

# Koch's Postulates, Carnivorous Cows, and Tuberculosis Today

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## Abstract

*With Koch's announcement in 1882 of his work with the tubercle bacillus, his famous postulates launched the rational world of infectious disease and an abrupt social change—strict patient isolation.*

*The postulates, so successful at their inception, soon began to show some problems, particularly with cholera, which clearly violated some of Koch's requirements. Subsequent studies of other diseases and the discovery of entirely new ones have so altered and expanded the original postulates that they now are little but a precious touch of history. The present additions and replacements of the original concepts are skillful changes that several authors have devised to introduce new order into understanding complex viral and prion diseases. In 1988, this knowledge, with the totally rational response of the British population and its cattle industry, was critical in promptly blocking the threatened epidemic of human prion disease.*

*In contrast, the recent upsurge of tuberculosis (TB) in the worldwide AIDS epidemic in developing countries, and the sudden increase in metabolic syndrome in wealthy ones, suggests the need for focused sociobiologic research seeking ways to affect the damaging lifestyle behavior of many less educated populations in both settings. The world awaits an equivalent of Koch's Postulates in sociobiology to explain and possibly avert large self-destructive behaviors.*

On 24 March 1882, a bitter cold night in Berlin, Dr. Robert Koch, speaking to the Berlin Physiological Society, a small group of savants that included the great European pathologist, Virchow, offered these words:

There have been repeated attempts to fathom the nature of tuberculosis, but thus far without success. The so frequently successful staining methods for the demonstration of pathogenic microorganisms have failed in regard to this disease, and to date, the experiments designed to isolate and cultivate a tubercle virus cannot be considered successful, so that... the direct demonstration of the tuberculous virus is a still unsolved problem.

Within the hour, world history was to be made by Koch's words about his isolation and transfer of specific organisms and resulting disease:

The aims of the study had to be directed toward the demonstration of some kind of parasitic forms which are foreign to the body and which might possibly be interpreted as the cause of the disease. This demonstration became successful, indeed, by means of a certain staining process, which disclosed characteristics and heretofore unknown bacteria in all tuberculous organs.

Koch closes with the description of his remarkable staining process:

With the exception of leprosy bacilli, all other bacteria which I have thus far examined assume a brown color with this staining method. The color contrast between the brown stained tissue and the blue tubercle bacilli is so striking that the latter which are frequently present only in very small numbers, are nevertheless seen and identified with the greatest certainty.<sup>1</sup>

With this critical discovery, Koch was able to methodically work out the role of these organisms in the development of tubercles and widespread tissue destruction, and recognize them as the cause of the characteristic pathology of tuberculosis in man, cattle, hogs, chickens, monkeys, and "172 guinea pigs, 32 rabbits, and 5 cats." Meticulous isolation of colonies over many weeks, and transfer of the organisms with characteristic resulting disease led to the famous Henle-Koch Postulates which at the time became the gold standard for proof relating a parasitic organism with a specific disease:

1. The organism must be shown to be invariably present in characteristic form and arrangement in the diseased tissue.
2. The organism, which from its relationship to the diseased tissue appears to be responsible for the disease, must be isolated and grown in pure culture.
3. The pure culture must be shown to induce the disease experimentally.
4. The organism should be re-isolated from the experimentally infected subject (this postulate was added after Loeffler).

In contrast with these brilliant disclosures, the century was coming to its end with tuberculosis widely regarded as an inevitable wasting disease which was thought to produce in its victims a refinement of the body, heightened artistic sensibilities, and ennoblement of the soul, notions that were romanticized in the arts and music of the nineteenth century. Mimi's death in Puccini's *La Boheme* and Satine in *Moulin Rouge* are only two of the dozens of TB nineteenth century celebrity deaths, including Guy De Maupassant (1893), Robert Louis Stevenson (1894), and Anton Chekov, a famous writer and physician, 1904.<sup>2</sup>

Despite delay and uncertainty led by Virchow's lifelong skepticism, Koch's work slowly changed the romantic perception of consumptive patients as tragically beautiful victims of a wasting disease to dangerously infectious carriers whose cough or sputum transferring as few as ten bacilli could be an ultimately fatal contact.

With this simple fact in hand, over time, public health measures were put in place involving isolation in sanatoria, masks, sputum, and the lowly spittoon. Although transmission was reduced, successful treatment lay far in the future.

