Annals of *Spiru Haret* University

Veterinary Medicine Series
Year XX, no. 20, volume 2, 2019

Publishing House of *România de Mâine* Foundation
Bucharest

A Publishing House classified by the *Ministry of National Education and Scientific Research, by the National Council of Scientific Research, Category C (areas Philology, Philosophy, History and Cultural Studies, Architecture and urbanism, Performing Arts)*

http://www.edituraf rm.ro

ISSN-L: 1454-8283; ISSN 2501-7780 (online)

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STRATEGIC ANTI-EPIZOOTIC BEHAVIOURS IN CLASSICAL SWINE FEVER (CSF) AND AFRICAN SWINE FEVER (ASF)

Elena-Luiza BĂDIC
Spiru Haret University, Faculty of Veterinary Medicine
256 Basarabia Blvd., 30352, Bucharest, Romania
e-mail: germius@yahoo.com

Abstract

The paper presents the strategic behaviour elaborated by ANSVSA and put into practice with DSVJ and the concessionary or free practice veterinarians in the case of outbreaks of African swine fever diagnosed in Romania in 2017 and 2018. The paper brings into discussion the differential diagnosis between the two infectious diseases' specific behaviours, as well as their official confirmation behaviour.

Keywords*: classical swine fever, African swine fever, ANSVSA, IDSA*

Strategic conduct in the case of CSF outbreaks is elaborated by ANSVSA.

Classical swine fever is a septicemic disease specific to domestic and wild swine. It first appeared in Ohio, USA in the year 1833, in England in the year 1862, and finally was diagnosed on the Bragadiru Farm, in Romania, by Dr. Rugler in 1893.

In 1896, the Veterinary Police Law Enforcement Agency for the first time presented Classical Swine Fever, as "Pig Pneumonia", next to the erysipelas, which was already known.

Suspicion of CSF

Etiology / Epizootic. Classical swine fever is produced by a single-stranded RNA virus from the Flaviviridae family and Pestivirus genus. The natural reservoirs for the virus are domestic pigs and wild boars, especially in the northern and western Balkan region. Circulation of infected animals is a common method of spread throughout Europe. Transmission occurs through both direct and indirect contact. Direct contact transmission includes direct contact between animals, through secretions, excrement, semen, and blood of the infected animals; and by visitors, veterinarians, and traders who enter pig farms. Indirect contact transmission occurs through unclean shelters, vehicles, clothes, tools and needles, through pig feed made from food waste which is insufficiently treated or goes without heat treatment; and lastly through transplacental infections. The pathways of infection are: digestive, conjunctival, all mucous membranes, skin lesions, insemination, or through contact with the blood of diseased animals.

Although other animal species are capable of mechanically spreading the disease by contact with infected excretions or materials, they are not able to develop clinical signs of the disease. Acute clinical signs include: hyperthermia (41ºC), anorexia, adynamia, multifocal hyperaemia and haemorrhagic skin lesions, conjunctivitis, cyanosis of the extremities, transient constipation, alternating with diarrhoea, occasional vomiting, dyspnoea, cough, ataxia, paresis and seizures. Acute infection typically occurs 5 months and 15 days from the first appearance of clinical symptoms in 100% of young pigs.

Clinical symptoms of a chronic infection include: apathy, whimsical appetite, pyrexia, prolonged diarrhoea, apparent remission with relapses and death, embryonic resorption, mummification and death of foetuses, preterm birth, and abortion. Clinical symptoms of a congenital infection include: congenital tremor, weakness, heavy development over several weeks or months, followed by death. Infected pigs are clinically normal, but with persistent viremia and no immune response.

Morpho-pathological aspects in acute form include: petechiae and ecchymoses spread unevenly, especially on the skin, lymph nodes, larynx, bladder, kidney, ileocecal valve; splenic marginal multifocal infarcts – characteristic, although not always present; hemorrhagic diathesis; hypertrophied, hemorrhagic lymph nodes; encephalomyelitis with perivascular thickening.

In chronic form: typhlitis and ulcerative colitis (ulcerative stitches); lymphoid tissue emaciation; hemorrhagic and inflammatory lesions – often absent.

In congenital form: errors in the formation of myelin in the CNS, cerebellar hypoplasia, micro encephalitis, pulmonary hypoplasia, hydrolysis, and other various malformations.

Alert levels at the suspicion of CSF

Level 0: The suspicion is not justified after the clinical examination corroborated with the data provided by the epidemiological investigation. It is not followed by further action.

Level 1: The lesions and the clinical signs are not typical, but the suspicion of the disease cannot be excluded only on the basis of the initial investigations. Suspicious animals are left alive and placed under clinical observation, the farm is placed under temporary control; send samples for laboratory diagnosis.

Level 2: The lesions and clinical signs suggest the presence of the disease, but are not specific. Suspicious animals are left alive and placed under clinical observation. There are restrictions on the movement of animals. Samples are sent for laboratory diagnosis. Optionally, a temporary control zone of 1 km may be required.

Level 3: The competent veterinary authority opens investigations on the farm, because the clinical signs implicate the evolution and spread of the disease. Sick and dead animals are isolated. There are restrictions on the movement of animals and sending samples for laboratory diagnosis. The holding is placed under temporary control; a temporary control area may be established.

Level 4: The disease is confirmed in the laboratory. Total restrictions are imposed on the holding. The protection zone and the surveillance zone are established. The animals on the farm are killed and neutralized. All the houses and spaces are mechanically cleaned and disinfected.

Confirmation of CSF Diagnosis in Romania

Clinical characteristic and lesional aspects

– Detection of Ag or viral genome of CSFV from dead or euthanized pigs from tonsils, spleen, or kidneys.

Two samples from lnn. retropharyngeal, parotid, mandibular or mesenteric and one ileum sample are sent to an appropriate laboratory. In the case of autolysed carcasses – long unopened or sternum bone. From pigs that have had fever or other signs of disease, blood samples are sent on blood thinners or blood for serum.

For pigs in the incubation period, the tonsils are indicated.

Packing and transport of samples – tight containers, 0-4 ° C, fast, according to Order no. 66/2005.

– Isolation and typing of CSFV by accredited methods: IFD (36 DSVJ laboratories); RT-PCR (10 DSVJ laboratories).

– Highlighting specific Ac in blood samples through competitive ELISA (30 DSVJ laboratories).

CSF Official Declaration

The protection zone of 3 km and the surveillance zone of 10 km are set up around the outbreak.

As of 2017, Romania and EU countries no longer perform immunoprophylaxis against classical swine fever. In case of outbreaks in Romania, ANSVSA may have necessary vaccination procedures, as well as emergency vaccination.

