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Commentary

Homo sapiens against Paenibacillus larvae: Global Beekeeping and "Worst-Case Scenarios" Play

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American foulbrood (AFB, *Pestis apium)* is a notifiable contagious bacterial disease of honey bee brood, *Apis mellifera*, that is globally propagated with huge environmental and economic impacts. There is no effective drugs or preventive medicine in terms of registered vaccines, serums, or genetically modified therapeutics that can eliminate the causative agent bacterium *Paenibacillus larvae*. In case of inadequate control AFB can be propagated for many years. Pharmacological control from the domain ad. us. vet. is not accepted in the same way by veterinary consensus in many countries. Antibiotic treatment is not effective because it does not affect spores that are the source of infection for honey bee larvae. Modern beekeeping practices in some countries with their technological solutions, ignored mechanisms of natural history and sustainability honey bee colonies and that is how they increase AFB incidence.

In the existing stage of development science and practice, it is almost impossible to talk about AFB disease eradication and all legal recommendations should relate to ways to ongoing control AFB. The intention of this opinion presentation or mental position is not to add more citations but rather to tackle common sense directing the more complex challenges that we ignore as veterinarians.

Beehive is a very complex and dynamic epidemiological unit of an animal species that existing in the form of social organism and where management practices does not look like in other animal farms. At the same time, one should imagine whether the apiary today is nothing but a specific farm unit that depends on the one who manages its sustainability. The farmer in this case is beekeeper. Veterinary medicine deals with problems of bee health, public health which can be affected by bee products, as well as the welfare of the bee community as an "equal" livestock branch. The epizootiology of AFB is complex. Complexity is in interactions between beekeepers, environment, bee community, agricultural practices, legislations, economic regulations and consumer perception.

Globally attitude towards this infectious disease, generally do not contribute to sustainable control. Entropy of control procedures and choice of strategies in maintaining bee health globally is so unjustifiably different that it seems that the legislative framework must first cope with the limited knowledge of basic scientific knowledge in the field of veterinary medicine and veterinary epidemiology. To understand this, it is recommended to visit the World Animal Health Information System (https://wahis.oie.int/#/home) *Paenibacillus larvae* disease maps. There are verified research recommendations (https://coloss.org/beebook/) and diagnostic procedures available globally (https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/) to serve all who need know more about the disease but they cannot change current practices in beekeeping managements. The golden era of honeybee research, beekeeping institutes with extremely applicable diagnostic technologies, amazing numbers of scientific papers, and global initiatives "to save bees" are present but still, in bee yards, some parts of the world are fighting against the centuries-old microbe with rude dogma.

The sum of control activities for AFB refers to the need for timely detection of suspected or disease typically changed bee communities. Underlying this need is an awareness of the risk factors involved in a scenario of good or bad disease control in an area through good veterinary and good beekeeping practices. Metaphylaxis is wildly accepted concept in some parts of the world in disease response. This is a treatment based on the fact that antibiotics used in hives, can prevent replication of the vegetative bacteria and suppress clinically visible

infection manifestations in the brood. This therapy does not affect the spores present in infected and treated colonies as the main infection source in an outbreak area. This is the perfect scenario for long-lasting interference with the bee gut microbiome and also a perfect way to develop Anti-Microbial Resistance among *Paenibacillus larvae* populations. There are no vaccine protocol or has not yet been developed in practice to control this disease so the total amount of money associated with pharmaceutical control of disease in some areas goes to antibiotics and disinfectants lately.

In addition to pollinating plants and making beekeepers happy, bees produce food for humans. This is a fact that burdens the producer in relation to product safety. At least that is part of the quality standard that producer or veterinary service in a country can organize. Medicine, even veterinary medicine, is nothing but a specific policy that regulates some relationships. Relations in the control of this disease are related not only to the knowledge of doctrines in the field of life sciences but also directly to the fact of how much a country is able to finance created control measures and how much such "state" manages the ownership function of protecting a national resource. Simply put, whether state leaves the control measures at the expense of the producer or wants and can transfer the measures at the expense of the state budget and thus manage the challenge of production. Once the state becomes the owner of a contagious disease, the prescribed measures are enforceable. However, this happens when costs control does not exceed the possibility of compensating payment from outbreak area.

The dogma is that antibiotics cure AFB. Control an infectious disease using antibiotics is a bad choice in the case of AFB. They mask the epidemiological facts with high ecological price. That is recognized literary available without the need for special review. When causative agent of AFB appears for the first time on site, its "bumps" enzootically and becomes present not only in the hive but also in environment. It is difficult to treat environment. The bee community environment is on the first level "apartment" in which they live, so the hive, or let's call it a farm unit, is one epidemiological unit. In doing so, the second envelope is the external environment on whose territory the infected community is located. Regardless of the number of hives, we are talking about a hotspot, an outbreak, in which the presence of bacteria spores that are very resistant to all- natural environmental conditions, is propagated. This is how a district is created. AFB, pestis of bees, the plague of the bee community can be propagated over the years with a more or less developed manifestation of the clinical pictures. If we like to draw attention between how *Bacillus anthracis* and *Paenibacillus larvae* are close in some ecological characteristics, then understanding the significance in the control of the most important infectious bee disease (historically speaking) would be more meaningfully organized.