Sheila Rothman, in her 1994 book, *Living In The Shadow of Death*, said:

A generation of physicians, social reformers, and philanthropists were convinced that confining the tubercular [insanatoria] would promote not only societal well-being by isolation of those with the disease, but also well-being by implementing a therapeutic regime. The sanatorium satisfied both the drive to coerce and cure. As concepts of bacteriology gained acceptance, the idea of caring for patients in a setting removed from the general populace was considered wise and necessary to prevent spread of the disease.<sup>3</sup>

Fresh air, rest, and good food were intuitively deemed important. Supplanting the sanatorium routine was the practice of “resting the lung” by pneumothorax. Collapsing the lung by introducing air into the pulmonary cavity was used successfully in 1834 after George Baglivi, in 1696, noted a general improvement in a tubercular patient who suffered a sword wound to the chest. With the wide establishment of sanatoria, and their often heroic routines of cold air, absolute rest and ultimately, thoracoplasty, even under the best conditions in the early nineteenth hundreds, fifty percent of those who entered were dead within five years.<sup>4</sup>

Although transmission of the disease was somewhat abated after Koch’s definitive announcement of mycobacterial spread, the carnage continued into the twentieth century. Today, one hundred years later, through ignorance, poverty, apathy, and the deadly interaction of TB with HIV, the disease continues to kill roughly 5000 people every day, nearly two million a year, making it the second leading cause of adult death by infectious disease,<sup>5</sup> despite the discovery after 1944 of effective antibiotics. Resistance of tubercle bacilli to multidrug regimes has become a serious problem, ironically matching the resistance of patients to treatment of their disease through their refusal or inability to follow their medication regime. Directly observed therapy (DOT) has been successful in several settings<sup>6</sup> with reported results of cure in as high as ninety five percent of cases.<sup>7</sup>

Aside from the effects of specific treatment, there has been a long history of controversy over whether specific public health actions limiting modes of bacterial transmission, such as mandatory reporting and isolation of patients, have been as effective in reducing case incidence as social and economic changes, reduction of poverty, improved housing and nutrition. A comprehensive review of this question will be found in reference eight, which is much more than an academic argument, as conclusions can affect future resource allocation for disease control.

## **Tuberculosis and AIDS**

People who are infected with HIV are especially susceptible to developing active TB. TB is the leading cause of death among people-living-with-HIV/AIDS (PLWHA) and one of the most common opportunistic infections they experience. The prevalence of HIV infection among patients in TB clinical settings is high, up to 80 percent in some countries. The US President’s Emergency Plan for AIDS Relief (PEPFAR)<sup>9</sup> is leading a unified US Government (USG) global response to fully integrate HIV prevention, treatment, and care with TB services at the country level.

The most important work in combating TB takes place through partnerships at the country level to support national health authorities, non-governmental organizations, and community- and faith-based organizations to strengthen and implement effective TB/HIV programs.

Accelerated activities include:

- Providing HIV testing for people with TB and improving TB diagnosis for PLWHA;
- Ensuring that eligible TB patients receive HIV/AIDS prevention, treatment, and care including antiretroviral treatment, cotrimoxazole, and isoniazid to prevent active TB;
- Improving TB infection control to prevent PLWHA from

coming in direct contact with someone with active TB;

- Implementing the WHO-recommended TB treatment protocol, Directly Observed Therapy- Short Course (DOTS), in order to ensure that patients complete their TB treatment;
- To respond to the increasing rate of smear-negative and extrapulmonary TB among PLWHA, implementing laboratory-strengthening activities (eg, enhanced capacity to detect both smear negative and extrapulmonary TB among PLWHA, external quality assessment, drug resistance surveillance, and rapid detection of TB drug resistance for clinical decision-making); and
- Supporting activities to address multi-drug resistant and extensively-drug resistant TB for TB/HIV patients, including rapid TB diagnosis and treatment.

PEPFAR also supports expanding the capacity of the local health workforce to deal with TB/HIV and improving supply chain management systems for TB/HIV medications and other commodities. In addition, it is essential to establish linkages between TB treatment and antiretroviral treatment so that people who are co-infected receive the medical attention they need.

## **Koch’s Postulates Revisited**

Very soon after the announcement of the postulates 128 years ago, it became apparent that in some cases, all the conditions for proving a causative agent and its transmission could not be met.