The strategic conduct, in the case of CSF outbreaks, is elaborated and implemented by ANSVSA, according to the EU procedures. African swine fever (ASF) is a systemic, contagious, febrile, CSF-like viral disease. It is transmitted by ticks with soft cuticle (Ornithodorus spp.), diagnosed in domestic pigs in Africa and wild and domestic pigs in Europe, Central and South America. So far, there have been no illnesses on other continents, but they are certainly possible.

The etiological agent is a double-stranded Arbovirus of DNA, with characters of Iridoviridae and Poxviridae, of the Asfarviridae family, much more resistant and stable in the environment than the CSF virus. It produces very high mortality rates and kills pigs of all ages. There is no treatment or vaccine against ASFV.

Some authors believe that pigs are accidentally affected, the virus naturally affecting only ticks. Cross-border ASF is transmitted via food waste from unsanitary ports, airports, and hotels. The main vector and reservoir of viruses are found in Africa.

Currently: located on the African continent, the Republic of Cape Verde, Madagascar and Sardinia (Italy).

In Central and East Africa: endemic to domestic pigs in Angola, Congo, Uganda, Zambia, Malawi and Mozambique.

In West Africa: endemic to two islands of the Cape Verde archipelago, Senegal, Gambia, Cameroon and Guinea-Bissau.

Since 1996, episodes have evolved in Ivory Coast, Benin, Togo, Nigeria, Ghana. Isolated outbreaks in Brazil have occurred on the South American continent.

In Europe – Italy (ASFV located on the island of Sardinia), Spain, Portugal (1999), Georgia, Armenia, Azerbaijan, Ukraine, Russia, Belarus (2016), Romania (2017).

Suspicion of ASF

Clinical aspects – largely similar to those in CSF, but with more expression of hemorrhagic syndrome.

Lesions: hemorrhagic lymphadenitis and hemorrhagic diathesis.

There are records of 100% morbidity and approx. 100% mortality in infections with very virulent strains; up to 60-70% morbidity and 30-70% mortality in infections with moderately pathogenic strains.

African wild pigs have viremia associated with subclinical or aborted forms, usually without mortality. Although mortality can vary practically unlimited – between 0 and 100%, low mortality is excluded in the recently infected pig herds.

In case of a suspected evolution of ASF, the specialized examining personnel and the technical or auxiliary staff will, in all cases, apply measures identical to those taken in the case of an already confirmed infection, in order to prevent the spread of any infection in the herd subject to examination.

Differential diagnosis of ASF versus CSF

Clinical and lesional differences are difficult to detect.

Duration of the febrile period: for almost the entire duration of the disease in CSF and only 12-14 days in ASF.

Dyspnoea and cough are sporadic in CSF and much more frequent in ASF, affecting more than 30% of pigs.

Nasal bleeding and nasal leakage can be detected, which are non-existent in CSF. Death occurs faster than in CSF, possibly even the day after the clinical onset.

Frequent abortion, 5-8 days after infection or 1-3 days after febrile onset, whereas in CSF abortions are sporadic.

In chronic forms – arthritis and cutaneous necrosis, which are non-existent in CSF. Hemorrhagic lesions are much more severe in ASF: hemothorax, hemopericardium and hemoperitoneum, which are non-existent in CSF.

It is very important that these differences be notified by the examiner, in order not to delay the procedures for combating and eradicating ASF and not to spread the disease from the outbreak.

Confirmation of ASF Diagnosis

The pathological materials are sent EXCLUSIVELY to IDSA with the labelling: "Animal pathological material. Perishable. Fragile. Do not open outside the African Pig Laboratory."

The suspicion / presence of ASF is officially notified to ANSVSA.

The disease is officially declared and I grade quarantine is instituted.

Tests used in ASF diagnosis:

a) e.g. virological: RT-PCR (molecular biology test), IFD, ELISA – Ag detection, Isolation and identification of Ag by hemadsorption and cytolysis (Malmquist and Hay);

b) serological: ELISA – Ac detection (screening test), ELISA immunoblotting (confirmatory test), IPT immunoperoxidase (confirmatory test), IFI – Ac detection (confirmatory test).

ASF Combat in Romania

If the ASF is officially confirmed on a holding, ANSVSA and DSVJ have the following: All pigs on the holding must be killed (euthanized) immediately under official control and in a way to avoid any risk of propagation of ASFV both during killing and during transport.

The carcasses of dead / euthanized pigs shall be destroyed by incineration or burial only under official supervision.

The meat of the pigs slaughtered during the period between the probable introduction of the disease into the holding and the application of the official measures shall, to the extent possible, be marked and subject to neutralization only under official supervision; the semen, ova or embryos shall be subject to neutralization only under official supervision; shelters, vehicles, equipment used, litter and debris likely to be contaminated must be mechanically cleansed and disinfected.

Protection and surveillance zones (restriction) of 10-30 km or more are established.

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THE INFLUENCE OF EARLY CASTRATION ON CANINE BEHAVIOUR

Aneta Laura TUDOR, Paul GRIGORESCU
Spiru Haret University, Faculty of Veterinary Medicine
256 Basarabia Blvd., 30352, Bucharest, Romania
e-mail: anetalauratudor@yahoo.com

Abstract

Many times owners ask for castration of animals around the age of six months, not because of the advice of the veterinarian, but because of the fact that the patient has some unwanted behaviours that the master hopes to diminish with sterilization. Of these, abnormal sexual behaviour, aggression, excessive barking, anxious temper, urination in unwanted places are the most common.

The study was conducted on 30 animals that were castrated at 5 to 7 months of age and was run for one year between March 2018 and March 2019 at FMVUSH Clinic and two private clinics in Bucharest, pursuing the evolution of unwanted behaviours at least 6 months post castration.

Keywords: *castration, excessive barking, urination in unwanted places*

Introduction

The study was carried out on a batch of 30 males of different sizes and breeds who were neutered after 5 months.

The owners asked for castration, first of all, due to repeated behavioural disorders of the puppies, on the first place being the aberrant sexual behaviour followed by the aggression with the family members, the excessive barking, the fear of noise, the anxiety of separation and the urination in places where they are not allowed.

The radical decision for the neutering surgery was taken after trying conservatory measures for training, avoiding family separation for a long time and all the factors of stress.

After all of these had no other influence on their unwanted behaviours, the owners decided to neuter their animals.

Methods and materials

The 30 dogs who participated in the study were: 2 from the Pekinese breed; 12 from the Bichon breed; 3 from the Yorkshire breed; 1 from the

Chihuahua breed; 1 from the Doberman breed; 2 from the German Sheperd breed; 9 mix breed.

