

Part 1. Biosafety and biosecurity

UDC 595.421:57.082.114:619:616.98:578.833.314:636.4

SOFT TICK SAMPLING AND COLLECTION

Pérez de León A.*, **Showler A.****

United States Department of Agriculture, Agricultural Research Service, Knipling-Bushland U.S.
Livestock Insects Research Laboratory, Kerrville, Texas, USA., e-mail: beto.perezdeleon@ars.usda.gov

Stegniy B. T., **Kucheryavenko R. O.**, **Kucheryavenko V. V.**, **Gerilovych A. P.**, **Filatov S. V.**

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine

Li A.

United States Department of Agriculture, Agricultural Research Service, Invasive Insect Biocontrol
and Behavior Laboratory, Beltsville, Maryland, USA

Teel P.

Texas A&M AgriLife Research, Department of Entomology, Texas A&M University, College Station, TX, USA

McVey S.

United States Department of Agriculture, Agricultural Research Service, Arthropod-borne Animal
Diseases Research Unit, Manhattan, Kansas, USA

Summary. Soft, or argasid, ticks are challenging to sample for or to collect because of their mostly cryptic behaviors that involve crevices, animal burrows, animal nest materials, and digging into soil. Soft ticks generally do not stay attached to their hosts for more than 30 min, hence, examination of living and dead host animals should not be expected to detect specimens in numbers that represent substantial proportions of the total soft tick population in a given area. Sampling provides foundational information that is important for efforts to develop soft tick surveillance programs. Methods applied commonly to sample soft ticks include: manual examination of habitat substrate material, aspiration of host nests or burrows, trapping using CO₂ or possibly other attractants such as pheromones and their analogs. Because African swine fever, caused by a virus, which is highly contagious and afflicts pigs and their close relatives, has been spreading from its usual range in Africa into the Ukraine, we discuss features of the disease and its soft tick (*Ornithodoros* spp.) vectors in order to indicate a contemporary situation involving the need for systematic argasid tick monitoring through sampling.

Keywords: argasid, attractants, collecting, soft tick, surveillance, sampling, African swine fever, trapping, pheromones, *Ornithodoros*

Argasid tick surveillance. There are many important reasons for doing reliable quantitative surveys, and sometimes it is important to determine all of the different tick species in a particular area (qualitative survey), or to determine the range and spread of soft tick species. It is likely that one survey techniques will not be sufficient to conduct an accurate quantitative and qualitative surveys, so a combination of techniques is usually needed. Accurate tick species population estimates are important in evaluating the effectiveness of various control tactics. In the instance of assessing chemical control methods, being able to determine numbers of ticks in their different life stages is essential in both treated and nontreated (control) areas.

Argasid tick distributions can change over time, and these changes are more challenging to predict than those of ixodids. Soft tick modeling is possible and it is based on the natural niche concept, accounting for the influences of climatic factors, nidicolous lifestyle, indiscriminate host feeding, and flexible developmental

cycle along diapause periods (Vial, 2009). Accurate knowledge of the distribution of ticks and the monitoring of changes in their distribution are important factors for defining of risk areas for tick-borne diseases and to establish adequate measures for tick control and the prevention of tick-borne disease. For this reason, long-term tick surveillance is a critical component for prevention of widespread and devastating tick-borne disease outbreaks of medical and veterinary significance.

Argasid tick sampling techniques. Each tick species requires optimum environmental conditions and biotypes for its development, which determine their geographic distribution and the pathogens they transmit (Parola and Raoult, 2001). Aspects related to the biology and ecology of argasid ticks, then, need to be taken into consideration to assess their presence in the environment (Uspensky, 2008; Sonenshine and Roe, 2014). These considerations bear relevance in the context of pathogen transmission and the epidemiology of tick-borne diseases (Vial, 2009; Manzano-Román et al., 2012). Certain safety precautions need to be observed when sampling some soft ticks because of their role as vectors of pathogens that affect humans.

* Corresponding author

** Contributed equally to this work

Examination of living or dead host animals.

Direct methods for tick surveillance are based on capturing and identifying specimens from vegetation, animal hosts, or other places frequented by ticks. While this works for many ixodid ticks, such methodology is not suited as well for soft ticks. This is mainly because many soft tick species are nidicolous or endophilous, and fast feeders. In such instances, it becomes necessary to investigate all possible soft tick refuges in the sampling area, which is impractical for large-scale studies (Oleaga-Pérez, Pérez-Sánchez, and Encinas-Grandes, 1990; Vial et al., 2006).

In some instances, soft tick collecting is attempted by examining the living or dead bodies of animals that host the target tick species. Animals have been collected from live-animal traps, jaw traps, shooting, road kills, and tranquilizer gun, and by checking game killed by hunters and as road kills (Clymer, Howell, and Hair, 1970; Semtner and Hair, 1973; Tugwell and Lancaster, 1962). Dead animals should be placed inside a container or bag as soon as possible so that ticks detaching from the animals will not be lost. Once the animal has been transported to a laboratory it can be thoroughly examined, and ticks can be removed and preserved for later species identification. It is advised to note the location of the animal body from which the tick was taken (Gladney, 1978). There is a variety of techniques for collecting ticks from wild animals, but special permits are usually required from government agencies, and these administrative requirements should be anticipated and completed before initiating the tick surveys (Gladney, 1978).

