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Indoor Dust Direct Examination (E.D.P.A.®) and biotic pollution in confined environments

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Abstract

Indoor biotic pollution is a condition in which living organisms cause infestations in confined environments. They are often represented by insects and mites of medical, agri-food, industrial or forensic interest, depending on the environment and on the point of view taken into consideration**.** Indoor infestations can generate several different problems, especially for people's health, due to the unavoidable interaction between humans and arthropods. The Indoor Dust Direct Examination (E.D.P.A.®) is a patented diagnostic method that enables to detect the traces left by insects and mites in confined environments and to isolate and identify the agent suspected to be the cause of an infestation by examining dust samples simply collected from the floor of every indoor area. Most cases of dermatitis of unknown environmental origin, for example, can be correctly diagnosed thanks to the E.D.P.A.®, that allows to identify the aetiologic agent, to discover where it is located, to discern if it is of indoor or outdoor origin and then to act with a targeted intervention to remove the cause, thus achieving spontaneous healing of dermopathy. In the agri-food field, the E.D.P.A.® enables to locate sites of larval infestation, to identify the species, to discover the origin of pests and to calculate the time of the infestation, in order to have the storage and the production sites of the factory under control. In the forensic field, the E.D.P.A.® can give also its contribution, allowing to find out if an indoor area of interest has been altered, by studying the biotic traces detected in the dust, such as arthropods, pollens, skin scales or other ones, discerning between their indoor or outdoor origin, evaluating their different concentrations, providing useful diagnostic data for further more detailed investigations. In conclusion, it is possible to successfully monitor the biotic pollution of any indoor environment for different purposes by examining dust samples with the E.D.P.A.® method.

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1. Introduction

Indoor infestations, when caused by insects and mites, can have very annoying effects to humans, such as respiratory or skin pathologies. In order to remove those problems it is therefore necessary to know the causes and to be able to act with targeted interventions on the environment (Principato, 2000; Principato et al., 1999).

Pathogenic arthropods present in confined environments are often almost invisible to the naked eye or are very small and hidden or camoufled in the dust. Until some time ago it was not possible to early unveil their presence indoor and one became aware of an infestation only when it had already reached an uncontrollable level. After many years of parasitological research, it was understood how many associations there may be between parasites living in domestic environments and some pathologies of unclear origin manifested by their inhabitants, above all those of dermatological nature. At the Urania Research Center an innovative and revolutionary method has been therefore developed and patented, enabling to highlight the traces left in the dust of any confined environment by pathogenic arthropods or pests: the Indoor Dust Direct Examination or E.D.P.A.® (Principato, 1999). At first this method was successfully applied to the medical field and later on also to the agri-food, industrial and forensic fields.

2. The Indoor Dust Direct Examination (E.D.P.A.®)

The E.D.P.A.® is performed on indoor dust samples collected manually by the patients themselves, by simply sweeping the entire surface of the floor of each room of their house, after shaking sheets, pillows, couch and chair upholstery. Dust collection has to be done preferably after a period of two or three days, during which no cleaning has been carried out, to get more significant and reliable samples of dust of the various house environments (Principato et al., 2014). The dust, placed by the patients into hermetic plastic jars, properly labelled with the name of each room, is then processed according to E.D.P.A.® as follows (Principato, 1998; Stingeni et al., 2017):

2.1 Sifting

The dust is sifted through 2 sieves having 5 mm and 1 mm wide meshes. These sieves allow to separate the material into 3 fractions:

- frustules of dust of large size (> 5 mm) for prominent sized arthropods (e.g., ticks);
- frustules of dust of medium size (1-5 mm) for medium sized arthropods (e.g., fleas, micro-hymenoptera);
- frustules of dust of small size (< 1 mm) for small sized arthropods (e.g., mites).

2.2 Dry examination

Direct observation of the material in a Petri dish is performed under a stereo microscope using different magnifications (0.75x-4x) to point out traces of arthropods (e.g., fragments of micro-hymenoptera, feces, eggs) and to study the acarological and entomological fragments. These fragments are collected by a flattened pin previously immersed in arabic

gum solution to make its surface sticky and placed in a Petri dish containing 80% lactic acid.