After the inhalation anaesthesia, the neutering surgery was done through the classical method, with an incision made before the scrotum; for the testicular cord ligature it was used a braided wire (Neocryl 3/0-0), braided wire and to close the incision, it was used PDS wire 3/0-0. The treatments with antibiotics were given for 48-72 hours.

Results and discussions

After the end of the vaccination period, at about 3 months, most of the owners requested detailed information about sterilization as they observed the behaviours mentioned above.

After consulting the patients, it followed a very detailed veterinary check, there were established new clear behavioural rules and the puppies were under observation.

The owner's training was based on several rules:

– The dog should not be allowed to behave like a human – to eat at the table, to sleep in bed with the owner, etc.

– The dog's problem should not be solved like a human one. E.g.: calming an anxious dog or the enthusiastic welcome of an agitated dog.

– Human feelings and emotions should not be attributed to the actions of the body language or facial expression of the dog.

Regarding the aggression that is thought to be the consequence of an "excess testosterone", the owners were trained to learn to be true "leaders of the pack".

Dogs become dominant because of the lack of firm management on the part of the family and this is why they will try to take control.

Without rules, limits and restrictions a dog does not know what to do; they must be applied firmly and consistently by all the family members.

It is very important that the owner never shows affection to the animal when it manifests unwanted behaviour.

In general, unwanted behaviours such as excessive barking or aggression were associated, in 8 of the dogs under study, to the fact that animals were "pack leaders" in the family.

Most of the time, the dog is considered strong if it gathers many objects around it: toys, bones or other things the animal considers his own.

They must be confiscated and the dog can only play under the conditions established by the owner.

As pack animals, most dogs are subordinate, not leaders in their groups.

Aggression in canine communities is unnatural; the dominant canine members of the group quickly show their superiority and calm down another aggressive dog.

Without a strong leader, dogs are pushed to a role that most do not want and cannot fulfil, so they attack everything.

Anxiety was observed in 4 of the 30 males.

Following the discussion with the owners, it was considered that they should not abuse the animals in any way or isolate them from the family for a long time during the day.

Also, the owners confirmed that all 4 puppies were taken from the nest around the age of 3-4 weeks – much too early –, missing a proper socialization by the way the mothers would have fed, cleaned and corrected them.

Tempering anxiety involves a long process of rehabilitation, a lot of patience and time on the part of the owner.

Excessive barking – behaviour observed in 6 of the dogs under study – can have multiple causes including energy accumulation, frustration, separation anxiety, and boredom.

It always needs careful observation at the exact moment of the unwanted behaviour and understanding its causes.

The behavioural straightening formula always includes: sufficient movement every day, constant discipline from all family members, affection and a firm and warm leadership.

Abnormal sexual behaviour – observed in 9 of the dogs – was manifested by the fact that in general they had a toy that played the role of sexual partner and showed aggression in confiscating the object.

Urination in places they are not allowed to was observed in 3 of the males and was often interpreted as a sexual manifestation of “marking the territory”, in all cases the owner being convinced that these “accidents” happened because they felt females in heat in the neighbourhood and if they sterilize them, this behaviour will vanish.

Following repeated discussions with the owners, the possible causes of all these inappropriate behaviours were explained and the overwhelming role of the adoptive family, both in terms of the rules to be imposed on animals, as well as strictly observing them.

Also, it was constantly recommended to work with people specialized in canine behaviour.

In the absence of results, they called a veterinarian to request the orhydectomy of 30 dogs, aged between 5.5 months and 6.5 months.

Surgical procedures were performed after preoperative analysis (Panel 1 and hemolysograms 5 DIFF); all the interventions were carried out without incident within or after surgeries, the recovery lasting up to 2-3 days.

The 30 males were observed for 6 months after the surgery.

Since the behaviour of the masters was the same in the sense of not being actively involved in the education of the males, we followed the evolution of the unwanted behaviours under the influence of the decrease of the testosterone level related to the orhydectomy.

The animals were initially divided into two groups:

- lot 1 made up of neutered dogs with the age of 23 weeks;
- lot 2 consists of neutered dogs with the age of more than 23 weeks.

The following results were observed:

- the only positive change was recorded in the first lot of dogs, who were less than 23 weeks of age, where urination in unwanted places was reduced by approximately 75%;

- in the second group, 25 weeks old, no decrease in this behaviour was observed.

In the case of the dogs that were neutered early, before 23 weeks, the owners reported even a slight worsening (10-15%) of aggression, excessive barking and aberrant sexual behaviour.

In males neutered after 23 weeks, there was no change in habits.

■ Age	■ Aggression ■ Excessive barking ■ Anxiety	■ Aberrant sexual behaviour	■ Urinating in unwanted places
■ < 23 weeks	■ ↑	■ ↑	■ ↓
■ > 23 weeks	■ No change	■ No change	■ No change

Conclusions

The results were recorded monthly and they were accompanied by the observation that none of the masters changed their behavioural routine towards the males or did not extend their methods of discipline, socialization, outdoor movement, etc.

The conclusion is that, regarding these unwanted behaviours, the exclusive impact of orhydectomy in their disappearance is totally insignificant, most being determined by innate reflexes and it is possible that it can be diminished in a longer time than the 6 months after surgery, while the animals were also observed with the active involvement of the owners in educating the new members of their families.

The study will continue with patient observation and recording of the results up to one year after surgery.

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THE QUALITY OF STRAY DOGS HOUSING, AN ESSENTIAL CONDITION IN PROVIDING THEIR WELFARE

Ioana ANDRONIE, Monica PÂRVU, V. ANDRONIE, Cristina RENTEA
Spiru Haret University, Faculty of Veterinary Medicine
256 Basarabia Blvd., 30352, Bucharest, Romania
e-mail: ioanaandronie@ymail.com

Abstract

The stray dogs' shelters must offer a minimum of comfort so that their welfare is not affected. This aspect causes the animals to develop non-adaptive behaviours over time, which will often lead to their failure to be accepted by future owners, thus remaining a long time in shelters. The purpose of the study was to point the importance of shelter housing conditions for stray dogs in providing their welfare. The assessment was carried out by observing and appreciating the characteristics of accommodation offered to stray dogs, their health and their behaviour during the visits in two private shelters. The results showed that the welfare of the animals was visibly affected in the pens where the number of dogs exceeded the regulated accommodation area. The overcrowding of accommodation spaces resulted in injuries to the body as a result of the aggressive behaviour manifested. The welfare of the animals was modified in the pens where the dogs were moved frequently from one pen to another, even though the accommodation area was more generous than the previous one, due to their contact with new individuals.