Jori et al. (2012) reported a study on the association between *Ornithodoros moubata* (Murray) ticks and warthogs (*Phacochoerus* spp.) and bushpigs (*Potamochoerus* spp.) which was conducted to determine whether wild pig/warthog populations are infected with relapsing fever. Samples were collected from free-ranging animals or by collecting samples from animals killed by hunters (Jori et al., 2012). Collection from free-ranging animals can involve a number of different animal collection techniques followed by immobilizing the animals with a tranquilizer. Small and meso-animals can be live-trapped and checked for ticks (traps can be placed in transects) (Semtner and Hair, 1973; Niebuhr et al., 2013; Labruna et al., 2014), and ticks have been collected from sleeping humans (Labruna et al., 2014). As an example of soft ticks on living hosts, *Argas vespertilionis* (Latreille) was collected manually from trapped Pipistrel bats, *Pipistrellus pipistrellus* (Schreber) (Hosseini-Chegeni and Tavakoli, 2013). Adults and nymphs of *Carios quadridentatus* Heath (Argasidae), a soft tick species associated with the New Zealand lesser short-tailed bat, *Mystacina tuberculata* Gray, become replete in 20–50 min and spend the rest of their lives in guano or crevices in the bat roost, hence, a researcher is much more likely to find larvae than other stages of the tick when bats are examined. Adults and nymphs of fowl ticks, *Argas* spp., are multihost parasites that hide during the day inside chicken houses or near roosting sites, and they engorge rapidly on hosts for only 20–45 min during the night (Diamant and Strickland, 1965). Hence, surveillance for adult and nymph fowl ticks generally requires searching in places where they conceal themselves during the day, but the larvae are commonly found on the host because they attach and feed continuously on one bird for 2–10 d (Gladney, 1978). Surveys for fowl tick larvae can be conducted by examining five birds for attached larvae under wings, on sides of the body, and on the inside of the thighs (Gladney,

1978). Small live-trapped rodents can be removed and placed in wide-mouth plastic jars containing cotton saturated with chloroform. The resulting dead animal can be removed, placed in a plastic freezer bag, labeled, sealed, and stored on ice (Clymer, Howell, and Hair, 1970; Tugwell and Lancaster, 1962). After the animal cadavers cool (detached ticks should be collected and stored), they are placed in a wide-mouthed jar with 50% ethanol which is then shaken vigorously. The animal can be removed from the ethanol and examined carefully for ticks that remain attached. The alcohol is filtered through paper toweling to recover ticks that dropped off while in the jar (Gladney, 1978).

Animals such as raccoons can be anesthetized with ether and released after a visual inspection (Clymer, Howell, and Hair, 1970). Larger animals can be examined by combing over a funnel that leads to a collection jar (Gladney, 1978). With larger animals, blood and mucus often drain out which falls into the funnels. A three-phase strainer is used to separate the bloody mixture from the ticks. The lower screen is 100-mesh screen wire, the second is saran cloth, and the top is 16-mesh screen wire. Ticks are removed by holding the strainer under running water until the blood is washed away (Gladney, 1978). Feral pigs were captured alive using fence traps or by cowboys using a lasso, then the captured pigs were tranquilized before examination for soft ticks (Cançado et al., 2013). Ticks are also collected from road kills, such as *Ornithodoros* spp. being collected manually from coyotes that had been stuck and killed by cars (Bermúdez, González, and García, 2013).

While it might seem intuitive to focus on host animals for collection of soft ticks, argasids usually feed on hosts for only a relatively short time, then drop off to hide in crevices, soil, and nesting material. Animal traps should be checked at least twice each day because ticks begin to leave a dead body after a few hours (Gladney, 1978). Some species, including species of *Ornithodoros*, feed nocturnally for as little as 10 min and are not found on hosts during daylight hours, further complicating efforts to collect them on the bodies of animals (Butler and Gibbs, 1984). In order to more accurately assess tick populations or to maximize specimen captures, using techniques to collect from such abiotic habitats is recommended (Gladney, 1978; Manzano-Román et al., 2012).

Examining soil, debris, and other substrates.

Ornithodoros coniceps (Canestrini) is an ornithophilic species and its life cycle is strictly connected with the pigeon (wild and domestic). It when not feeding, it inhabits cracks and crevices where pigeons nest. These places include ancient towers, bell towers, old houses, ruins, ledges, and attics (Khoury et al., 2011). Manual collection of *O. coniceps* in one study consisted of picking up visible ticks moving on the wall using forceps (Khoury et al., 2011). Similarly, the bat tick, *O. mimon* Kohls, Clifford et Jones, was collected from internal walls of human dwellings, beds, ceilings, and attics (Labruna et al., 2014). *Ornithodoros rostratus* Aragão and *O. brasiliensis* Aragão, known colloquially at 'ground ticks' because both species live buried in sand or soft land near host habitats, mainly parasitize rodents, pigs, *Conepatus* sp. (skunk), *Tayassus* sp. (javelina) (Martins et al., 2011). Sampling for ground ticks has been conducted by examining cellars, stables, and primitive human habitations (Martins et al., 2011). Adult *O. guaporensis* Nava were collected manually from a rocky fissure inhabited by bats in the Amazonian forest, Bolivia (Nava et al., 2013).

Soil, debris, nesting material, and other nonhost substrates in which soft ticks hide can also be examined manually using forceps (Latif and Walker, 2004). The material can be examined where it was found, or it can be collected and examined later (Niebuhr et al., 2013). Sampling for *O. erraticus* in Portugal was accomplished by removing the dust and contents of crevices and holes in stone walled pens and the wooden or tiled roofs over a white cloth, which was examined for soft ticks (Caiado et al., 1990). The pavement of the pig sties was also dug out, especially around the walls and in the resting places of the pigs, and the soil was examined for soft ticks (Caiado et al., 1990). *Ornithodoros capensis* Neumann has been found in the nest material of brown pelicans, *Pelecanus occidentalis* L., and the ticks, which cause nest desertion by the pelicans, are readily obtained by visual examination of pelican nest material and manually removing the ticks from it (Keirans, Hutcheson, and Oliver, 1992). Similarly, *Argas arboreus* Kaiser, Hoogstraal et Kohls was manually collected from nests of cattle egrets, *Bubulcus ibis* (L.) (Mumcuoglu et al., 2005).

A debris-filtering method was developed to filter topsoil, bedding material, and other debris of varying size and composition to collect adult *O. megnini* ticks found within and around locations frequented by ungulates and other hosts (Niebuhr et al., 2013). The method involved three screens with hole sizes of 1.3 × 1.3 cm, 0.6 × 0.6 cm, and 0.3 × 0.3 cm affixed within wooden frames and stacked in order of hole size (largest on top) to serve as the filter apparatus (Niebuhr et al., 2013). Soil sieving was also used to collect adult *O. rostratus* and *O. brasiliensis* (Martins et al., 2011). In a mark-recapture study on *Argas reflexus* (F.), which parasitizes pigeons, *Columba livia* (Gmelin), the ticks were trapped in house attics using smooth V-shaped metal gutters attached to the attic walls (Dautel et al., 1994).

