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The Great PCR and HPAI Mistake

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Abstract

Despite Kary Mullis PhD saying that PCR testing cannot tell if there is disease present, or if an individual is infectious, it has become a commonly used test in veterinary medicine for certain infections. This review paper explains why clinical diagnosis and use as a standalone technique in research constitutes inappropriate usage of PCR and risks establishing false information as fact. Combined with the misinterpretation of results due to a seeming ignorance of the high incidence of false positives where disease prevalence is low, this is leading to the over-diagnosis of Highly Pathogenic Avian Influenza in poultry and other species, and potentially other diseases too with serious implications and consequences depending on policies. When it comes to HPAI and other notifiable disease policies we must not abandon common sense nor lose sight of what is an achievable end goal that doesn't cull unnecessarily and maintains farming viability going forward.

Keywords: PCR, HPAI

The Polymerase Chain Reaction (PCR) test was developed by Dr Kary Mullis for which he was awarded a Nobel Prize. He developed it **for use within a clean laboratory environment only** to help **detect the existence of a known sequence** of DNA/RNA present only in the tiniest amount **amongst other known sequences** that it would be virtually impossible to detect by any other means. I've added bold enhancement to this explanation to accentuate a crucially important subtlety that it is essential to grasp in order to understand why PCR is totally inappropriate for disease diagnosis in a field clinical situation.

As Dr Kary Mullis explained, PCR testing was not developed for and should NOT be used to diagnose disease and infer whether an individual is infective.

When the original genetic material includes the looked-for sequence, each cycle of the PCR process doubles the starting amount which means it increases exponentially at each cycle, i.e. 1 becomes 2 becomes 4 becomes 8 becomes 16 etc. There is research (1) that explains that if a positive PCR test result is only obtained by detecting the looked-for sequence following amplification of the original sample through more than 23 PCR test cycles, then the original sample did not contain sufficient genetic material to be considered infectious, assuming it came from a whole viable organism. For COVID testing, and many of the PCR tests now being inappropriately used to diagnose disease, the PCR test is being cycled more than 40 times. If you are presented with a PCR Test result it is therefore clini-

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cally meaningless without knowing this Ct number that denotes how many cycles were performed.

It is crucial to understand that PCR only detects the presence of a selected short sequence of genetic material, not the whole sequence of the whole organism. It will therefore detect this sequence whether the organism it came from was viable at the time of swabbing, whether it was a residual fragment having already been recently dealt with successfully by the immune system or had been otherwise broken down and shed by another individual and was an incidental finding on/in the individual being swabbed. PCR cannot differentiate whether the detected primer sequence came from the intended target organism or an entirely different organism. It will detect the sequence whether it includes a mutation and originated from another organism or not and cannot distinguish the difference.

People, animals and plants exist in a microbiome soup of bacteria, viruses, protozoa, fungi etc – including not just whole organisms but fragments of them too according to their lifecycle stage, immune interference, and/or influences from other elements of the microbiome that are in competition with each other. All of these pathogens are constantly mutating at differing rates and will exist in different variant forms; some that we know about, and others that we don't. We certainly don't have all possible genetic sequences for the whole microbiome within a swabbed area, let alone know which detected sequence belongs to which individual organism in the microbiome!

With the best swabbing technique and will in the world, any swab taken from a person or animal will inevitably be a sample of the microbiome soup (plus contamination) at that area. It will contain microorganisms of all types of unknown genetic sequences and will contain other cells too – from the individual being swabbed plus whatever other sloughed cells they might have on them or have breathed in from the environment. I would expect that unless the person doing the swabbing is wearing a Hazmat suit there will also be their cells and other contamination too. Under those circumstances, cycling a PCR test 40+ times has a significant chance of a false positive, by which I mean the result will be clinically insignificant.

Question: If we consider how this relates to HPAI now, which of the following 8 veterinary viruses is Highly Pathogenic Avian Influenza (**Figure 1**)?

Answer: I don't know if any of them are, but if one/all happen to contain the looked-for nucleotide sequence known to be present in HPAI then they will be diagnosed as such. The tested individual (bird/cattle/person) will not only be diagnosed as having Highly Pathogenic Avian Influenza (HPAI) but will be highly infectious too. The same principle applies to any other pathogen potentially being looked for using PCR. If there is a cull policy in place, then that individual and potentially the whole farm will be euthanised based on the above test result.