In current practice, by assessing the responses of animals to the environment in which they live, we can identify the factors that can impair the welfare of an animal such as the conditions offered by shelters.

Keywords*: housing quality, welfare, stray dogs*

Introduction

Lately, the increase in the number of stray dogs in our country has led to the development of programs and campaigns organized in order to capture them from the public space, sterilization and microchipping, as well as programs to promote their adoption. In order to meet these, public and private shelters have been established, that offer a certain level of comfort to the animals, so that their welfare is not affected.

Today, in determining the level of welfare, animal breeding science will lay particular emphasis on the use of animal-based measures, such as cortisol levels, heart rate, heart rhythm, changes in body temperature and even their immune status. Behavioural manifestations are also relevant in assessing canine welfare by measuring fear, anxiety behaviour, frustration, stereotypes, but also the response given by animals in interacting with strangers. [1, 4] In the environment in which they live, the animals can make choices against this and the other individuals, which are not always in their long-term interest, thus an assessment of the emotional state is required – pain, fear, suffering, illness, etc. Results of scientific studies suggest that good welfare can be best assessed by measuring resources and appreciating behavioural responses that may be influenced by cognitive processes and the level of physiological indicators. [6]

In shelters, stray dogs that do not have an adequate level of welfare develop abnormal behaviours over time, which will lead to non-acceptance by the people who want to adopt them. It is known that future owners want to adopt dogs that interact positively with humans and do not exhibit abnormal behaviours. In areas with poor management and low adoption rate, most of these animals spend the rest of their lives in limited conditions, which negatively affects their welfare. Most often this is due to the inadequate accommodation surface, the lack of enrichment of the environment, the inadequate feeding and adaptation facilities and the low degree of adaptation to the living environment. Evaluation of the stray dog behaviour in shelters is increasingly used to know their ability to relocate, which contributes to the increased adoption rate. [1, 3, 8].

Under the same shelter conditions, stray dogs may exhibit different behavioural responses due to individual variability, as they may sense the same stressors differently. Stray dogs that live in the same environmental conditions for long periods of time can go from one level of welfare to another according to their physiological and behavioural requirements. [1]

Materials and methods

The objective of the study was to quantify the importance of housing conditions for stray dogs in providing their welfare. The research was carried out in two private shelters for stray dogs, of small capacity, located in the southern part of the country, for a period of 12 months, during which we tracked the accommodation conditions and their behaviour, as a starting point in the assessing their welfare level. The two shelters aim to save, rehabilitate and place abandoned animals for adoption.

Housing conditions

Shelter (A) has 11 pens with an accommodation area of 6.25m² each, built directly on the ground and fenced with wire mesh fence. To limit air currents, fences are doubled with plastic tarpaulins. In each of them there is a wooden cage, with two compartments, with an area of $1.80m^2$ with straw bedding. In the pens there are stainless steel bowls and plastic material for feeding; one for each animal. The water containers are made of plastic material, fastened to the fence so that they are not emptied by animals.

Shelter (B) has 12 paddocks with an area of 6m² each with concrete floor covered with a material that provides the efficiency of sanitation operations. On the floor there are wooden pallets for dogs resting. The walls of the paddocks are fenced with an iron mesh fence, except the one behind the paddock, which consists of concrete. The dividing walls are doubled with plates made of opaque material to limit the dog's interaction. In each paddock there is a large wooden cage with an area of 2m² and straw bedding. All the paddocks have roofs.

Dogs observed in the two shelters are fed once/twice daily, water is provided ad libitum, and during feeding they are supervised by employees or volunteers to prevent unpleasant incidents such as food theft. The hygiene of the pens/paddocks is performed regularly and whenever it is needed. The feeding facilities hygiene programs are maintained after each feeding. The canine youth from shelter B benefits from more exercise than adult dogs. At least twice a day (eight hours in summer and four hours in winter) these are left in the paddock arranged for play on the Association's land (100 m² fenced land). During the warm season, they benefit from places with water for their cooling. Animals are internally and externally dewormed, vaccinated, neutered, and in case they need medical care they are transported to the veterinary practice with which the shelter collaborates.

Animals

Shelter (A, n: 47) has 54 mixed-breed dogs, females (87%) and males (13%) captured from the street, between the ages of 4 months and 6 years. A number of four dogs are housed in the pens of the shelters according to age and sex.

Shelter (B, n: 19) recently established, has 25 mixed-breed dogs, females and males, between 3 months and 3 years old, housed on average two dogs per paddock.

In the two shelters, dogs between six months and 6 years were taken into observation (n: 66).

The level of stray dogs welfare was assessed based on the four principles of welfare, namely, adequate housing, good health and natural behaviour displayed, described in the Welfare Quality® (2009) project for farm animals. Thus, for proper shelter and good feeding, we assessed the conditions offered by shelters from the point of view of their location, the surface of the insured accommodation, the number of existing cages, the mode and frequency of applying the hygiene measures, the presence of feeding and adaptation facilities and the freedom of movement of dogs. [7] We monitored by direct observation the behaviour of dogs throughout the whole study, following the main behavioural manifestations described in next table. [1]

Behaviour	Description
Sociable	Seeks contact/interaction, kind towards humans and other dogs.
Playful	Cheerful, obvious playful, calling other dogs to play.
Quiet	Does not interact with others, slightly depressed.
Restless	Restless, extremely excitable.
Aggressive	Attentive to any movement / noise, curious.
Nervous	Noisy, draws attention, excessive barking.

The data obtained were processed statistically (ANOVA) in order to compare the growth conditions of the two shelters taken in the study.

Results and discussions

The results obtained show that in the two shelters studied the surface of the facilities (pens, paddocks) was organized for each age category of the animals. The surface of the playground arranged in shelter B is made as such to ensure the possibility of daily movement for dogs up to the one year of age, outside the paddocks where they were housed. In this shelter, at the time of assessment, the number of animals was smaller than in shelter A, the area of the swamps was thus more generous (figure 1), and the number of dogs housed in the paddocks was of two dogs, which approached the requirements to which the accommodation applies.

For dogs in shelter A, the resting place in the pens was not comfortable enough compared to shelter B, due to the size of the cages which was in contradiction with their number. This generated the manifestation of aggressive behaviour for occupying the resting place, especially for the last ones accommodated in these pens. From the point of view of the bedding material, it was provided in sufficient quantity and also hygienically maintained.