A major drawback to examining soil, debris, and other substrates for soft ticks is that it can be laborious and time consuming. Also, because of the small size of larval and nymphal stages, and their dark coloration which makes them difficult to see, individuals can go undetected in the sample substrate. Therefore, examination of soil and debris is not a desirable sampling method for large-scale studies.

Vacuuming. Trapping ticks inhabiting animal burrows can be challenging. Nocturnal burrow dwelling ticks are secretive, hiding or digging into the soil when disturbed (Uspensky 2008; Anderson and Magnarelli, 2008). Their presence on a host is limited to periods of feeding, which may be completed in as little as ten minutes. After taking a blood meal, they rapidly return to their hiding place. The use of vacuum sampling devices has facilitated surveying tick distribution in the burrow habitat (Butler et al., 1984). Vacuum devices allow the removal of live ticks from the burrow habitat in significant numbers when other survey methods are unable to determine their presence (Butler et al., 1985). For collecting *Ornithodoros* spp. near animal burrows and swine holding areas, a vacuum system was used employing a modified ECHO power blower (model 202) mounted in an aluminum case on an internal door which housed a nylon collection bag (Butler et al., 1985). The vacuum produced by the blower pulled air from the burrow through a 3-m-long crushproof vacuum hose 4 cm in diameter (Butler et al., 1985). Specimen collections of *O. coniceps* have been performed by various methods, including battery-operated aspirators that permitted collection of ticks along corners and cracks that could not be reached

manually (Khoury et al., 2011). In a study that involved searching for *Ornithodoros sonrai* Sautet et Witkowski on pigeons in Senegalese villages, a portable gasoline-powered vacuum cleaner adapted for burrow-dwelling ticks was used (Vial and Martins, 1984; Vial et al., 2007).

Advantages of vacuum collecting include ease of finding fuel for running the vacuum, the equipment is relatively inexpensive, a fuel-powered vacuum can be run autonomously which is conducive to long field missions, the suction tube can probe and collect ticks from deep fissures and burrows, all stages of the tick are captured, and it is known where the ticks were immediately before being captured. Disadvantages include the possibility of the operator being bitten by ticks and being exposed to fuel fumes (wear protective clothing to avoid both), and although vacuuming is more rapid for collecting ticks than manual examination of substrates, it is slower than collecting by using CO₂ as an attractant (no empirical comparison, however, has been reported for vacuum collecting versus manual and CO₂ collecting).

Traps and attractants. Carbon dioxide (CO₂) emanating from the host is a chemoattractant for *Ornithodoros* ticks (Garcia, 1962; Nevill, 1964; Khoury et al., 2011). Dry ice has been used as a source of CO₂ in devices used to lure *Ornithodoros* ticks for different purposes (Adeyeye and Butler, 1991; Vredevoe n. d.). Larvae of *O. coriaceus* were collected with a CO₂ trap while none could be obtained by the continual handpicking method (Hokama and Howarth, 1977). Schwan et al. (2009) used a white terry cloth towel wrapped around small blocks of dry ice to attract ticks. Each trap was taped to the end of a 1-m-long stick and placed in recesses of a room. One collection trap involves using compressed CO₂ gas in a small (e. g., 2.27-kg) cylinder (Niebuhr et al., 2013). Two pieces of 8-m-long clear vinyl tubing with a 0.635-cm diameter attached to a brass Y-valve connected to a gas flow regulator on the cylinder. Using a 22-gauge needle, two holes were made at opposite sides of the tubing in 1-m increments, and each end was sealed with a metal eye-bolt (which allowed for each end of the tubing to be staked to the ground or hung if desired) (Niebuhr et al., 2013). Once a trap was set, 16 'sample squares' of white fabric per trap were placed over each pair of perforations to allow ticks to attach and subsequently be collected. The sample squares were comprised of white 200-thread count cotton fabric affixed to metal frames (0.33 × 0.33 m). In one study the CO₂ cylinder was opened for 30 min with a flow rate of CO₂ from the regulator into the tubing at ≈ 0.28 m³/h during each use (Niebuhr et al., 2013).

The CO₂ collection method allowed to evaluate the involvement of *O. erraticus* in the maintenance and transmission of African swine fever virus (ASFV) in Portugal (Caiado et al., 1990). Fifty-gram chunks of dry ice placed at various distances from tick-infested gopher tortoise burrows used to assess *Ornithodoros turicata* (Dugès) responses at various distances from tick-infested gopher tortoise burrows collected ticks up to eight meters away from the burrows in 2 hours (Adeyeye and Butler, 1991). Differences in attraction were not detected in a 1-h period using 500 to 2,000 ml CO₂/min (Adeyeye and Butler, 1991). Higher numbers of *O. coniceps* nymphs, and adult males and females were collected with a CO₂ trap as compared to employing an aspirator (Khoury et al., 2011). It must be noted, however, that factors such as season, ambient temperature, tick population dynamics, and host availability influence how many, and the life stage of specimens attracted

by CO₂. A convenient and effective CO₂ soft tick trapping device was a stainless-steel tray (30 × 45 × 8 cm) carrying a polystyrene plastic (Styrofoam) cup of ~ 500 ml capacity which was filled with solid CO₂ pellets (Caiado et al., 1990). The traps were placed in the ground with soil or other bedding material covering them to their top edges and left for 1–24 h depending on the severity of local infestation, season, and ambient temperature (activity of soft ticks decreases at temperatures < 15 °C), and the eventual presence of vertebrate hosts using the premises for the night (Caiado et al., 1990).