Hygienic bee behavior of any selective range, currently, have no practical infection regulating proof significance against AFB in field conditions. Worker bees that clean the spores of infected larvae in the late or early stages of infection are actually just transmitters of the causative agent. So, creation genetically superior bees in relation to the trait of cleaning for this disease have to be evaluated in real conditions not just laboratory setups. Experiments that have tried to prove the difference between hygienically developed traits in the control of AFB should be meaningfully proofed. Simply "cleaning up" the spores is very different from cleaning up a frozen or punctured larva that is not infected with *Paenibacillus larvae*, which in clinical chronic cases with AFB infections would be just a pure spore load.

Antibiotics usage seeks to mask the visibility of the clinical presence of the AFB disease. The infectious agent is present in a given area, infections are masked and the causative agent acquires genetic resistance to a particular antibiotic. Such a harsh denying attitude towards knowledge of pathogens results in various bad practices that give the infection even more on bad outcomes. According to the types of antibiotics used, some strains of *Paenibacillus larvae* acquired resistance to the used antibiotics, such as penicillin, streptomycin, geomycin, lincomycin and tylosin and we can talk about resistant clusters of *Paenibacillus*, which in medical classifications for beekeeping, could be considered as honey bee superbug. There is a lot of evidences about disorder of the bee microbiome, dysbiosis caused by the use of antibiotics, but the reasons for the "abuse of the use" of antimicrobial formulations are ignored. This is certainly a direct detrimental effect on the health of the bee as a social organism. It is difficult to prove that the use of probiotics in the forms intended for bee nutrition helps to restore once disturbed physiological status of the short-lived insect, which is a part of the dynamic social organism, honey bee community.

Identifying the typical clinical picture of AFB in the field is not difficult. The clinical form of the disease phase for those less proficient can go unnoticed, most often in the early and very late stages of the disease. Once established on territory, resilient spore forming bacterium, remains long potential danger for bees. Methods of environmental "remediation" are not clearly defined and are very difficult to implement efficiently. In favorable conditions dormant spores can be easily transformed into an effective vegetative form and depending of dynamics in bee community development they will continue infectious mission.

Bees have not been the focus of veterinary medicine in many countries so this omission in understanding concept of significance has deep roots; financially, the value of services to veterinarians in the case of beekeeping services are underestimated in relation to the income provided by working with other animal species. Failure of beekeeping practices and self-help directions in controlling AFB are common misconceptions. There are differences between "animal health and animal welfare" practices on the continents because the experiences with this infection were shaped by the time when historically countries encountered the plague on their own territory. Knowing how to combat the spore-causing bacterial agent and in particularly demanding cohabitation communities such as the bee community is a high attitude that should be harmonized with the best possible practices to disease control.

Question is how much money should be available for control, how much we are willing to take risks by doing or not doing something. The established perspectives of disease control so far are only a part of the experiential screen of the time in which measures were prescribed and proposed, and they are not based on clear scientific answers and experimental actions. Knowing how complicated the control of AFB is, the Republika Srpska in Bosnia and Herzegovina, has introduced a preventive method of controlling the presence AFB spores in honey samples since 2010-2021, whether it is a honey sample from bee colonies or a bulk sample of honey from group beehives. It should certainly be said that the use of antibiotics in hives has been banned for more than twenty years. Based on the free diagnostic service provided to beekeepers, we have significantly improved control of this disease by facing the fact of how difficult it is to influence awareness and changing practices among beekeepers. It takes time to change bad practices. It was necessary to prepare a better legislative framework in order to create the conditions for the adopted good practice to apply and for that application to be comprehensive because without comprehensiveness and in a large percentage there is no way to control disease. The result of certainly intensive information campaigns on the importance of disease control has contributed to the fact that Bosnia and Herzegovina in the Republika Srpska is actually the only territory in the region where the veterinary service has the possibility for preventive action before clinically typical images of the AFB appear clinically. That surveillance program brought a lot of data.

We have verified that two different pheno- genotypes of *Paenibacillus larvae* ERIC I and *Paenibacillus larvae* and ERIC II can be found in apiaries in a narrow area. None of them give off a special smell or stench that could be taken as a pathognomonic sign of suspected infection. Simply put: stench is not a characteristic of AFB and thus cannot be taken as a subjectively recognized symptom of disease. The consequence of not knowing the distinction between European Foulbrood (EFB) and AFB has led to confusion among beekeepers globally. What strongly and typically stinks in hives and apiaries with rotten broods are pathological putrefaction processes caused by a complex of bacteria, of which the sporogenic bacillus, *Paenibacillus alvei* is particularly interesting in the complex of clinical pictures of EFB syndrome in our region. In exceptional, rare cases, mixed infection with EFB and AFB agents can be found, in which case the odor is not part of the AFB pathology complex.