2.3 Liquid examination

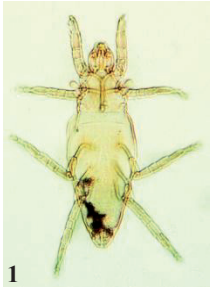
The sieved material is immersed in a saturated solution of NaCl for about 20 minutes (flotation). If the sieved material contains particular substances such as flour, soot or very fine dust, it is immersed in pure ethylic alcohol for about 1 hour (sedimentation). The sediment is first observed under a stereo microscope (0.75x-4x) and then under an optical microscope (4x; 10x). Organic fragments are then taken by a curved pin, adequately flattened on its extremity, and placed in a Petri dish containing 80% lactic acid to clarify entomological fragments and organic material for their identification.

2.4 Identification

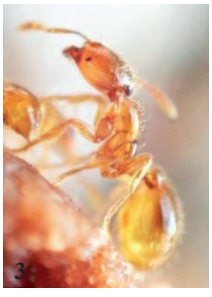
Arthropods fragments are extracted under a stereo microscope and then placed in a drop of Berlese solution on a slide. They are then studied under a phase contrast microscope to highlight the morphological features useful to identify the species.

3. Medical field

Among the fields of application of the E.D.P.A.®, the medical sector is the most relevant. One of the emerging health problems is in fact connected to the presence in a confined environment of pathogenic arthropods for humans. These arthropods often stealthily sneak into the houses attracted by the carbon dioxide that humans emit by breathing; other times humans themselves introduce them into the house, for example, by bringing firewood inside. Infested sites are often woodworm eaten wooden furniture, beams, parquet or other small objects kept in the house, which can be colonized by a fearsome mite, *Pyemotes ventricosus* (Fig. 1) (Principato & Polidori, 1993).



Figs. 1-2. *Pyemotes ventricosus* and its lesions on human skin



Figs. 3-4. *Solenopsis fugax* and its skin lesions

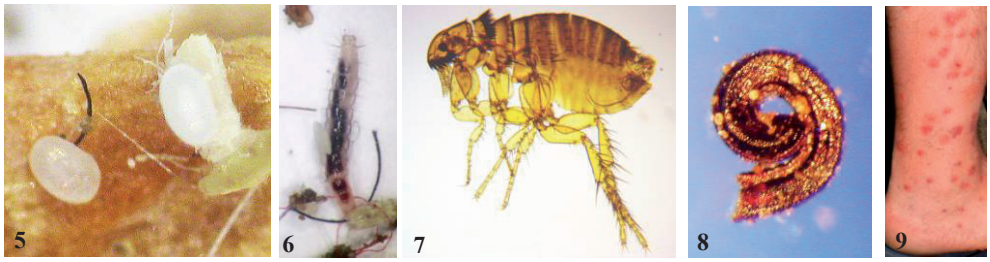
Sometimes environmental conditions may encourage multiplication of arthropods: this is the typical case of mites of the genera *Glycyphagus* and *Lepidoglyphus,* which live in conditions of high environmental humidity and feed on molds. They are strongly allergising and with their urticating bristles can cause dermatological and respiratory problems.

The most frequent clinical picture in humans with entomodermatosis is papular urticaria, characterised by erythematous-oedematous and papular lesions, that are

frequently centered by tiny vesicles, clinical picture preferably named “strophulus” (Fig. 2), instead of papular urticaria, which is frequently used to indicate skin diseases with different aetiologic and pathogenic mechanisms. Other clinical pictures are erythematous and escoriated papules, urticaria and scabies-like lesions, erythematous papules and pustules, vesicle-bullous lesions (Fig. 4). Only rarely can the lesions appear such as varicella-like and erythema-multiform like eruption (Principato et al., 2014; Stingeni et al., 2017). Several cases of dermatitis of unknown environmental origin could be correctly diagnosed with the E.D.P.A.®, which allowed to identify the aetiologic agent, to discover where it was located, to discern if it was of indoor or outdoor origin, and then to act with targeted interventions on the environment to remove the cause, thus achieving spontaneous healing of human dermopathy (Principato, 2005). With E.D.P.A.® it was possible even to diagnose cases of scabies at distance, by detecting the presence of the mite responsible for this disease (*Sarcoptes scabiei*) in the skin scales present in the dust of patients' bedrooms.