In 1996, Fredericks and Relman summarized the problem in a landmark article in *Clinical Microbiological Reviews*:<sup>10</sup>

The critical elements of Koch’s postulates include a specific association of the microbe with the disease state; scientific concordance of microbiological, pathological, and clinical evidence; isolation of the microbe by culture on lifeless media; and reproduction of disease by inoculation of the cultured organism into a host. These stringent criteria provide a framework for thinking about the proof of microbial disease causation. For diseases like tuberculosis, these postulates have been quite successful. Koch was able to visualize *Mycobacterium tuberculosis* in diseased to reproduce the disease in animals upon inoculation from pure culture. Animals and people without disease were found not to have *M. tuberculosis* in tissues. However, even Koch was aware of the limitations imposed by these postulates. He believed that cholera and leprosy were caused by specific visible microbes, but he could not fulfill all of the postulates for disease causation. Although *Vibrio cholerae* was isolated from patients with cholera in the time of Koch, it was also isolated from healthy subjects, thereby defying the specificity of association demanded by Koch’s second postulate.

Scientists have been no more successful today than a century ago in culturing the etiologic agent of leprosy, *Mycobacterium leprae*. The inability to isolate *M. leprae* in pure culture prevents the fulfillment of Koch’s third postulate. Nonetheless, Koch stated, ‘Therefore, we are justified in stating that if only the first two conditions of the rules of proof are fulfilled, i.e., if the regular and exclusive occurrence of the parasite is demonstrated, the causal relationship between parasite and disease is validly established.’

The limitations of Koch’s postulates, evident in the 1800s, are even more pronounced today. Organisms such as *Plasmodium falciparum* and herpes simplex virus or other viruses cannot be grown alone, i.e., in cell-free culture, and hence cannot fulfill Koch’s postulates, yet they are unequivocally pathogenic. Similarly, certain microbes such as human immunodeficiency virus (HIV) exhibit a host range that is restricted to humans; they cannot produce typical disease in other hosts, thereby making impossible or unethical the final fulfillment of the third postulate.

In contrast to the beliefs of Koch and those of his era, we are well aware today that microbial pathogens often cause subclinical infection. For example, the vast majority of patients exposed to *M. tuberculosis* will simply develop a silent infection accompanied by microscopic forms of pathology, marked by the presence of a positive tuberculin skin test, and will not go on to develop active disease.<sup>10</sup>

Although Koch's postulates were of incalculable value in their first application, new knowledge has required major changes. Fredericks and Relman presented this revision in their 1996 review:

1. A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.
2. Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
3. With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.
4. When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.
5. The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
6. Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific *in situ* hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.
7. These sequence-based forms of evidence for microbial causation should be reproducible.

In Fredericks' and Relman's summary, some of the reasons for these additions to the original postulates are that:

Many viruses do not cause illness in all infected individuals, a requirement of postulate #1. An example is poliovirus, which causes paralytic disease in about 1% of those infected. Further compromising postulate #1 is the fact that infection with the same virus may lead to markedly different diseases, while different viruses may cause the same disease. Postulates #2 and #3 cannot be fulfilled for viruses that do not replicate in cell culture, or for which a suitable animal model has not been identified.

The application of nucleic acid-based methods of microbial identification has made Koch's postulates even less applicable. Polymerase chain reaction and high-throughput sequence analyses have revealed a great deal about microbes that are associated with pathology or disease, but proving causation has become even more difficult as the number of uncultivable viruses rapidly multiplies. Nucleic acid based

detection methods are so sensitive that they detect small numbers of viruses that may occur in the absence of disease. The use of these new methods has led to revised versions of Koch's postulates that are fundamentally sound: both hepatitis C virus and human papillomaviruses were convincingly shown to be causative agents of hepatitis and cervical cancer, respectively, long before methods were developed for propagation of the viruses in cell culture.

## **Carnivorous Cows**

Complicating current revisions and additions to Koch's postulates is the unique concept of prion diseases.<sup>11</sup>

Belay et al write:

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of animal and human brain diseases that are uniformly fatal and often characterized by a long incubation period and a multifocal neuropathologic picture of neuronal loss, spongiform changes, and astrogliosis. Investigators believe the etiologic agents of TSEs are abnormal conformers of a host-encoded cellular protein known as the prion protein. Prion diseases do not characteristically elicit an immune response by the host, and the mechanism of brain damage is poorly understood. However, progressive neuronal accumulation of the disease-associated prions may damage neurons directly, and diminished availability of the normal prion protein may interfere with the presumed neuroprotective effect of the normal prion protein, contributing to the underlying neurodegenerative process.

