

## *Mad Cows & Heresies: Prions*

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**20 March 1996 -- British government announces a deadly new disease killed 10 young Britons and that the victims may have contracted the disease from eating infected beef.**

**Mad Cow Disease or Bovine Spongiform Encephalopathy (BSE) -- slowly destroys brain cells and is ALWAYS fatal.**

**World-wide ban on British beef and the near collapse of the \$8.9 billion beef industry.**

**European Union demanded slaughter of 4.7 million British cattle.**

**Cost British government over \$12 million to compensate farmers, import milk, buy cattle for new herds.**

**At the molecular level, BSE is nearly identical to “variant Creutzfeldt-Jakob disease (vCJD)”.**

**At least 24 Europeans have died of vCJD.**

**USDA banned the importation of cattle or meat products from countries that have BSE.**

*No cases of BSE or Mad Cow Disease have originated in the US ??????????????*

**BSE and CJD are members of a group of neurological diseases known as spongiform encephalopathies.**

**These lethal diseases have long incubation times (up to 20 -- 30 years).**

**Lead to progressive neurodegeneration.**

**Infected brain tissue resembles a sponge with proteinacious deposits.**

**Victims of the disease lose motor function, become demented, and eventually die.**

**CJD arises spontaneously and randomly at a rate of 1 per million humans per year.**

**A less common form of CJD is inherited as an autosomal dominant trait.**

**Transmitted CJD is the strangest form. Can be transmitted through corneal or nervous tissue grafts or by injection of growth hormone derived from human pituitary glands.**

**Kuru, a CJD-like disease of the Fore people of New Guinea, was transmitted via ritualistic cannibalism.**

**The epidemic of BSE in Britain occurred because diseased cows and sheep were processed and fed to other cows and sheep as a protein supplement.**

**For many years, spongiform encephalopathies defied analysis.**

**Difficult to study -- require injection of infected brain tissue into brains of experimental animals.**

**Infectious agent is NOT a virus or bacterium and infected individuals DO NOT develop antibodies.**

**No treatment for the disease and the only way to make a firm diagnosis is after death.**

**In 1980s, American scientist, Stanley Prusiner purified the infectious agent and concluded that it only consisted of protein.**

**Called it a “Prion”**

**Much doubt by other scientists because could not believe an infectious agent did not contain DNA or RNA.**

**Prion hypothesis is now widely accepted and in 1997 Stanley Prusiner received a Nobel Prize.**

*If prions are composed only of proteins, how do they work??????*

**The protein that makes up a prion (PrP) is also a normal protein that is synthesized in neurons and found in the brains of adult animals.**

**The difference between a normal PrP and that of a prion is in the folding of the protein.**

**Normal noninfectious PrP fold into alpha-helices whereas infectious PrP form beta-helices.**

**Once normal PrP molecule contacts a prion PrP, the normal protein is somehow unfolded and refolded into the abnormal PrP conformation.**

**This process spreads among all normal PrPs.**

**Normal PrP is a soluble protein that is easily destroyed by heat or enzymes that digest proteins.**

**Infectious PrP is insoluble in detergents and resists both heat and proteinase digestion.**

**Infectious PrP forms deposits in the brain, causes death of neurons, and is nearly indestructible.**

**Therefore, spongiform encephalopathies can be considered diseases of protein secondary structure.**

*Several Remaining Questions:*

- 1. How many humans are infected but do not show symptoms?**
- 2. Can humans or animals act as asymptomatic carriers of prion disease?**
- 3. Do some people have a genetic susceptibility to the disease?**
- 4. Can prions exist in other parts of the body besides the brain and spinal cord?**
- 5. Can CJD be spread via blood transfusions, from mother to fetus, or by surgical instruments?**
- 6. Can we develop diagnostic test and therapy for BSE and vCJD?**

# Forensic Sciences & DNA

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**With advent of PCR, genetic markers can be examined from splattering of blood or semen left at a crime scene, skin left under the fingernails or a victim, or the cells from a single hair shaft.**

**When a DNA profile from tissue found at a crime scene matches that of a suspect, it DOES NOT prove that the tissue belongs to the suspect; instead it excludes all those who have a different pattern.**

**BUT, if it does match it is very powerful evidence!!!!**

**DNA from other organisms can also be useful for human forensics!!**

**May 2, 1992 a Phoenix, AZ woman was strangled and her body dumped near an abandoned factory in the desert.**

**Investigators discovered a pager near her body.**

**When questioned, the man that owned the pager admitted to being with the woman, but claimed he had not been near the factory and that the woman must have stolen his pager.**