Avian Influenza "detection" by PCR is currently happening in the USA on all types of farms with pressure to increase how widespread and how frequently screening will be carried out. This will inevitably lead to a high number of false positives.

If we look at the HPAI situation in more depth, our understanding of the virus itself may not be as reliable as we would like to think. We need to keep a proper perspective on what we are trying to achieve with a realistic and achievable end goal in focus. To date, there are no confirmed cases of human HPAI infection from eating eggs, though handling contaminated fresh raw eggs could potentially pose a risk if proper hygiene is not followed, **and** if the person is severely immuno-compromised (2).

Survival in the environment

When it comes to how long HPAI survives in the environment, there would appear to be no justification whatsoever for banning restocking for a year as is currently the rule in the UK. Similarly, HPAI survival in the chicken feed is finite too which again indicates that the requirement to destroy the feed is OTT unless the normal life of the food is less than the delay a shorter stocking ban as determined by the following research evidence would dictate.

Highly pathogenic avian influenza (HPAI) viruses, particularly H5N1, can persist in the environment depending on temperature, humidity, and the type of surface or medium. Here's a summary of evidence on its environmental persistence:

Survival in water

HPAI viruses can survive in water for weeks to months, particularly at low temperatures. Provided water drinkers are replaced and pipes flushed with a suitable disinfectant I don't think this is relevant to the survival on the farm overall.

- Cold water (≤4°C or 39°F): Studies have shown that H5N1 can persist for more than 30 days in cold water (e.g., lakes, ponds, or drinking water sources for birds). Provided there are no ponds or lakes on the property this is irrelevant. Even

for a lake, I would imagine that the dilution factor alone would make it nigh on impossible for infection to come from this source.

- Warmer water (22°C or 72°F): Survival decreases significantly, with the virus persisting for about 4 days. During summer months, ponds and small shallow lakes may well reach these temperatures assuming they've lasted that long to get to summer. Again, if there's no direct access for the chickens to bodies of water such as these on the farm, this is irrelevant.

There was one study in 2014 by Brown et al. (3) who found that HPAI H5N1 could remain infectious in distilled water for similar lengths of time to LPAI (Low Pathogen Avian Influenza) which has more implications for the wild bird population than for commercial poultry as discussed later.

Survival on surfaces (4)

- On porous surfaces (e.g., feathers, soil, straw), HPAI can persist for several hours to days. Days. Not weeks or months, and certainly far less than the stocking ban year.
- On non-porous surfaces (e.g., metal, plastic), it survives longer, sometimes up to 48 hours at room temperature. If anybody has ever been inside a commercial poultry barn, the temperature from all the birds' body heat is easily what I would consider to be room temperature. Barns have heating systems for when young birds first arrive, and their combined body temperatures are not yet sufficient to generate enough heat to keep the barn warm. Farmers want energy from feed to go into growing (meat) or producing eggs and not keeping warm, so heat can be controlled to optimise this. There is therefore facility to warm barns to eradicate HPAI in surfaces after a barn has been cleared of birds.
- At 4°C, survival extends significantly - potentially weeks. Poultry barns don't stay at 4°C for extended periods of time if at all, unless rested between batches during the winter.

Survival in soil and faeces

- In bird faeces, HPAI can survive for up to 35 days at 4°C and for a week or less at 20°C. This is one of primary routes of infection from wild birds to domesticated poultry flocks.
- Soil provides some protection, with survival depending on moisture content and organic material.

Survival in carcasses

Decomposing bird carcasses can harbour infectious HPAI for several weeks, particularly in cold conditions. Carcass decomposition in warmer temperatures speeds up viral degradation. Again, this is perhaps relevant for wild birds that can potentially act as a source of infection to water and/or living birds that may feed on carrion. This is irrelevant to a farming situation.