Knowing the genetic variations of causative agent and recognizing the clinical picture of the disease is very important for those who are trying to control the disease. An aggravating fact in knowing the course of clinical picture of different phenotypes and genotypes of *Paenibacillus larvae* refers to the existing ban on experimental field research in some countries on a real model of the bee community. Unlike some other bacterial diseases, and specifically for the life of the bee community, in many countries, no field experiments are allowed. This is a missing piece in disease mosaic visibility. Otherwise, it would bring better determination eco-genotype specificity pathogenic effects in course real-time infection.

In the host-pathogen relationship, each genotype may have differences concerning the time of incubation in the hive, the virulence or pathogenicity of the process, as well as the possibility of chronic forms of subclinical infections. Spatial and temporal disease propagation have not been experimentally scientifically confirmed. The course of our proposed action AFB control was: "test and neutralize", not "ignore and treat". Diseased hives (laboratory positive and clinically positive) are burned under veterinary inspection supervision. Other "clinically uninfected" communities in outbreak area were screened bacteriologically for spore presence in honey hive samples from brood chambers and based on the results they were managed usually through artificial swarming recommendation. Clinically negative hives in an infected apiary are monitored for the presence of spores in the honey of each hive until the infectious pressure is reduced to a level when it becomes negligible. The biggest question was and still is: for how long this process of reducing infectious pressure can take by enforcing effective regulations. As an example: If inspection procedures are not able to suppresses AFB within two years, the apiary should be closed completely because either there is no good beekeeping practice or there is no good veterinary practice, so the legislator or the state is not obliged to pay for inadequacy due to inadequacy those who implement the measures. This is just one option that could be a template for sustainable risk management in preventive control measures.

Good veterinary practice and good beekeeping practice in the apiary are esoteric terms for insufficiently harmonized procedures of both. Talking about good practices in a country that does not have control over the AFB spores of all wax processing and bee food production facilities is far from what is called good practice. And that is an issue in majority countries in the world. Use antibiotics in AFB control is a mistaken harmful control mechanism without significant effect on spore load in honey. Honey dilution process on the market can change the MRL limits of some antibiotic's residues but spores in even minimal level of dilutions are there. Communities that have American foulbrood suffering from the intensity of infection or destruction of the organization of the bee community may still have yields in honey and other bee products. American foulbrood is not a zoonosis unless bee products unusually enter the human body through medical misuse of products through intravenous intake. Such honey is contaminated with *Paenibacillus larvae* spores as in hive contamination or extraction procedures. Antimicrobial resistance information is transmitted through honey in which there are spores, which are then obtained through food intake (honey) to the human digestive tract. Eating honey with spores and other products (edible) that contain spores will not trigger a chain of infection in humans when the spores are ingested orally. Thus, tissue tropism and the type of organ that encounters the antigen determine the fate of contamination or infection. Human digestive tract, unlike that of the bee, is a very complex and numerous cohabitation complex called the microbiome. It is not much known about how ingested spores passing all human body environment niche non host species. Question is: are they potentially have opportunity to share information code with other microorganisms that structure the microbiome of the human digestive tract? Of course, this process is not obvious and of course there are no elements of emergencies for humans, but certainly with shares in genetic information between bacteria, there is a possibility of constructing very significant bad scenarios for humans and their health. Can spores superbug *Paenibacillus larvae* be a source of resistance gene donation and is it of any significance? Translational medicine discipline needs to understand dynamics of passage of honey spores along the digestive tract to the final destination. This "digestive corridor" is likely to be able to disintegrate and destroy the genetic packaging of the life form of a sporogenic bacterium. But what if another scenario is possible? And then question remains on how microbiome of *Homo sapiens* reacts to random contamination *Paenibacillus larvae*. Can shield around spores become permeable and let the immortal bacterial DNA flow into the digestive and immune processes. Can gen of bacterial resistance be integrated into a part of the genome of a commensal bacterium of human microbiome?

Honey bee animal welfare is not a hot spot in veterinary legislations but using and abusing antibiotics to cure ignorance is a direct violation veterinary profession. Animal welfare is important for animal husbandry and beekeepers and veterinarians have to know that is not just production, pollination, profit but insects need of life quality. Physical and social environment is largely changed in global settlements where we live. It is necessary to understand bad practices and change the whole environment event. We need to save honey bees with tools that we already have. The complex concept behaviors of bees are related to their health and welfare status. And that can improve their sustainability as an endangered species. Honey, bee bred, pollen, royal jelly are not just food they are a medicine that hu-

mans consume. If we do not see the intelligent impacts from veterinary practices it is a question how safe is food or drug in this context. Veterinarians urgently have to discuss and change today's ignorance on bad beekeeping and veterinary practices in response to *Paenibacillus larvae* infections.

Finally, the question arises: Why the already obviously acquired knowledge about the agent cannot be harmonized to better health protection and honest animal welfare?

Picture 1: *AFB suspected brood pattern that is actually EFB case.*

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