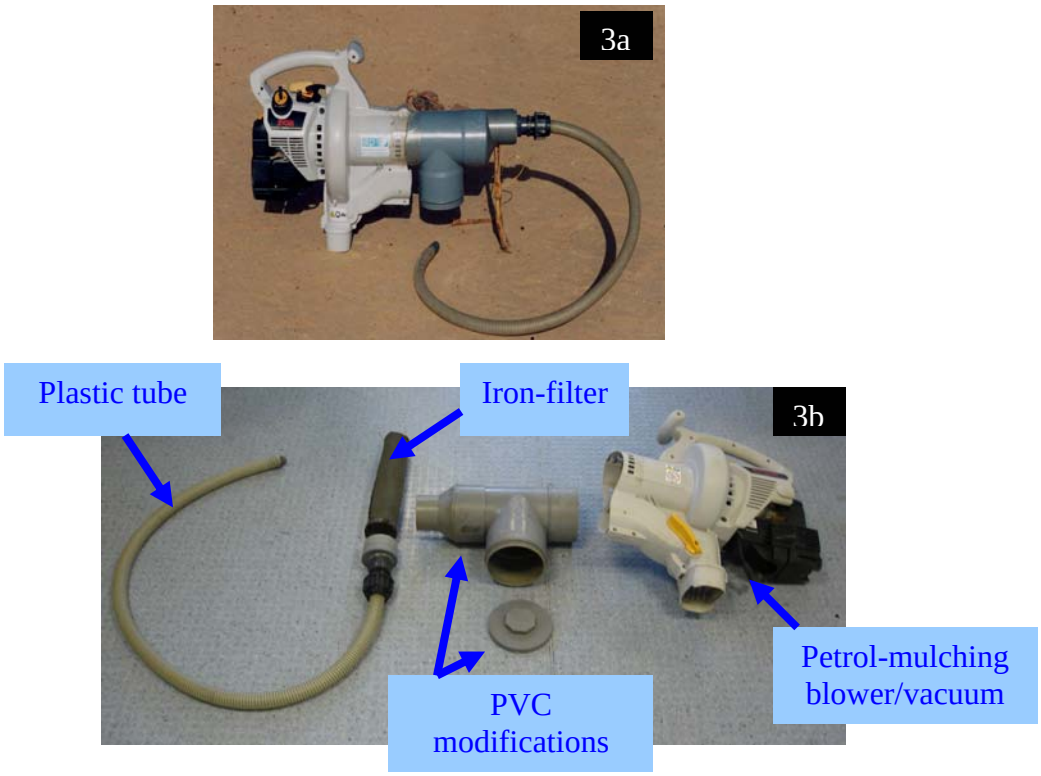
This method was compared to the manual collection in Portugal in order to find *O. erraticus* in pigsties which experienced ASF outbreak a few years before this study (Caiado *et al.*, 1990). While the manual method had a 10% success, carbon dioxide traps provided *O. erraticus* specimens in 70% of visited pigsties.

Cleaning vacuum aspirating

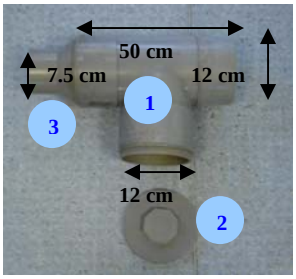
This method has been first described by …. It has been more recently adapted by Vial and Diatta in Senegal in order to collect domestic and wild specimens of the soft tick *Ornithodoros sonrai* transmitting tick-borne relapsing fever to human and maybe ASFV to domestic pigs.

Different adaptations have been tested in order to provide an efficient model. A petrol-mulching blower/vacuum (RYOBI) that can be bought in any gardening shops is used. It is operating thanks to petrol/oil mixture (100 ML of oil per 5 L of petrol; use 2 cycle engine synthetic oil; mix oil and petrol in a special petrol can and not directly in the engine petrol tank). PVC modifications and flexible plastic tube have been added in order to examine deep burrows or cracks to collect ticks (Figure 3). An iron-filter was fixed on the plastic tube and entered the PVC modifications in order to keep/sort large particles of litter and ticks as well.

Figure 3: Modifications on petrol-mulching blower/vacuum (RYOBI). Assembled (3a) and disassembled (3b).

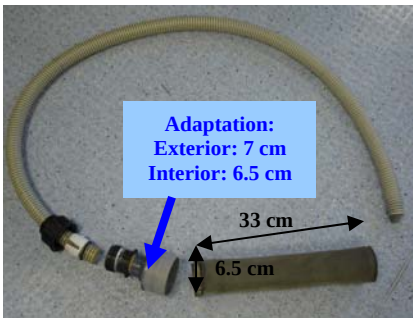


PVC modifications are built with PVC connections used for plumbing. They are composed of:



1) A principal tube with a lateral extension. Turned towards the ground during the operating of the vacuum, this extension allows the collection of smallest particles of litter that passed through the iron-filter.
2) A lid with screw allowing emptying the content of the extension without disassembling the whole.
3) A terminal triangle tube adapting PVC modifications to the plastic tube allowing the aspiration.