Research

As you might expect, there has been lots of research into Avian Influenza and specifically HPAI as well as LPAI. For reasons previously explained, PCR testing is being inappropriately used and interpreted in clinical situations and unless confirmed by virus isolation I question the validity of much of the research that our supposed knowledge is based upon. For example, there was a study that assessed feed samples from commercial poultry facilities that experienced HPAI H5N2 outbreaks. Some feedstuffs tested positive for Avian Influenza Virus RNA via real-time RT-PCR but were negative when subjected to virus isolation tests. This was assumed to indicate the presence of AIV genetic material without evidence of viable, infectious virus. How do the researchers even know if what the PCR test detected originated from an AI virus?!! I would contest that they don't know whether the PCR result was a false positive from another origin altogether, or a snippet of Avian Influenza which was unviable. Without the virus isolation it would no doubt have been assumed that the feed was infectious. It begs the question as to how good our knowledge of HPAI is at all if we're "seeing" viable virus where it isn't present in reality?

A potential example of this issue is a recent preprint paper (5) that discusses the possible role that airborne spread of HPAI might have in the epidemiology of the disease taking into consideration meteorological data and sampling at different geographical locations. Researchers relied totally on PCR to detect HPAI with no confirmatory virus isolation. No attempt was made to determine whether what had been sequenced was from a viable virus capable of initiating infection. Consequently, the suggested conclusion is that HPAI can be spread by the wind with obvious implications for biosecurity, exclusion zones and barn ventilation. It goes against everything that has been long established for the viability of viruses outside the body and their survivability in the environment. If extrapolated to other situations it makes any 2m rule as introduced in the UK during

COVID even more laughable than it already is. The problem is that no common sense is being brought to bear, and the fact that the collected data goes against all the past observations on viral spread is being ignored based on the trust being put into PCR. The biggest problem is that policies may well be introduced on the back of it that are equally ludicrous. If airborne transmission were as possible as the preprint suggests then why wouldn't all barns on a property be equally infected at the same time from the original source, and why wouldn't it spread between all barns very quickly? As brilliant physicist Richard Feynman once said, if a theory doesn't match the observation, then the theory is wrong.

The problem with HPAI is that it is highly infectious with high mortality. However, without virus isolation confirmation we cannot trust or rely on PCR testing alone in a clinical situation. Without sufficient birds with significant symptoms present on a farm, I don't consider that PCR can be relied upon as a screening tool unless we know the false positive rate. Even then, what work has been done to look at the likelihood of birds with a respiratory infection other than HPAI testing positive under PCR for HPAI? I would include LPAI in this for which we already know there can be cross-reactivity. Any healthy birds tested that give a positive PCR result are automatically assumed to have HPAI which precludes any other possible cause.

If there is an outbreak of respiratory disease in a barn on a farm, and birds start dying quickly and it spreads quickly between other birds in the barn, then it's not unreasonable to check for HPAI. I get that and cannot disagree with doing so. If there is a high probability that a significant number of the birds in the infected barn are going to die, and survivors are going to be compromised in terms of production such that they are no longer commercially viable then I can see why a fast cull of that barn only may be justified on the basis of minimising the spread to any other barns on the farm. Biosecurity measures can be taken to ensure there is minimal risk of passing infection on to healthy barns.

What I'm struggling with is the current total farm cull policy including many thousands of healthy birds when there is nothing in place to control the wild bird source which acts as a reservoir for domesticated birds. To be clear, I'm not advocating the mass slaughter of wild birds, especially waterfowl who are regarded as the main reservoir and who seem more resistant to expressing HPAI than our domesticated poultry and other wild birds.

However, we need to address the Dumbo in the room! HPAI is not going away unless we discover a way of controlling it in the wild population, and I don't see any possibility of that happening. If we carry on as we have started just because HPAI is a notifiable disease in the UK, we seem to be heading down the same road we took with F&M in 2001 where thousands of healthy cattle, sheep and goats were slaughtered unnecessarily. There isn't even an epicentre that could be ring fenced to stop it spreading. Over time we will have decimated our National Flock of poultry but will be no further forward in controlling HPAI. Wild birds have no boundaries and will fly wherever they like. The reservoir in the wild will remain in some sort of equilibrium such that HPAI never goes away but will likely attenuate to a less highly pathogenic form over time.

What's the end goal?

We must ask what is the achievable end goal that we are aiming for here? Vaccination for HPAI would be ineffective and is not a viable option because the HPAI virus mutates quickly and would escape the protection, plus it is a huge expense. It arguably weakens the immune system to other infections too.

We need to explore other solutions to protect our domesticated poultry, and we need to be very much more aware of using PCR alone both clinically and for clinical research, or else our knowledge of what we're dealing with is as potentially inaccurate as our ability to diagnose accurately.