In the year 2017, in house dust samples of n. 270 individuals suffering from dermatitis examined at Urania Research Center, the presence of mites above all of the genera *Pyemotes* (33.3%), *Glycyphagus* (16.2%) and *Tydeus* (14.8%) was detected and in other n. 202 house dust samples there was recorded the presence of insects, above all of the genera *Scleroderma* (13.8%), *Solenopsis* (17.3%) (Fig. 3) and *Ctenocephalides* (9.9%) (Figs. 5-9).

The examination of the dust samples through the E.D.P.A.® enabled to understand the origin of the arthropods. For example, *Pyemotes ventricosus* and *Scleroderma domesticum* turned out to be associated with woodworm eaten wooden furniture, beams or parquet, *Glycyphagus domesticus* with wall molds and environmental high humidity (75%-80% RH), *Tydeus molestus* with plants, *Ctenocephalides felis* with the presence of domestic animals and *Solenopsis fugax* with food storage. The confirmation that each one of the arthropods detected through the E.D.P.A.® was the real cause of dermatological problems was demonstrated by the patients’ quick healing after the targeted house disinfestation.



Figs. 5-9. *Ctenocephalides felis*: 5. Egg 6. Larva 7. Adult 8. Feces 9. Skin lesions by *C. felis*.

4. Agri-food and industrial field

The possibility of adapting the E.D.P.A.® to pest control systems to avoid economic losses of agri-food companies has been also studied. The E.D.P.A.® is, at present, the only industrially applicable method enabling to unveil the presence of creeping arthropods and outbreaks of their larvae, which cannot be detected through other methods currently in use, such as pheromone or light traps.

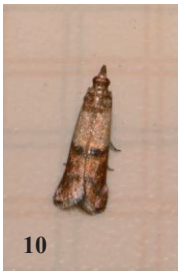
The E.D.P.A.®, by identifying larval outbreaks of pathogenic insects and mites and pests even before they damage food products or human health, allows to treat the environment in a targeted way, only where necessary, with less environmental impact and more significant

economic savings, a matter of primary importance for companies. (Principato et al., 2004; Consonni, 2005).

The monitoring carried out in a pet food factory with E.D.P.A.® from 17 fixed points gave as a result the identification of larval outbreaks of some pests, such as *Plodia interpunctella* (Figs. 10, 11, 12), *Ephestia kuehniella*, *Tribolium confusum*, *Oryzaephilus surinamensis*, *Stegobium paniceum*, recording also the presence of a pest of recent spread in Italy: *Necrobia rufipes*. Where the infestation was low, no corrective action was taken; where the infestation was high and in the sites where outbreaks of larvae were found, a targeted treatment was performed using pyrethroids. In a ham factory and in a dairy the examination of samples was useful to identify the species of pests: *Acarus siro* in the dairy (Figs. 8, 9) and *Tyrophagus putrescentiae* (Figs. 10, 11) in the ham factory. Moreover the infestation rate could be checked, which turned out to be strongly lowered after the application of ALISTAG™ (https://www.alistag.it), a food grade coating agent able to provide a protective coating to cured hams and cheeses, favouring their natural aging process (Principato et al., 2018).