Advantages to making CO₂ collections are that the traps can be left unattended, and the equipment is easy to handle and assemble, the materials used in the apparatus is inexpensive, the exposure of research personnel to tick bites is low, large number of ticks can be collected regardless of their life stage, and the method simulated vertebrate hosts by exuding CO₂. Disadvantages of using CO₂ to collect soft ticks include scarcity of dry ice in some places, dry ice storage at room temperature is relatively short (3 d), hence, it is not conducive for long periods in the field. The method is also less effective in deep burrows (e. g., warthogs) than in shallower burrows, and the precise place where the soft ticks were residing is not determinable. Despite the disadvantages of the CO₂ collection method, a study conducted in pig sties by Caiado et al. (1990) showed that the success rate was 70% in contrast to only 10% when sampling was restricted to manual means.

Other attractants to soft ticks have been tested. A mixture of guanine hydrochloride and diatomaceous earth in saline was used as an attractant in bioassays, causing 53.1–95.7% assembly, and the attractant was mixed with acaricides to reduce their repellency and enhance their efficiency in bioassays (Gothe, Week, and Kraiss, 1984; Dusbábek et al., 1997). A modification of a synthetic analog of an assembly pheromone was used as follows: a mixture of 5 mg of guanine hydrochloride and 5 mg of diatomaceous earth as pheromone carrier (1:1 w/w) was dispersed in 220 µl of 0.85% NaCl solution (Dusbábek, Jegorov, and Šimek, 1991). In the instance of the soft tick *Argas walkerae* Kaiser et Hoogstraal an assembly pheromone was used to attract ticks to filter paper discs impregnated with the pyrethroid flumethrin (Gothe, Week, and Kraiss, 1984).

Serology. The challenges to soft tick surveillance indicate a need for serological tests (e. g., ELISA) as an indirect method. Such serological methods detect specific antibodies against tick salivary proteins in serum samples taken from hosts. Development of this approach requires resolution of several factors, including 1) the host species to be sampled (domestic animals are preferred if available), 2) demonstration that the tick species induces a humoral immune response, 3) characterization of the response in terms of the number of tick bites needed to induce detectable antibody levels, and how long antibodies remain at detectable levels after the last tick bite, and 4) which antigen should be used and its sensitivity and specificity (Manzano-Román et al., 2012). Serological tests have been developed for *O. erraticus* in southern Europe and for *O. moubata* in Africa. Such tests could help to identify vectoring tick species populations that can be targeted for control, possibly eliminating the diseases that the tick species transmits. While tick salivary gland extract for the two *Ornithodoros* species are suitable antigens for serological surveillance, the method has some drawbacks that include being difficult to standardize, time-consuming collection, poorly known composition, and possible inclusion of nonspecific antigens that could result in confounding cross-reactivity.

Other sampling methods. Additionally, techniques utilizing the natural fluorescence of ticks when exposed to ultraviolet light allow observations on nocturnal behavior to be made (Butler and Gibbs, 1984; Latif and Walker, 2004).

African swine fever and its *Ornithodoros* spp. tick vectors. Several soft tick species in the genus *Ornithodoros* are vectors of ASFV in nature, or known to be susceptible to infection (Kleiboeker and Scoles, 2001). African swine fever (ASF) caused by ASFV is considered one of the most serious transboundary swine diseases because of its high lethality for pigs, its crippling socio-economic consequences, its propensity for rapid and unanticipated international spread, and the absence of either treatment or vaccine (FAO, 2009). Presently the *Ornithodoros* is moving into the Ukraine, the northern range limit of *Ornithodoros* spp. in the Palearctic region being 47°N (Filippova, 1966). However, there is no surveillance for soft ticks and the pathogens they transmit in the Ukraine. The Ukraine is surrounded by territory in which ASFV is present and therefore has legitimate concern for the introduction of ASF. Recent developments in Eastern Europe indicate that further geographic expansion of ASF is likely to occur, requiring increased prevention and vigilance to protect swine populations and the associated business and livelihoods (FAO, 2012).

In the United States, ASF is considered a high-consequence foreign animal disease. As such, ASF is classified in the first of three tiers with other diseases because it poses a significant threat to animal agriculture at the national level by having the highest risks and consequences (APHIS, 2013). Native soft tick species and the exploding feral swine population pose risks for the emergence of ASF in the United States. Some soft tick species native to the United States have been shown to be competent ASFV vectors in the laboratory. Wild pigs, like the wild boar, that are native to Europe and feral hogs that are abundant in the United States represent a potential reservoir population for the virus (Jori and Bastos, 2009), which is a risk for the emergence of ASF in new parts of the world. Establishment of an endemic infection in these regions would make eradication difficult or impossible. An ecological approach is required to evaluate the potential for temporal and spatial interactions between soft ticks and feral swine, which may provide a pathway for spillover of ASF into the United States if the disease emerged in the southern transboundary region.

The upsurge of ASF in many areas of the world has the potential to cause a continuing panzootic crisis. The Ukraine shares borders with the Russian Federation and Georgia, both of which are experiencing ASF epizootics. By initiating a surveillance program in wild pigs and soft ticks, the Ukraine will be able to enhance its veterinary services infrastructure by developing a detection system for the emergence of ASF, and the development of an emergency response that will maximize the chances of eradicating outbreaks if they were to occur. Similar systems would be applicable to the United States and other countries with feral swine populations.

It is considered that because of their long life (up to 15 years) and strong resistance to starvation and persistence of infection for at least 5 years, ticks of the *O. erraticus* (Lucas) complex can be important in maintaining local foci of ASFV, which can lead to endemicity, in regions encompassing Trans Caucasian Countries and the Russian Federation (EFSA ..., 2010a; Boinas et al., 2011). *Ornithodoros* ticks can feed on pigs, from which the vectors can be infected. The epidemiological role played by soft ticks becomes

important where pigs are managed under traditional systems, including old shelters/sties with crevices.

The European wild boar (*Sus scrofa* L.) is distributed throughout the Ukraine. Wild boars are as susceptible to ASFV infection as domestic pigs. Wild boar populations are a risk for the introduction of ASF to the European Union (Blome, Gabriel, and Beer, 2013; De la Torre et al., 2013). In Eastern Europe the current distribution and density of *Ornithodoros* ticks, whether feeding on pigs or wild boar, and their ability to maintain ASFV or transmit the virus to suids remain largely unknown. There is an urgent need for more research in those areas (FAO, 2013).