Prion diseases attracted much attention and public concern after an outbreak of bovine spongiform encephalopathy (BSE) occurred among cattle in many European countries and scientific evidence indicated the foodborne transmission of BSE to humans. The classic form of Creutzfeldt-Jakob disease (CJD) was first reported in the 1920s, decades before the first BSE cases were identified in the mid-1980s. About 10%–15% of CJD cases occur as a familial disease associated with pathogenic mutations of the prion protein gene, and about 85% of classic CJD cases occur as a sporadic disease with no recognizable pattern of transmission. The stable, almost predictable, occurrence of the disease in many areas of the world, primarily in the elderly, led to the speculation that sporadic CJD may occur from *de novo* spontaneous generation of the self-replicating prions, presumably facilitated by somatic random mutations. Beginning in the 1970s, iatrogenic person-to-person transmission of the CJD agent was reported in a small percentage of CJD patients.

This iatrogenic spread involved the use of contaminated corneal and dura mater grafts, neurosurgical equipment, and cadaver-derived human growth hormone. At present, the number of iatrogenic CJD cases is on the decline as a result of public health preventive measures implemented as the various modes of transmission were identified.

## **Etiologic Agent of Prion Diseases**

"Most of the earliest studies done to identify the agents of TSEs focused on describing the causative agent of scrapie, a prion disease of sheep known to have been occurring in Europe for centuries. Lack of suitable laboratory models or cell culture systems had limited the efforts to characterize the scrapie agent. However, the successful transmission of scrapie to mice in 1961 greatly facilitated the identification and characterization of the scrapie agent. Several theories had been proposed to describe its characteristics. Owing to the transmissibility of the agent, retention of its infectivity after filtration, and the long incubation period before disease onset, scrapie was thought to be caused by a slow virus. The possibility that the agent could be a viroid was considered also. However, no viral particles or disease-specific nucleic acids were identified in association with scrapie infection. Resistance of the scrapie agent to radiation, nucleases, and standard sterilization and disinfection agents, and its inactivation by procedures that modify proteins led to

the suggestion that the scrapie agent is not a virus but, instead, might be composed primarily of a protein. In 1966, Alper et al. suggested the possibility that the scrapie agent could replicate in the absence of nucleic acids. Pattison & Jones also investigated this possibility and suggested that the scrapie agent might be a basic protein or associated with such a protein, thus igniting a controversy among many of their contemporaries. In 1967, Griffith carefully outlined the potential pathways by which such a protein agent could support its own replication.”

### **Generating a Prion with Bacterially Expressed Recombinant Prion Protein**

Fei Wang, Xinhe Wang, Chong-Gang Yuan, and Jiyan Ma wrote:

The prion hypothesis posits that a misfolded form of prion protein (PrP) is responsible for the infectivity of prion disease. Using recombinant murine PrP purified from *Escherichia coli*, we created a recombinant prion with the attributes of the pathogenic PrP isoform: aggregated, protease-resistant, and self-perpetuating. After intracerebral injection of the recombinant prion, wild-type mice developed neurological signs in ~130 days and reached the terminal stage of disease in ~150 days. Characterization of diseased mice revealed classic neuropathology of prion disease, the presence of protease-resistant PrP, and the capability of serially transmitting the disease; these findings confirmed that the mice succumbed to prion disease. Thus, as postulated by the prion hypothesis, the infectivity in mammalian prion disease results from an altered conformation of PrP.<sup>12</sup>

Prions are specific proteins found mainly in the nervous system, where – in their normal forms – they may have important functions. For example, studies on sea slugs, *Aplysia*, suggest that prions have a crucial role in memory formation (Kausik et al., 2010)<sup>13</sup> Infectious prions are abnormal (aberrant) forms of prion proteins that replicate inside the host by forcing normal proteins of the same type to adopt the aberrant structure. This has a domino effect whereby a small number of aberrant prions can affect many normal ones and eventually lead to disease. As the aberrant prions form amyloids — aggregates of protein — in the cells, the cells die, creating holes in the brain.