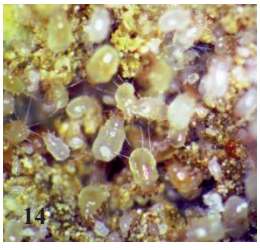
All that emphasises how the use of E.D.P.A.® in the agri-food productive chain can allow to obtain the following advantages:

- to identify the infesting species and their development outbreaks;
- to monitor the infestation after the corrective actions carried out specifically;
- to reduce the use of biocides;
- to control the frequency of disinfestation and treatment sites;
- to reduce the risk of infestations and damage to food products and raw materials;
- finally, but not less important, to identify also the possible presence of pathogenic arthropods (e.g., *Glycyphagus domesticus, Lepidoglyphus destructor, Cephalonomia gallicola*), which can be responsible for occupational diseases, in particular entomodermatoses, in the sector operators.



Figs. 10-11. *Plodia interpunctella* adult and larva

Fig. 12. Feed infested by *P. interpunctella*



Figs. 13-14. Cheese infested by *Acarus siro*

Figs. 15-16. Ham infested by *Tyrophagus putrescentiae*

The systematic use of E.D.P.A.®, therefore, has shown not only an immediate effectiveness in identifying the infesting species and the rate of environmental infestation, but also a long-term efficacy, highlighted by a reduction of the infestations as a result of highly targeted corrective actions with the consequent reduction of the risks of expensive damage to goods and humans.

Through E.D.P.A.® it is possible to make an excellent environmental prevention also in hotels, trains, ships, for example, with a constant monitoring of *Cimex lectularius* (bedbugs), whose onset can be unveiled even before this insect can reproduce to excess, making itself evident to the customers.

5. Forensic field

The study of house dusts carried out over the years has led to refine the E.D.P.A.® technique, so that its application can be taken into consideration in fields other than those strictly parasitological ones for which it was initially developed. One of those of particular interest is the forensic field, where this technique can be used to help investigators in identifying important traces on the crime scene in confined environments, discerning their indoor or outdoor origin from the different concentrations of the population of mites and insects or of pollens and allows to find out if an area of interest has been alterated (Principato et al., 2016).

To this aim, in September-December 2017, the dusts collected in 20 houses located in the area of Perugia (Central Italy) were examined at the Urania Research Center. For each house the dust of 2 bedrooms were analysed, for a total of 40 samples. In 22 samples the presence of the so-called "mite of the mattress" (*Dermatophagoides farinae*) was detected, with a charge ranging between 82 and 110 mites/g of dust. In 11 of 22 positive rooms, new samples were taken for three days (one sampling every 12 hours) after having washed the entire floor surface, while in the remaining 11 rooms only the central part of the floor was washed and the dust samples were collected separately from the washed surface and from the uncleaned perimeter. The results obtained were particularly interesting: the E.D.P.A.® was negative in the 36 hours following the cleaning in the 11 fully washed rooms and in the samples collected at the central area of those washed only partially; afterwards the *Dermatophagoides* mites were found again in increasing numbers, up to a maximum of 43 mites/g at the end of the third day. On the other hand, the sampling performed on the unwashed internal perimeter showed a similar concentration of mites (75-98 mites/g) compared to the initial sampling. An area of the cleaned floor can therefore be easily highlighted for an alteration of the traces or, at least, for their non-homogeneity. A comparison can be done between the density of the acarofauna and of the other traces present in different areas of the crime scene, in particular around the victim or, in any case, on the site where the crime is supposed to have been committed and along the perimeter of the room. This may provide diagnostic data able to clarify if the crime scene has been altered by environmental clean-up operations. The various dust samples can also contain skin scales or other traces that may have different origin and that, once isolated by means of the E.D.P.A.®, can be made available to the investigators for further and more detailed investigations.

6. Conclusions

In conclusion, the E.D.P.A.® (Indoor Dust Direct Examination), enabling to detect the presence in any confined environment of pathogenic or infesting arthropods, even at their

larval stages, allows to do monitoring for different purposes by examining dust samples. These can be sent to Urania Research Center (http://www.edpa.it) from all over the world, since the collected material does not undergo damage during shipping, nor it alters for even long delivery times.

The advantage in adopting this test in any indoor area is to ensure environmental safety under the parasitological profile, being able to make prevention and to have the situation under control.

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