There are approximately 36 species of *Ornithodoros* ticks in the world that show these general characteristics: i) nidicolous lifestyle, ii) indiscriminate host feeding and short bloodmeal duration, and iii) flexible developmental cycles via diapause periods (Uspensky, 2008; Vial, 2009). Methods applied commonly to sample these ticks include: handpicking, aspiration of host nests or burrows, baiting and trapping using CO₂. Although handpicking ticks in their natural habitat may be considered a crude surveying method, in some cases this is the only practical approach to encounter some soft tick species (Robert, 2002). Continued research on the application of serological methods for surveillance will help monitor tick occurrence and their involvement in the epidemiology of ASFV (Ravaomanana et al., 2011). There have been several reports of surveillance of *Ornithodoros* spp. vectors conducted among domestic pig populations in Portugal and Spain (Boinas et al., 2011; Caiado et al., 1990; Oleaga-Pérez, Pérez-Sánchez, and Encinas-Grandes, 1990; Pérez-Sánchez et al., 1994) and in the ASF-endemic/epidemic regions of Africa among both domestic pig populations and warthogs (Ravaomanana et al., 2010; Vial et al., 2007; Haresnape, Lungu, and Mamu, 1987). Although *Ornithodoros* tick have been reported in the Caucasus region, their current species composition and distribution remains to be fully understood (EFSA ..., 2010a; Diaz et al., 2012). The *Ornithodoros* species present in the Trans Caucasus Countries and the Russian Federation reportedly fall within the *O. erraticus* group and their vector capacity and ability for ASFV remain to be tested (EFSA ..., 2010b).

The global spread of ASF is a concern for the United States. The four species of *Ornithodoros* soft ticks from North America and the Caribbean Basin that have been experimentally infected

with ASF virus (ASFV) are: *O. coriaceus* Koch; *O. turicata*; *O. parkeri* Cooley and *O. puertoricensis* Fox (Hess et al., 1987). ASF outbreaks have occurred in Central and South America, and the Caribbean. *O. turicata* is considered a potential vector of ASFV in north central Florida (Butler and Gibbs, 1984).

The term 'feral hog' tends to be used generically to refer to Eurasian wild boars, domesticated hogs that have become feral, and their hybrid offspring. Feral pigs, used here as a synonym for feral hogs, have been shown to be highly susceptible to experimental infection with ASFV. The problem is such at the Texas-Mexico border that Mexican officials planned to cull 50,000 feral hogs from the United States invading Mexico and affecting 3,700 acres of farmland. Life history traits of feral hogs and soft ticks facilitate their ecological interaction in time and space. Feral pigs are burrowing animals. Soft ticks tend to live in the burrows of their primary hosts. In Florida, *O. turicata* fed readily on caged piglets that were placed at the aprons of burrows inhabited by the ticks and gopher tortoises (Adeyeye and Butler, 1989). Preliminary serological data indicates infection with *B. turicatae*, which suggests that feral swine in Texas are exposed to *O. turicata*, which is the biological vector of that causing agent of human tick relapsing fever (Sanders, 2011). The infestation with soft ticks of feral pigs crossing the border between Mexico and the United States in the ecosystem comprising south Texas provides a potential pathway for the introduction of ASFV into the national herd of hogs and pigs that as of March 2012 included 64.9 million head, which is vital to an industry yielding an expected total of 23.3 billion pounds of pig meat in 2012. Texas' national ranking in terms of total number of pigs produced hovers between 15th and 18th. Over 48,000 hogs are raised annually for show.

We tested the hypothesis that feral pigs may share the same habitat with *O. turicata* by looking for the ticks in area where they were found to infest other hosts like *Neotoma* rats. Using CO₂ as bait, we attracted and collected *O. turicata* from a rodent nest that was likely inhabited by *Neotoma* rats in the northern part of the Rio Grande Plains ecological area in Texas. It must be noted that *Ornithodoros* ticks have been found to infest feral swine in Brazil (Cançado et al., 2013).

References

- Adeyeye, O. A. and Butler, J. F. (1989) 'Population structure and seasonal intra-burrow movement of *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows', *Journal of Medical Entomology*, 26(4), pp. 279–283. doi: 10.1093/jmedent/26.4.279.
- Adeyeye, O. A. and Butler, J. F. (1991) 'Field evaluation of carbon dioxide baits for sampling *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows', *Journal of Medical Entomology*, 28(1), pp. 45–48. doi: 10.1093/jmedent/28.1.45.
- Anderson, J. F. and Magnarelli, L. A. (2008) 'Biology of ticks', *Infectious Disease Clinics of North America*, 22(2), pp. 195–215. doi: 10.1016/j.idc.2007.12.006.
- APHIS. (2013) *High-consequence foreign animal diseases and pests*. Factsheet. United States Department of Agriculture, Animal and Plant Health Inspection Service. Available at: http://www.aphis.usda.gov/publications/animal_health/2013/fs_hc_diseases.pdf.
- Bermúdez, S. E., González, D. D. and García, G. S. (2013) 'Ticks (Acari: Ixodidae, Argasidae) of coyotes in Panama'. *Systematic and Applied Acarology*, 18(2), pp. 112–115. doi: 10.11158/saa.18.2.2.
- Blome, S., Gabriel, C. and Beer, M. (2013) 'Pathogenesis of African swine fever in domestic pigs and European wild boar', *Virus Research*, 173(1), pp. 122–130. doi: 10.1016/j.virusres.2012.10.026.
- Boinas, F. S., Wilson, A. J., Hutchings, G. H., Martins, C. and Dixon, L. J. (2011) 'The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal', *PLoS ONE*, 6(5), doi: 10.1371/journal.pone.0020383.
- Butler, J. F. and Gibbs, E. P. J. (1984) 'Distribution of potential soft tick vectors of African swine fever in the Caribbean region (Acari: Argasidae)', *Preventive Veterinary Medicine*, 2(1–4), pp. 63–70. doi: 10.1016/0167-5877(84)90049-7.
- Butler, J. F., Holscher, K. H., Adeyeye, O. A. and Gibbs, E. P. J. (1984) Sampling techniques for burrow dwelling ticks in reference to potential African swine fever virus vectors. In: Griffiths, D. A. and Bowman, C. E. (eds.). *Acarology VI*, 2, pp. 1065–1074. Chichester, West Sussex, England: Ellis Horwood. ISBN 978-0-8531-2603-4.
- Butler, J. F., Wilson, D. D., Garris, G. I., Koch, H. G., Crum, J. M. and Castellanos, V. E. (1985) 'Survey for potential soft tick (Acari: Argasidae)