The overcrowding of the pens in this shelter has led to poor hygiene of the dogs as compared to those in shelter B; they also suffered from this point of view. The ratio of clean-skinned dogs was clearly higher in shelter B (89%) compared to A (53%), where at certain times of the visits hygiene of the pens was poor, there being dirty animals with faeces and urine (figure 2). The differences were very significant ($p \leq 0.01$).

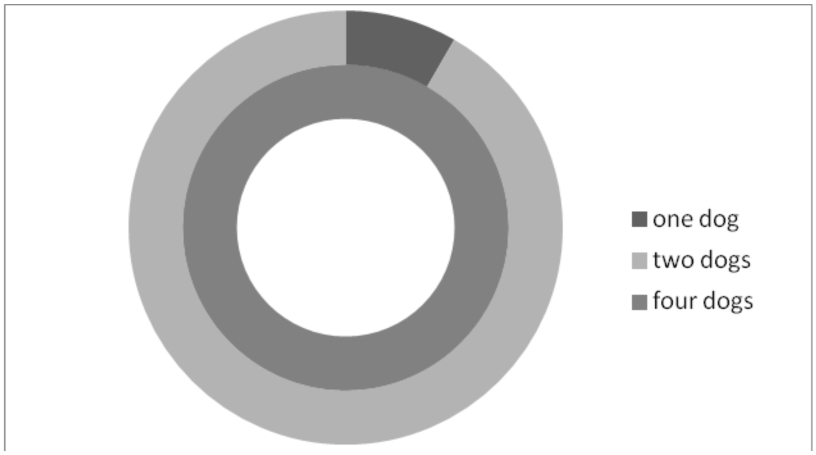

Figure 1. *Average of dogs (number) occupying the paddocks/pens of the two shelters*

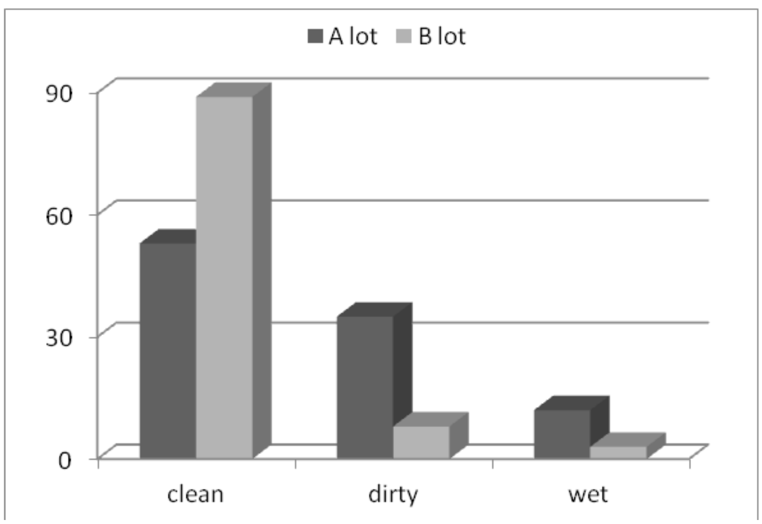

Figure 2. *Assessment of dogs body hygiene (%)*

It is noteworthy that the relatively small accommodation area for dogs in shelter A also led to a much smaller ratio of daily behavioural manifestations than those in shelter B.

In shelter B, the positive behavioural manifestations (figure 3) were 91.8% (joy at the sight of foreign people, socialization, play less aggression, restlessness or agitation) both in the paddocks and on the surface of the playing area, compared to 59.9% in shelter A ($p \le 0.01$).

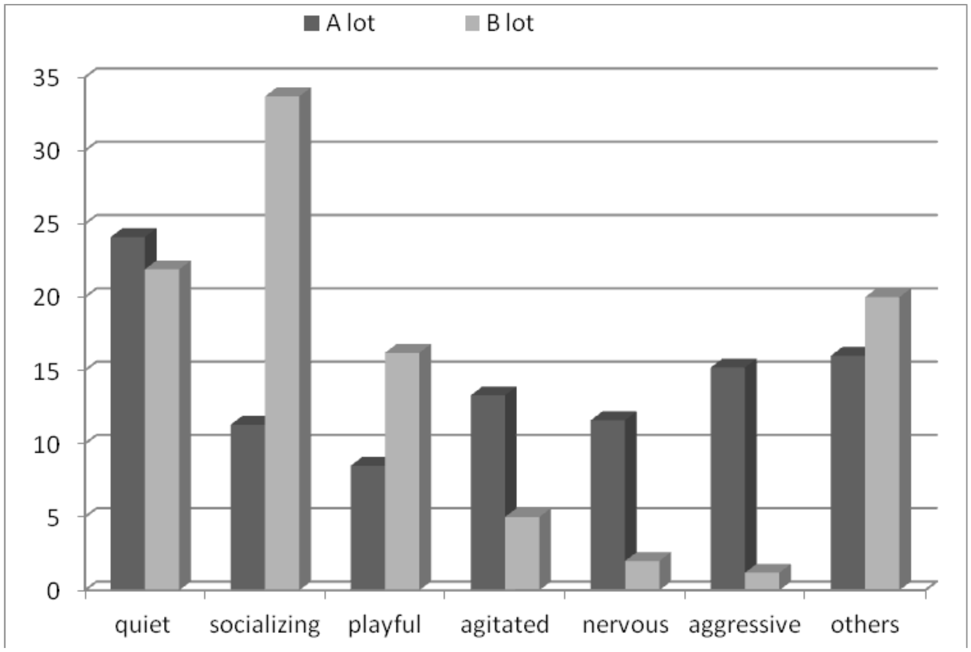

Figure 3. *Incidence (%) of the behaviour manifestation in the two shelters*

In shelter B, almost all dogs were sociable and friendly, did not show aggressive behaviour towards visitors, some even enjoyed their presence. Only one case was noticed, an 11-year-old dog, abandoned by the owner, who did not interact with the other dogs or humans and left the cage only at the time of feeding. Even if the socialization and its integration have been tried, it was not successful until the time of the study. This dog was housed alone in the paddock.

The overcrowding of the paddocks in shelter A has increased the number of aggressive manifestations near the feeders or when the animals were fed. The state of agitation of the dogs was present in 18.3% of the visits in the two shelters and due to the increased frequency of moving the animals from one pad/paddock to another (lot A), but also from the failure to adapt to the "detention" system for some of them (lot B). Some researchers say that interpreting the signs of acute stress can be relatively easy, while the signs of chronic stress require further research before they can include measures to assess the quality of life and welfare of dogs. [2]

The change of environment and the contact with the new animals also generated aggression, with a high incidence of head injuries (especially of the ears) in shelter A compared to shelter B.