Bigger Agenda

The wider issue is the use of PCR testing to diagnose HPAI in other species such as cattle as they have been doing in the USA. An Ohio report(6) that gave 150 veterinarians in the USA a $50 gift voucher to get tested for HPAI antibodies found that only 3 tested positive (2%) none of which had been actively working with any "confirmed cases" of HPAI in cattle, and yet despite this, the report concluded that these positive results were suggestive of recent HPAI infection despite having not experienced any symptoms. This is a prime example of why not to subject yourselves or your animals to testing when asymptomatic. Whilst there have been no repercussions that I'm aware of here, it leaves the door open to potential consequences that could affect others as well as you. Who knows how selective these antibodies were for just HPAI? How did these veterinarians contract HPAI if it was? Without symptoms, how is it a problem?

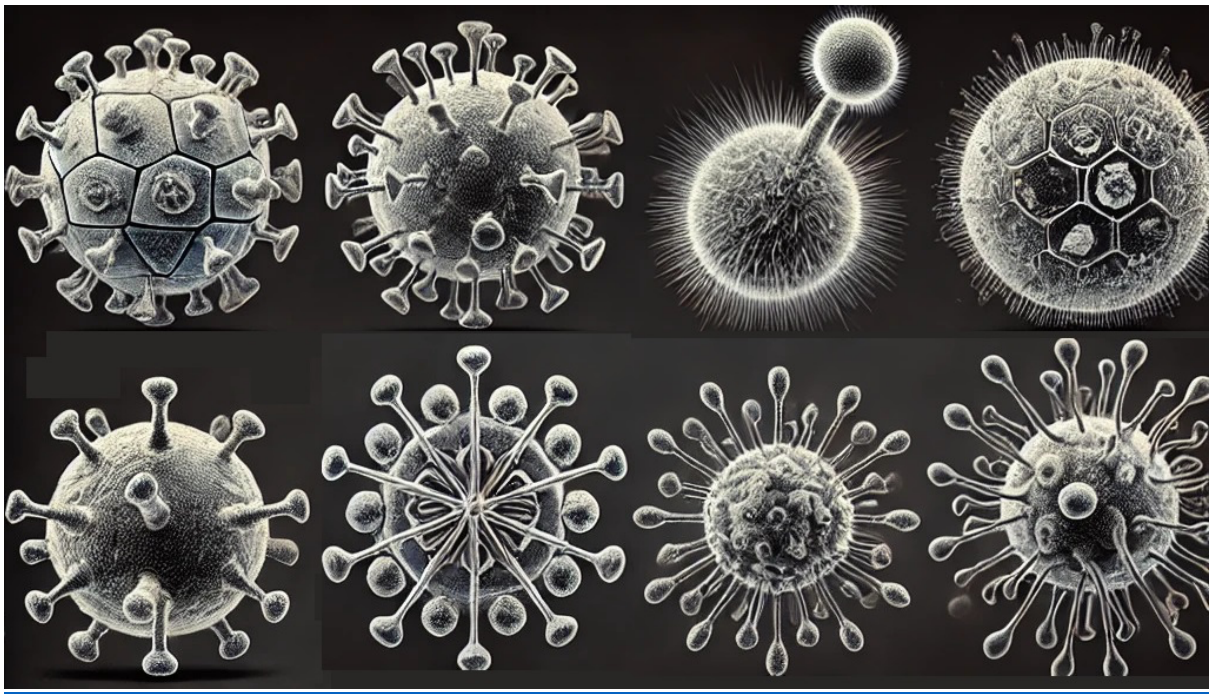
How much of what is going on the UK with HPAI

is DEFRA/APHA wanting to be seen to be doing something I don't know, but alongside videos I've seen where some of those involved in the F&M cull of 2001 have been put on notice, I can't help but consider that this whole HPAI issues is indicative of the wider agenda to scare people away from eating meat, dairy, eggs and to pressurise more farmers into giving up livestock farming. Regardless of that, authorities need to communicate clearly what their vision of a realistic end game looks like, and how it will be achieved without decimating not only farming, but our food security too.

Conflict of interest declaration

The author declares no conflicts of interest.

Figure 1. Image generated using ChatGPT from the prompt "Draw a stylised selection of 8 different veterinary viruses as if taken by an electron microscope"



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