- vectors of African swine fever on the island of Hispaniola'. *Experimental and Applied Acarology*, 1(1), pp. 63–72. doi: 10.1007/bf01262200.
- Caiado, J. M., Boinas, F. S., Melo, M. A. and Louzã, A. C. (1990) 'The use of carbon dioxide insect traps for the collection of *Ornithodoros erraticus* on African swine fever-infected farms', *Preventive Veterinary Medicine*, 8(1), pp. 55–59. doi: 10.1016/0167-5877(90)90022-a.
- Cançado, P. H. D., Faccini, J. L. H., Herrera, H. M., Tavares, L. E. R., Mourão, G. M., Piranda, E. M., Paes, R. C. S., Ribeiro, C. C. D. U., Borghesan, T. C., Piacenti, A. K., Kinas, M. A., Santos, C. C., Ono, T. M. and Paiva, F. (2013) 'Host-parasite relationship of ticks (Acari: Ixodidae and Argasidae) and feral pigs (*Sus scrofa*) in the Nhecolândia region of the pantanal wetlands in Mato Grosso do Sul'. *ISRN Parasitology*, 2013, article ID 610262. doi: 10.5402/2013/610262.
- Clymer, B. C., Howell, D. E. and Hair, J. A. (1970) 'Animal hosts of economically important ticks (Acarina) in East-Central Oklahoma', *Annals of the Entomological Society of America*, 63(2), pp. 612–613. doi: 10.1093/aesa/63.2.612.
- Dautel, H., Kahl, O., Scheurer, S. and Knülle, W. (1994) 'Seasonal activities of the pigeon tick *Argas reflexus* (Acari: Argasidae) in Berlin, Germany'. *Folia Parasitologica*, 41(2), pp. 155–160. Available at: <http://folia.paru.cas.cz/pdfs/fol/1994/02/11.pdf>.
- De la Torre, A., Bosch, J., Iglesias, I., Muñoz, M. J., Mur, L., Martínez-López, B., Martínez, M. and Sánchez-Vizcaíno, J. M. (2013) 'Assessing the risk of African swine fever introduction into the European Union by wild boar'. *Transboundary and Emerging Diseases*, 62(3), pp. 272–279. doi: 10.1111/tbed.12129.
- Diamant, G. and Strickland, R. K. (1965) *Manual on Livestock Ticks*. USDA-ARS, 91-49. Washington, DC: USDA-ARS.
- Diaz, A. V., Netherton, C. L., Dixon, L. K. and Wilson, A. J. (2012) 'African swine fever virus strain Georgia 2007/1 in *Ornithodoros erraticus* ticks', *Emerging Infectious Diseases*, 18(6), pp. 1026–1028. doi: 10.3201/eid1806.111728.
- Dusbábek, F., Jegorov, A. and Šimek, P. (1991) Artificial assembly pheromone of argasid ticks (Ixodoidea: Argasidae). In: Dusbábek, F. and Bukva, V. (eds.). *Modern acarology: Proceedings of the VIII International Congress of Acarology, České Budějovice, Czechoslovakia, August 6–11, 1990*, 1, pp. 59–68. Praha: Academia. ISBN 978-8-0200-0364-5.
- Dusbábek, F., Rupeš, V., Šimek, P. and Zahradníčková, H. (1997) 'Enhancement of permethrin efficacy in acaricide-attractant mixtures for control of the fowl tick *Argas persicus* (Acari: Argasidae)'. *Experimental and Applied Acarology*, 21(5), pp. 293–305. doi: 10.1023/A:1018407307532.
- EFSA Panel on Animal Health and Welfare (AHAW). (2010a) 'Scientific Opinion on African Swine Fever'. *EFSA Journal*, 8(3), 1556. doi: 10.2903/j.efsa.2010.1556.
- EFSA Panel on Animal Health and Welfare (AHAW). (2010b) 'Scientific opinion on the role of tick vectors in the epidemiology of Crimean-Congo hemorrhagic fever and African swine fever in Eurasia'. *EFSA Journal*, 8(3), 1703. doi: 10.2903/j.efsa.2010.1703.
- FAO. (2009) *Preparation of African swine fever contingency plans*. Penrith, M. L., Guberti, V., Depner, K. and Lubroth, J. (eds.). FAO Animal Production and Health Manual, 8. Rome. Available at: <ftp://ftp.fao.org/docrep/fao/012/i1196e/i1196e00.pdf>.
- FAO. (2012) 'African swine fever (ASF) recent developments and timely updates — Worrysome dynamics: steady spread towards unaffected areas could have disastrous impact'. *Focus on*, 6. Rome. Available at: <http://www.fao.org/docrep/016/ap372e/ap372e.pdf>.
- FAO. (2013) 'African swine fever in the Russian Federation: risk factors for Europe and beyond'. *EMPRES Watch*, 28. Rome. Available at: <http://www.fao.org/docrep/018/aq240e/aq240e.pdf>.
- Filippova, N. A. (1966). *Fauna of USSR. Arachnids. Vol. 4, iss. 3. Argasid ticks (Argasidae) [Fauna SSSR. Paukoobraznye. T. 4, vyp. 3. Argasovyie kleshchi (Argasidae)]*. Moskva, Leningrad: Izdatel'stvo AN SSSR. [in Russian].