Results of some studies have shown that differences in breed, sex and age of animals can partially explain the variability in the onset and prevalence of behavioural indicators of poor welfare in stray dogs in shelters. [5]

Conclusions

In the current context of managing the population of stray dogs, it is very important that the shelters ensure a minimum of accommodation conditions, especially from the point of view of the accommodation surface and the enrichment of the environment, to ensure an adequate level of their welfare.

The welfare of the stray dogs was visibly affected in shelters, when their number exceeded the recommended accommodation area. Overcrowding has led to the impossibility of proper feeding of all animals in the pen/paddock, to injuries on the body as a result of the aggressive behaviour manifested. The state of stress appeared frequently in the paddocks with more than two dogs, in which the dogs were moved frequently even though the accommodation area was larger than the previous one. The welfare of these dogs became poor when new individuals arrived, when the behaviour of establishing the group hierarchy is clearly visible. The efficiency of the hygiene was doubtful in the shelters where the materials from which the pens/paddocks are built did not meet the needs of the animals.

The welfare of stray dogs in shelters is largely dependent on the sheltering conditions ensured.

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BOVINE TUBERCULOSIS – LEGISLATIVE ASPECT, DETECTION, TRANSMISSION

Violeta-Elena SIMION, Adriana AMFIM, Mădălina BELOUS, Monica PÂRVU
Spiru Haret University, Faculty of Veterinary Medicine
256 Basarabia Blvd., 30352, Bucharest, Romania
e-mail: simion.violeta.elena@gmail.com

Abstract

The World Health Organization (WHO) reports a reduction in 2019, a reduction in the epidemic of tuberculosis (TB), and a decrease in the incidence rate of these diseases in the last ten years. However, this disease continues to affect humans and animals alike, being far from being eradicated. Bovine tuberculosis (bTB) caused by Mycobacterium bovis, is an important milk-borne zoonosis that affects cattle production and endangers human health.

In the veterinary medical field, the control and diagnosis of bovine tuberculosis is regulated by a series of normative acts regarding the control and eradication actions of this disease.

In Romania, the method of testing bovine and goat for the detection of tuberculosis is that through tuberculin skin test, an action regulated by a series of normative acts. This article presents updates in the early detection and identification of susceptible and positive cows at the bTB, through non-invasive measures. These involve taking samples of air, faeces, and dermal tissue followed by analysis of volatile compounds from the collected samples.

Keywords: *bovine tuberculosis (bTB), legislative regulations, diagnosis, non-invasive screening*

Introduction

The World Health Organization (WHO) recently announced in its 2019 report, a reduction in the tuberculosis epidemic (TB), and a decrease in TB incidence rate of 4.7% during 2017-2018, being considered one of the fastest in all WHO regions, for the European Region 30 cases (2634) per 100,000 inhabitants being estimated [1]. However, TB remains one of the diseases that continue to affect humans and animals, regardless of species and regardless of continent, being considered among the top infectious diseases in the world with the main cause being a single infectious agent, with lethal effects [2].

Tuberculosis is caused by a group of closely related bacterial species, called Mycobacterium tuberculosis complex, which is also the leading cause of human tuberculosis. Other members of the complex that can cause tuberculosis include: M. bovis, M. caprae with a wider range of hosts, being the main cause of tuberculosis in many animal species, including humans – M. pinnipedii, M. orygis, M. suricattae, M. mungi, M. canetti and more rarely M. africanum (predominant in humans) [3-8]. It is estimated that in the pre-antibiotic era, M. bovis was responsible for about 6% of tuberculosis deaths in humans [9-10]. Eradication of it, although currently controlled in more and more countries, with diagnostic and treatment protocols implemented, continues to be a challenge for specialists because of the lack of detection, to some sources of insufficiently controllable contamination (e.g. wild animals) but also due to another "disease" of our century: drug resistance (as in the case of antibiotics, of deworming, for example) – multidrug resistant TB (MDR-TB) [11-12]. Drug resistance can be classified as primary when encountered in a patient who has never received anti-tuberculosis treatment and acquired, which occurs as a result of previous specific treatment. The WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) have replaced the term primary resistance with the term drug resistance among new cases and the term acquired resistance, with the term drug resistance among previously treated cases [13-14]. The WHO strategy is that by 2050, TB will no longer be considered a public health problem [15-16]. It has been estimated that the lifetime risk of disease for an individual with confirmed latent tuberculosis infection is 5-10% most developing the disease in the first 5 years after the first infection, this being the pathogenic mechanism of active TB in the countries with low incidence of tuberculosis [17].

Bovine tuberculosis (bTB) is caused by M. bovis and M. caprae, both of which cause infectious diseases that can lead to significant productivity problems and impair animal health. Tuberculosis due to these pathogens is a zoonotic disease with a complex epidemiological pattern which includes transmitting the infection to and from: humans, pets and wildlife. While cattle may be affected by M. bovis, M. tuberculosis only produces asymptomatic or localized infections, as with other mycobacteria. Specialized breeds, with large milk productions, are the most frequently affected categories. Treatment for TB in animals, although possible, is not recommended, and in the case of cattle, it is forbidden [18-21]. M. bovis has one of the largest ranges of hosts (humans, cattle, wildlife including felines, ruminants, primates, rodents, etc.) of all known, diagnosed pathogenes worldwide [19-29]. Susceptibility to Mycobacterium tuberculosis is relatively high in humans, other primates and

Guinea pigs. Cattle, rabbits and cats are sensitive to M. bovis and are quite resistant to M. tuberculosis. Pigs and dogs are sensitive to both M. bovis and M. tuberculosis [30].

The World Health Organization recently estimated that a quarter of the world's population has latent tuberculosis infection (LTBI), estimation made generally based on Tuberculin Skin Test TST/test Mantoux and not on the basis of Interferon-Gamma Release Assay (IGRA). Cohen and col. (2019) do a systematic review and meta-analysis of the results from 3,280 studies published between 2005 and 2018, to estimate LTBI, based on the IGRA and TST test results. The estimation was made at regional and global level, thus classifying the countries according to the incidence (with low, intermediate and high tuberculosis incidence) for which a collected estimate was calculated for each area, using a random effects model. Thus, the results published in 88 studies from 36 countries with 41 IGRA (n = 67167) and 67 estimates for TST (n = 284644) were evaluated. The overall prevalence of LTBI was 24.8% (95% CI; 1.7-30.0%) and 21.2% (95% CI; 17.9-24.4%), based on IGRA and a reduction of TST of 10 mm respectively. Using a new strategy, using prevalence survey in 351,811 people, prevalence estimates correlated well with WHO incidence rates ($Rs = 0.70$, $p < 0.001$). The tests are considered useful for identifying persons eligible for preventive therapy, such as immune-compromised patients (HIV infected, immunosuppressive medication, anti-TNTα etc.) [31] and for the control of the group of recently infected persons who are a potential source of active tuberculosis, their monitoring and evaluation of treatment. In latent tuberculosis (ILTB) infection, persons, although not clinically manifesting radiological changes or characteristics of active tuberculosis, are at increased risk of progression to active disease [32].