Prions are the only known case of self-propagating pathogenic proteins, and they are able to cause severe illness even though they seem to be just protein molecules. Unlike bacteria, viruses or other known pathogens, they have no information encoded in nucleic acids (DNA or RNA) about how to invade and replicate within the host. There is still a veil of mystery around prions and exactly how they replicate, cross the blood-brain barrier, and cross the species barrier (ie, infect different species of host).

It was in the 1960s that investigators first found that TSE disease-causing agents appeared to lack nucleic acids. Tikvah Alper suggested that the agent was a protein. This idea sounded heretical because all other known disease-causing agents contained nucleic acids and their virulence and pathogenesis were genetically determined.

To prove that a TSE is caused by prion protein, purified aberrant prions must be used to transmit the disease. In February 2010 this was done, adding further evidence for the protein only hypothesis.<sup>12</sup>

### **Control of Bovine Spongiform Encephalopathy (BSE)**

*BSE was first recognized in the United Kingdom in 1986, where it caused a large outbreak among cattle. The leading hypothesis for the origin of BSE is cross-species transmission of scrapie to cattle via the feeding of meat and bone meal that was contaminated by the inclusion of scrapie-infected sheep parts. Spontaneous occurrence of the disease in cattle, much like sporadic CJD in humans, has also been*

*hypothesized. Although the origin of BSE remains controversial, it is widely accepted that the practice of using rendered BSE-infected carcasses for cattle feed had amplified the outbreak until a ruminant feed ban was instituted in 1988. Because of concerns about cross-contamination of cattle feed with prohibited material intended for other species, a specified bovine offal ban (also known as specified risk material ban) was introduced in 1990 to remove the known infectious parts of cattle from all animal feed. Although a dramatic decline in the BSE outbreak was registered in response to this feeding ban, over two million potentially infected cattle were butchered and consumed in the United Kingdom. Some risk of human disease remains since as little as ten mg. of infected material has been shown to be infectious in experimental animals.<sup>11</sup>*

### **Conclusions: Optimal Use of Public Health Resources**

Koch’s postulates, useful as they were when announced in 1882, have had a rough ride intellectually, both at their inception and in their application to the many diseases to which they were applied. After millennia of ignorance, superstition, and abject fear of tuberculosis as inevitable, Koch’s sudden explanation and proof of bacterial transmission brought strict patient isolation. Unrelated social changes were occurring at the time, with widespread improvement in housing, crowding, sanitation, and nutrition, all of which were important in subduing the disease. Much later, the most critical development of all, the antibiotics, produced a further remarkable dip in TB mortality—offset since 1985 by the current surge of AIDS/TB and highly resistant TB strains. There are still about five thousand deaths a day worldwide from TB, largely related to associated AIDS infection.

In some countries, while control of TB/AIDS by technological barriers to disease transmission such as viricidal jells, condom use, syringe exchange programs, and anti-HIV drug regimes have been useful, superstition, family structure, sexual practices, and chaotic economic and political forces (ie. in sub Sahara areas) all negate technical gains. War, rape, genocide, and poverty breed public health disaster.<sup>14</sup>

Rational clinical intervention needs at least some base of education and social stability in a target population. Without security, sanitation, nutrition, birth control, and other major changes in family lifestyle that control the role of women, it is unlikely that the disease patterns in developing countries will greatly improve. Even reasonably successful drugs and vaccines are of little use in a melee of apathy, genocide, rape, and starvation. In many regions, the most vicious problems are cultural and economic, and until these are realistically addressed, medical interventions offer little hope of success.

Today we know far more about the details of epidemiology, molecular biology, and pharmacology than we do about the obtuse human behavior that often prevents their clinical application. To affect world health, religious issues, social structure, political failure, and poverty demand focused attention.

Increased research support of sociobiologic studies of self-destructive populations is needed to teach us how to alter behaviors that block the application of rational health principles. Our need for understanding why people behave as they do lies not only in chaotic third world settings, but in rich nations whose populations approach a sixty percent obesity rate and a metabolic syndrome epidemic.

Informative research results analyzing public health failures would far outweigh their costs in health care expenditures and lives saved. The ultimate laboratory is the village, the town, and the metropolis.

In each, to study why people act as they do in blocking the obvious measures that would enhance their health and lives, would add enormously to human welfare.

Perhaps it is time for a Koch's Postulates equivalent to explain the crippling impedance of human behavior.

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