- Garcia, R. (1962) 'Carbon dioxide as an attractant for certain ticks (Acarina: Argasidae and Ixodidae)', *Annals of the Entomological Society of America*, 55(5), pp. 605–606. doi: 10.1093/aesa/55.5.605.
- Gladney, W. J. (1978) Ticks (Acarina: Argasidae and Ixodidae), pp. 102–118. In: Bram, R. A. (ed.). *Surveillance and collection of arthropods of veterinary importance*. Agricultural Handbook, 518, XVI. Washington, DC: USDA-APHIS.
- Gothe, R., Weck, P. and Kraiss, A. (1984) 'Zur pheromonal induzierten Kommunikation von *Argas* (Persicargas) *walkerae* und biologisch-chemischen Bekämpfung durch kombinierten Einsatz eines Pheromons und Pheromon-Analogs mit Flumethrin', *Zentralblatt für Veterinärmedizin Reihe B*, 31(1–10), pp. 161–179. doi: 10.1111/j.1439-0450.1984.tb01292.x.
- Haresnape, J. M., Lungu, S. A. M. and Mamu, F. D. (1987) 'An updated survey of African swine fever in Malawi', *Epidemiology and Infection*, 99(3), pp. 723–732. doi: 10.1017/s0950268800066589.
- Hess, W. R., Endris, R. G., Haslett, T. M., Monahan, M. J. and McCoy, J. P. (1987) 'Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin', *Veterinary Parasitology*, 26(1–2), pp. 145–155. doi: 10.1016/0304-4017(87)90084-7.
- Hokama, Y. and Howarth, J. A. (1977) 'Dry-ice (CO₂) trap for efficient field collection of *Ornithodoros coriaceus* (Acarina: Argasidae)', *Journal of Medical Entomology*, 13(4–5), pp. 627–628. doi: 10.1093/jmedent/13.4-5.627.
- Hosseini-Chegeni, A. and Tavakoli, M. (2013) '*Argas vespertilionis* (Ixodida: Argasidae): a parasite of pipistrel bat in Western Iran'. *Persian Journal of Acarology*, 2(2), pp. 321–330. Available at: <http://www.acarology.ir/Chegeni%20and%20Majidi,%20Vol.%202,%20No.%202,%202013.pdf>
- Jori, F. and Bastos, A. D. S. (2009) 'Role of wild suids in the epidemiology of African swine fever', *EcoHealth*, 6(2), pp. 296–310. doi: 10.1007/s10393-009-0248-7.
- Jori, F., Vial, L., Penrith, M. L., Pérez-Sánchez, R., Etter, E., Albina, E., Michaud, V. and Roger, F. (2013) 'Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian ocean', *Virus Research*, 173(1), pp. 212–227. doi: 10.1016/j.virusres.2012.10.005.
- Keirans, J. E., Hutcheson, J. J. and Oliver, J. H., Jr. (1992) '*Ornithodoros (Alectorobius) capensis* Neumann (Acari: Ixodoidea: Argasidae), a parasite of seabirds, established along the southeastern seacoast of the United States'. *Journal of Medical Entomology*, 29(2), pp. 371–373. doi: 10.1093/jmedent/29.2.371.
- Khoury, C., Bianchi, R., Massa, A. A., Severini, F., Di Luca, M. and Toma, L. (2011) 'A noteworthy record of *Ornithodoros (Alectorobius) coniceps* (Ixodida: Argasidae) from Central Italy'. *Experimental and Applied Acarology*, 54(2), pp. 205–209. doi: 10.1007/s10493-011-9429-5.
- Kleiboeker, S. B. and Scoles, G. A. (2001) 'Pathogenesis of African swine fever virus in *Ornithodoros* ticks'. *Animal Health Research Reviews*, 2(2), pp. 121–128. doi: 10.1079/AHRR200133.
- Labruna, M. B., Marcili, A., Ogrzewalska, M., Barros-Battesti, D. M., Dantas-Torres, F., Fernandes, A. A., Leite, R. C. and Venzal, J. M. (2014) 'New records and human parasitism by *Ornithodoros mimon* (Acari: Argasidae) in Brazil', *Journal of Medical Entomology*, 51(1), pp. 283–287. doi: 10.1603/me13062.
- Latif, A. A. and Walker, A. R. (2004) *An introduction to the biology and control of ticks in Africa*. Available at: <http://www.alanwalker.com/assets/PDF/tick-biology-africa.pdf>.
- Manzano-Román, R., Díaz-Martín, V., de la Fuente, J. and Pérez-Sánchez, R. (2012). Soft ticks as pathogen vectors: distribution, surveillance and control. In: Manjur Shah, M. (ed.). *Parasitology*, 7, pp. 125–162. InTech. ISBN 978-953-51-0149-9. doi: 10.5772/32521.
- Martins, J. R., Doyle, R. L., Barros-Battesti, D. M., Onofrio, V. C. and Guglielmone, A. A. (2011) 'Occurrence of *Ornithodoros brasiliensis* Aragão (Acari: Argasidae) in São Francisco de Paula, RS, Southern Brazil',