Legal framework in veterinary medicine

For the veterinary medical field, the control and diagnosis of bovine tuberculosis (bTB) is managed within the Institute of Diagnosis and Animal Health where the National Reference Laboratory for Tuberculosis and Paratuberculosis operates, designated by Order no. 205/2007 regarding the approval of the national reference laboratories, and their attributions.

At the level of the European Union, a number of structures work and carry out activity in this field such as European Reference Laboratory for Bovine Tuberculosis, The Laboratorio de Vigilancia Veterinaria (VISAVET), Facultad de Veterinaria, Universidad Complutense de Madrid, Spain, established by a series of normative acts – Regulation (EC) No. 882/2004 of the European Parliament and of the Council of 29th of April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules and

Commission Regulation (EC) No. 737/2008 of 28th of July 2008 designating the Community reference laboratories for crustacean diseases, rabies and bovine tuberculosis, laying down additional responsibilities and tasks for the Community reference laboratories for rabies and bovine tuberculosis and amending Annex VII to Regulation (EC) No. 882/2004 of the European Parliament and of the Council.

At the same time, the National Veterinary Health Authority (ANSVSA) carries out the activity for the verification of the actions of detection and control of tuberculosis to animals of economic interest based on a specific procedure which applies at the level of the animal health section within the directions for food safety.

A series of normative acts regulate the control and the actions of eradication of this disease: Order no. 35/2016 regarding the approval of the Methodological Norms for the application of the Program of actions for surveillance, prevention, control and eradication of diseases in animals, of those transmissible from animals to humans, animal protection and environmental protection, identification and registration of cattle, pigs, sheep, goats and equidae as well as the methological norms for the implementation of the Program of supervision and control in the field of food safety; Order no. 49/2010 on the qualification of bovine holdings in relation to bovine tuberculosis and enzootic bovine leukosis; Order no. 104/2005 for the approval of the veterinary sanitary norm regarding the criteria for national plans for the accelerated eradication of brucellosis, tuberculosis and enzootic bovine leukosis; Order no. 105/205 on the approval of the veterinary sanitary norm which introduces national measures for the eradication of brucellosis, tuberculosis and bovine leukosis; Order no. 106/2015 on tuberculosis technique, interpretation of results and documents to be completed at the time of the action.

Non-invasive VOC sampling for the TB contamination in dairy cows

In Romania, the method of testing bovine and goat for the detection of tuberculosis is that through tuberculin skin test, an action regulated by a series of normative acts, as presented above. This article presents updates in the early detection and identification of susceptible and positive bTB cows, through non-invasive measures. These involve taking samples of air, faeces, and dermal tissue followed by analysis of volatile compounds from the collected samples.

The technique of sampling the expired air, dermal tissue (skin) and faecal matter implies the use of a certain device, as presented below, according to a strict protocol for sampling and under strict conditions of safety (nitrile gloves, protective masks N95, etc.). The cleaning of the sampling equipment is done with antituberculocidal disinfectant (6% acetic acid, for 30 minutes) and then rinsed very well with water and finally with methanol. For filtration, 0.2 µm (Polytetrafluoroethylene) filters, resistant to acids, bases and solvents and stable at high temperatures, which prevent the passage of bacteria, not viruses, into the Tenax tube used. For aspiration of volatile organic compounds (VOC), a pump (SKC AirChek) is used, attached to the entire sampling device (Figure 1).

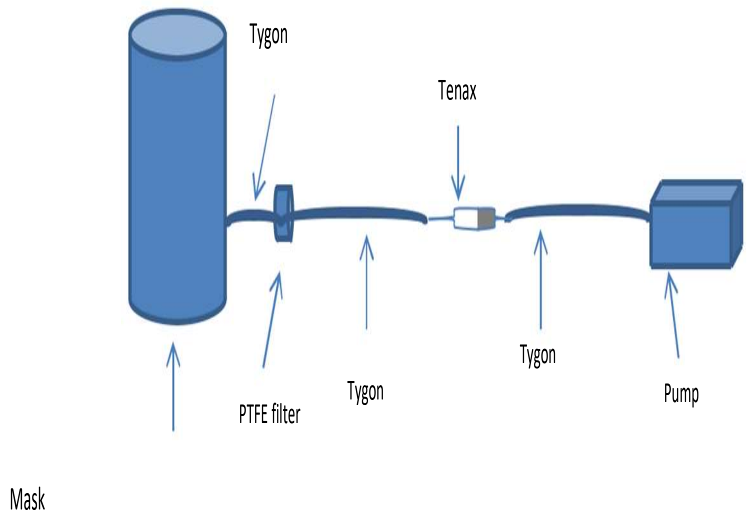

Figure 1. *Schematic of VOC sampling device assembly*
(source: *Instruction Guide for Volatile Organic Compound Collection from Cattle, "bTB-Test" project*)

The pump is operated with 1 l/min, for 1 minute, after which the Tenax tube related to the collected sample is removed, it is sealed and identified (no. sample, investigated animal, date, place of sampling). The tubes are conditioned until the moment of analysis at a temperature of 4˚C. For the collection of faecal matter the samples are taken manually from the animal's rectum. These can also be conditioned to storage at 4˚C, until the VOC is collected in laboratory conditions, preferably within maximum 24 hours after harvesting. For analysis, the faecal samples (5g) are brought to room temperature (approximate 25˚C), are introduced in a glass container and pumped with about 200 ml air/min, for 10 minutes, in order to collect VOC. After each analysis, we proceed as when taking the VOC sample from breath, sealing and labelling the Tenax tube. A similar procedure is also applied for collecting VOC from the skin of the animal, from the lateralcervical area, after a preliminary preparation of the skin, pumping 1l/min for 4 minutes.