Plastic tube is built with thick reinforced hosepipe of 2.5 cm of diameter and 1.5 m long. It is



fixed to the rest of the material by screw-connections and joins of plumbing. The exterior diameter of the terminal connection is 7.5 cm to adapt to PVC modifications and the interior diameter of the terminal connection is 7 cm to adapt to the iron filter.
The iron-filter is 33 cm long and 6.5 cm of diameter. It is built with double iron netting correctly soldered. Netting mesh has to be enough thin to keep ticks inside the filter but enough large to let sand passing through.

All parts of the system (vacuum and adaptations) fit into each other by themselves or with screws allowing easy assembly/disassembly. It is possible to reinforce the assembly between the vacuum and the PVC modifications by scotching them together (Figure 3a). Instructions to operate and maintain the petrol-mulching blower/vacuum are indicated in the operator manual of the vacuum. It is essential to carefully and regularly (every day or every 2 days) clean the whole system as indicated because it might be rapidly incrusted by dust that brake down the vacuum. Prepare a set of material to use and repair the vacuum on the field (screwdrivers to disassemble the system, brushes to clean, alternative spark plug, spark wrench, emery cloth to clean spark plug, alternative starter rope that may brake down very often, protection mask because of fumes…) (Figure 4). During the aspirating, do not aspire at the maximum speed during a too long period (maximum 5 min per aspiring session) to prolong the lifetime of the vacuum; the vacuum is originally used to aspirate leafs and not heavy sandy soil.

Figure 4: Material to clean and repair the petrol-mulching blower/vacuum.

In the field, the petrol-mulching blower/vacuum is used by introducing the plastic tube inside burrows, cracks or holes that are supposed to be inhabited by soft ticks and by aspirating the content of the habitat (sand, litter, dust…) (Figure 5a). The content of the iron-filter and the PVC extension is laid out in a white tray and exposed to the sun to make soft ticks moving and leaving sand (soft ticks are photophobic and do not support warm temperatures) (Figure 5b). Each specimen is then collected individually with entomological flexible tweezers.

Figure 5: Sampling using a petrol-mulching blower/vacuum (5a) and collection of soft ticks by laying out the aspirated content on trays exposed to the sun (5b).

Advantages of the method:

- Sampling material is easy to find in any country, except the petrol-mulching blower/vacuum that can be bought by European partners and sent to the field.
- The material is inexpensive (300 euros for the vacuum and maintenance material and then petrol only).
- The vacuum operates with petrol and is totally autonomous. It allows field missions during long periods.
- The 1.5 m plastic tube extension allows the examination of deep cracks and burrows, especially warthog burrows.
- A large number of ticks can be collected (even larval stages).
- By this method, we exactly know where the collected soft ticks come from.

Disadvantages of the method:

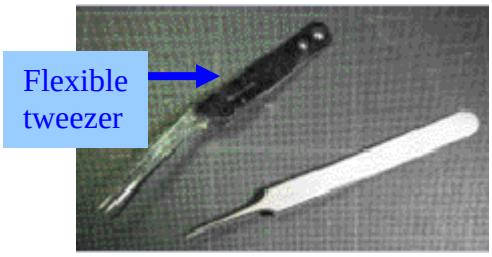
- The operator may be exposed to tick bites (important to ware protective clothes).
- The operator is exposed to petrol fumes (important to use protective mask).
- The method is more rapid than manual collection but less rapid to carbon dioxide trapping.

We did not compare the successful of cleaning vacuum aspiring with manual collection and carbon dioxide trapping.

Collection and storage of soft ticks

Because they have flexible cuticle, soft ticks are very frail especially engorged specimens. Their collection should be done carefully with entomological flexible tweezers (Figure 6).

Figure 6: Entomological flexible tweezers to collect soft ticks.



To assure the best conservation of the virus inside ticks and to avoid DNA degradation, collected ticks should be kept alive (difficult with warm conditions in the field) or directly stored in liquid nitrogen. The degree of conservation depends on the instantaneousness of the death and the storage in cold conditions. When scientists return to the lab, they can store samples in -80°C freezers and send samples in dry ice in polystyrene packs (be careful that the transfer of samples do not exceed 2-3 days). Because this process of storage is not easy in the field, another method is being tested by alcohol storage and conclusions will be soon broadcasted to field partners.

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