- Neotropical Entomology*, 40(1), pp. 143–144. doi: 10.1590/s1519-566x2011000100022.
- Mumcuoglu, K. Y., Banet-Noach, C., Malkinson, M., Shalom, U. and Galun, R. (2005) 'Argasid ticks as possible vectors of West Nile virus in Israel', *Vector-Borne and Zoonotic Diseases*, 5(1), pp. 65–71. doi: 10.1089/vbz.2005.5.65.
- Nava, S., Venzal, J. M., Terassini, F. A., Mongold, A. J., Camargo, L. M. A., Casás, G. and Labruna, M. B. (2013) '*Ornithodoros guaporensis* (Acari, Ixodida: Argasidae), a new tick species from the Guaporé River Basin in the Bolivian Amazon', *Zootaxa*, 3666(4), doi: 10.11646/zootaxa.3666.4.10.
- Nevill, E. M. (1964) 'The role of carbon dioxide as stimulant and attractant to the sand tampan, *Ornithodoros savignyi* (Audouin)', *Onderstepoort Journal of Veterinary Research*, 31(1), pp. 59–68.
- Niebuhr, C. N., Breeden, J. B., Lambert, B. D., Eyres, A. I., Haeefe, H. J. and Kattes, D. H. (2013) 'Off-host collection methods of the *Otobius megnini* (Acari: Argasidae)', *Journal of Medical Entomology*, 50(5), pp. 994–998. doi: 10.1603/me13020.
- Oleaga-Pérez, A., Pérez-Sánchez, R. and Encinas-Grandes, A. (1990) 'Distribution and biology of *Ornithodoros erraticus* in parts of Spain affected by African swine fever', *Veterinary Record*, 126(2), pp. 32–37. doi: 10.1136/vr.126.2.32.
- Parola, P. and Raoult, D. (2001) 'Ticks and tickborne bacterial disease in humans: an emerging infection threat', *Clinical Infectious Diseases*, 32(6), pp. 897–928. doi: 10.1086/319347.
- Pérez-Sánchez, R., Astigarraga, A., Oleaga-Pérez, A. and Encinas-Grandes, A. (1994) 'Relationship between the persistence of African swine fever and the distribution of *Ornithodoros erraticus* in the province of Salamanca, Spain', *Veterinary Record*, 135(9), pp. 207–209. doi: 10.1136/vr.135.9.207.
- Ravaomanana, J., Jori, F., Andriatsimahavandy, A., Roger, F., Albina, E. and Vial, L. (2010) 'First detection of African swine fever virus in *Ornithodoros porcinus* in Madagascar and new insights into tick distribution and taxonomy', *Parasites and Vectors*, 3(1), pp. 115. doi: 10.1186/1756-3305-3-115.
- Ravaomanana, J., Jori, F., Vial, L., Pérez-Sánchez, R., Blanco, E., Michaud, V. and Roger, F. (2011) 'Assessment of interactions between African swine fever virus, bushpigs (*Potamochoerus larvatus*), *Ornithodoros* ticks and domestic pigs in north-western Madagascar', *Transboundary and Emerging Diseases*, 58(3), pp. 247–254. doi: 10.1111/j.1865-1682.2011.01207.x.
- Robert, L. L. (2002). *Guide to pest surveillance during contingency operations*. Armed Forces Pest Management Board Technical Guide, 43. Washington, DC: Defense Pest Management Information Analysis Center, Forest Glen Section, Walter Reed Army Medical Center. Available at: <https://www.iaff.org/hs/disasterrelief/resources/AFPMBTechGuide43.pdf>.
- Sanders, D. (2011) *Ticks and tick-borne pathogens associated with feral swine in Edwards Plateau and Gulf Prairies and Marshes ecoregions of Texas*. A Dissertation Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. College Station, TX: Texas A&M University. Available at: <http://repository.tamu.edu/bitstream/handle/1969.1/ETD-TAMU-2011-05-9135/SANDERS-DISSERTATION.pdf>.
- Schwan, T. G., Raffel, S. J., Schrupf, M. E., Webster, L. S., Marques, A. R., Spano, R., Rood, M., Burns, J. and Hu, R. (2009) 'Tick-borne relapsing fever and *Borrelia hermsii*, Los Angeles County, California, USA', *Emerging Infectious Diseases*, 15(7), pp. 1026–1031. doi: 10.3201/eid1507.090223.
- Semtner, P. J. and Hair, J. A. (1973) 'Distribution, seasonal abundance, and hosts of the Gulf Coast tick in Oklahoma', *Annals of the Entomological Society of America*, 66(6), pp. 1264–1268. doi: 10.1093/aesa/66.6.1264.
- Sonenshine, D. E. and Roe, R. M. (eds.) (2014) *Biology of ticks*. 2nd ed. New York: Oxford University Press. ISBN 978-0-19-974405-3 (Vol. 1), ISBN 978-0-19-974406-0 (Vol. 2).
- Tugwell, P. and Lancaster, J. L., Jr. (1962) 'Results of a tick-host study in northwest Arkansas', *Journal of the Kansas Entomological Society*, 35(1), pp. 202–211. Available at: <http://www.jstor.org/stable/25083246>.
- Uspensky, I. (2008) Argasid (soft) ticks (Acari: Ixodida: Argasidae). In: Capinera, J. L. (ed.). *Encyclopedia of Entomology*, 1. 2nd ed. Dordrecht: Springer. ISBN 978-1-4020-6242-1. doi: 10.1007/978-1-4020-6359-6_10334.
- Vial, L., Durand, P., Arnathau, C., Halos, L., Diatta, G., Trape, J. F. and Renaud, F. (2006) 'Molecular divergences of the *Ornithodoros sonrai* soft tick species, a vector of human relapsing fever in West Africa', *Microbes and Infection*, 8(11), pp. 2605–2611. doi: 10.1016/j.micinf.2006.07.012.
- Vial, L., Wieland, B., Jori, F., Etter, E., Dixon, L. and Roger, F. (2007) 'African swine fever virus DNA in soft ticks, Senegal', *Emerging Infectious Diseases*, 13(12), pp. 1928–1931. doi: 10.3201/eid1312.071022.
- Vial, L. (2009) 'Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution', *Parasite*, 16(3), pp. 191–202. doi: 10.1051/parasite/2009163191.
- Vial, L. and Martins, C. (1984) *Different methods to collect soft ticks of the genus Ornithodoros transmitting African Swine fever virus (ASFV) in the field*. [Online] Available at: [http://www.researchgate.net/publication/237349445_Different_methods_to_collect_soft_ticks_of_the_genus_Ornithodoros_transmitting_African_Swine_Fever_virus_\(ASFV\)_in_the_field](http://www.researchgate.net/publication/237349445_Different_methods_to_collect_soft_ticks_of_the_genus_Ornithodoros_transmitting_African_Swine_Fever_virus_(ASFV)_in_the_field).
- Vredevoe, L. V. (n. d.) *Background information on the biology of ticks*. [Online] Available at: http://entomology.ucdavis.edu/Faculty/Robert_B_Kimsey/Kimsey_Research/Tick_Biology. [Accessed: 20th May 2014].