Transmission of the infection (animal-animal, animal-human, human-animal)

In cattle, the evolution of tuberculosis infection is insidious, extends over several months and can even evolve throughout the life of the animal. Clinical manifestations may often not be obvious, even under internal injuries. Nearly 90 years ago, Williams and Hoy (1930) suggested in a study that out of every 1000 cattle, 100 were infected, but only 3 were clinically manifesting the disease. M. bovis is an extremely resistant mycobacterium in the environment, surviving in faeces 5 months in winter, 4 months in autumn, 2 months in summer, almost 2 years in soil (depending on certain environmental conditions), and 4 months in the manure stored underground [33]. However, these results are relative; microclimate conditions, temperature, precipitation, the number of mycobacteria as the source of contamination influence the degree of contamination of the cows, in the pasture. Also, a probable source of contamination of cows is their contact on grassland, with wild animals. Donnelly and Nouvellet (2013) show that badgers can be sources of transmission of bTB to cows, but “how much is due to them” and “how much is due to other factors” is difficult to establish. The results showed that only 6% (CI: 1%-25%) of the badger population (Meles meles) can transmit the disease; it follows the path of interspecies transmission, in proportion of 52% (CI: 9%-100%) and can reach up to 54% (CI: 3% to 66%) at 10 months, at slaughter [34].

The research has shown, since the first decade of the 20^{th} century, that at least 10 mg of bovine tuberculosis bacilli are needed to cause food-borne infections, while 0.01 mg (a dose 1,000 times lower) causes infection by inhalation. Dean et al. (2005) showed that <10 viable bacilli are sufficient to cause confirmed tuberculosis pathology. This study clearly demonstrated that 1 CFU of M. bovis (containing 6 to 10 viable bacilli) is capable of causing bovine tuberculosis. This infection in this case led to a pathology whose severity was equivalent to that observed in animals that received much higher doses (up to 1,000 CFU) [35]. The respiratory tract is accepted as the main method of infection spread to all species. However, it is clear that there are other less common methods of spread, such as oral, professional, congenital and through wounds [35-40]. An important source of contamination (for humans but also for animals that consume it, for example cattle) is milk from cattle with bTB. Analysis of milk in countries without bTB eradication programs continues to show similar levels of M. bovis detection [41-42]. Even in countries with a bTB eradication program, where unpasteurized milk is administered as regular feed to farm cattle, a high prevalence of infection will indicate the probable presence of one or more cows with M. bovis in milk, which will require an adequate epidemiological investigation [43]. M. bovis transmission between animals depends on a number of factors, including the frequency of excretion, the route of infection, the infectious dose, the period of contact with the source of infection, and the sensitivity of the host [44]. This perspective is also supported by studies in cattle, which have indicated that bacterial contamination is, at best, transient and involves an extremely small number of bacilli [45]. Also, in humans and badgers the risk of transmission increases as the disease progresses, and these species usually become extremely infectious only when the disease is advanced and a large number of organisms are excreted [46-47]. Little et al. (1982) demonstrated the transmission between naturally infected badgers and calves housed with them after a period of 6 months [48]. Field experience and current evidence indicate that cattle in the early stages of the disease do not routinely transmit M. bovis to the contact animals, infection [49-53]. Currently, they are recognized as reservoirs for M. bovis, wildlife species such as ruminants and cervids, and as species that can contact the bacillus through the consumption of infected carcasses, a series of carnivorous animals such as: wolf, fox, panther, bear, lion, rodents, etc. Transmission of TB to endangered species, such as chimpanzees [54] caused by Chimpanzee bacillus and rhinos by M. orygis [55], are examples of the challenges TB presents in the conservation of wildlife. M. orygis was first reported to the zoological antelope, initially considered as M. bovis, and subsequently confirmed as a subspecies and which has been isolated from many other animal species, including humans, from a wide geographical area [56].

People are infected by M. bovis, usually through milk, dairy products or meat from an infected animal. The pasteurization process, by rapidly heating and then cooling the milk, eliminates M. bovis from dairy products. Although it can affect people of any age, people with weakened immune systems, for example, those with HIV, are at increased risk. The infection may also occur from direct contact with a wound during slaughter of the contaminated animal, in hunting or by inhaling bacteria from the air exhaled by animals infected with M. bovis. Direct transmission from animals to humans through the air is considered to be rare, but M. bovis can be spread directly from person to person, by coughing or sneezing. Most people have a very low risk of being infected with M. bovis. People at higher risk include people who work with cattle, bison or cervidae (for example, deer or elk) or products from these animals, such as skins, milk or meat. bTB control and the role of the "human component" was supported by Fekadu et al. (2018) [57] cited by Byrne et al. (2019) [58] in a study on raw meat consumption by workers in a slaughterhouse in Ethiopia who, although aware of the risk of transmission of bTB and aware of public health measures, did not change their behaviour.

Transmission of infection from humans to animals is also possible. Contacting M. tuberculosis in cattle produces an infection that usually disappears rapidly, but animals can give positive reactions to tuberculosis, for a short period, which disappears with the removal of the source of infection [59-60]. In the situation where the infected animal is young and the herd was declared, in the previous test, negative for tuberculin, it is possible that the source of infection is human. The personnel from the farm with active TB can contaminate the animals by transmitting the mycobacteria through sputum, urine or faeces [61-63]. Also, some pets may have a risk of contamination with M. tuberculosis from positive owner with TB. Michael and Huchzermeyer (1998) report a case of contamination in a monkey species (Callithrix jacchus) raised as a pet in a household where staff was treated for TB 8 years prior to this episode. In fact, non-human primates are most threatened at this time by the risk of contamination of mycobacterial infection in humans [64]. Development of disease transmission models, diagnostic tests by which the differentiated animals can be accurately distinguished from the vaccinated ones need to be validated now and the research is still in the beginning, although the results are encouraging [65-66].

Conclusions

Tuberculosis remains even more than 135 years after the identification by Robert Koch of the Mycobacterium tuberculosis bacillus, one of the diseases that continue to affect humans, pets and wildlife. Its approach, from the perspective of human and veterinary medicine, is centred on the necessity to limit the transmission of the human disease, from the domestic and wild animals to humans, and from humans to animals.

New methods of detection such as the one presented in this study on the analysis of volatile compounds from respiratory, skin and faecal samples from susceptible or positive TB cows, establishing new reference standards for diagnostic tests, are necessary and useful at this time.

TB control strategies must be based at the same time on the protection of wild animals, the conservation of endangered species and the maintenance of the planet’s biodiversity as they are the host or tuberculosis transmission source.

It is necessary to continuously inform and educate the population regarding the control of the transmission of TB to other animals or to the domestic animals, in order to eradicate this zoonosis.

Acknowledgement

"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement Volatolomics test for the diagnosis of bovine tuberculosis No 777832."

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