**A search of his pick-up truck revealed two seed pods from a palo verde tree.**

**The palo verde tree is native to the deserts of the southwest US.**

**Investigators wondered if it could be proven that the seed pods in the truck had fallen from a palo verde tree near the woman's body.**

**Investigators contacted a molecular biologist at the University of Arizona.**

**This scientist first proved that individual palo verde plants were genetically different from each other -- this first step was critical!**

**Next, in a blind experiment, this scientist was given the two seed pods found in the truck plus seed pods from 12 additional palo verde plants in the same area, including seeds from the tree nearest the body.**

**The results of his genetic analysis matched the seed pods in the truck to only 1 of the palo verde trees in the vicinity and it was the tree closest to the body.**

**The likelihood of a random match was less than 1 in 1,000,000/**

**Example 2 -- Cat Hair.**

# **Genetic Testing & Screening**

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**With the completion of sequencing the human genome, we will know the DNA sequence of all human genes.**

**Expected to revolutionize medicine as it will open the door to treating genetic diseases at their source by replacing or correcting defective genes.**

**However, the lag between identification of a gene and development of a new treatment is likely to be measured in decades rather than years.**

**In the meantime, it is already possible to test individuals to see if they carry mutant alleles that impart a high risk of developing a genetic disease.**

**Although genetic testing has the potential to save or extend lives, at the same time it raises difficult questions for which the answers are not always clear.**

***Example -- BREAST CANCER***

**Breast cancer is the most common type of cancer among woman and the second leading cause of cancer deaths (after lung cancer).**

**Each year > 180,000 new cases are diagnosed and about 43,000 women die of breast cancer.**

**The likelihood of developing breast cancer is low before age 35 but the risk increases after that.**

**If a woman lives a long life, her risk of breast cancer is about 12% .**

**About 5 -- 10% of the cases of breast cancer are familial.**

**Familial breast cancer tends to develop earlier (30 -- 40 years old) and is more often bilateral (both breasts) and multifocal (involving several separate tumors).**

**Study of familial breast cancer led to the identification of two genes, when mutated, impart an increased susceptibility to breast cancer.**

**These genes are BRCA1 and BRCA2.**

**Several other genes have also been identified but the effect of cancer is less with these.**

**Mutations in BRCA1 and BRCA2 greatly increase the risk of developing breast cancer over the lifetime risk in the general population (risk = 56% -- 85%).**

**In addition, mutations in BRCA1 result in a 10-fold increased risk of ovarian cancer.**

**The role of BRCA1 and BRCA2 in the origin of breast cancer is indirect.**

**The protein products of both normal genes appear to be involved in repair of DNA damage.**

**BRCA1 also behaves as a tumor suppressor gene.**

**Why mutations in these genes increase the likelihood of breast cancer (and ovarian cancer for BRCA1) is unclear but may be related to physiological response of these tissues to hormones.**

**Woman in high risk families have the option of being tested to see whether they carry the mutant alleles for either of these genes -- if so, they carry a lifetime risk of breast cancer of 85%; if not, they still have a 12% risk.**

**Good reasons for a woman in a high-risk group to be tested:**

- 1. Negative test result provides considerable peace of mind.**
- 2. Will be secure in knowledge that she will not pass the mutation to her offspring.**

**It is the consequence of a positive test that makes the decision difficult.**

**For a woman with BRCA1 or BRCA2 mutations there are two courses of action -- neither very appealing.**

- 1. Close medical surveillance, including frequent breast exams and mammography with the goal of catching it early, when it is more treatable.**
- 2. Surgical removal of breasts, and in the case of BRCA1, the ovaries.**

**Unfortunately, no middle ground between these two options.**

**Recent studies suggest that women in high risk groups that undergo a bilateral prophylactic mastectomy reduce their risk of breast cancer by 90%.**

**However, it is unclear if the personal and psychological costs of such extreme surgery are outweighed by the increased life expectancy.**

**Other fears:**

**A positive test result may lead to discrimination in employment and to higher insurance premiums, or a total loss of coverage.**

**Issues raised in genetic testing of breast cancer susceptibility are likely to be played out again and again with other genes that predispose individuals to other cancers and diseases.**

**Already genes that increase the risk of cancers of the prostate, colon, skin, and lungs are available and more will be soon.**

**Several genes have been identified that increase the risk of late-onset disorders including, Alzheimers disease, Parkinson disease, and heart disease.**

**We are on the verge of having the ability to predict diseases based on genotypes years before they might occur.**

**We must learn how to extend the use of this new technology while gaining the wisdom to know when to test and when not to test.**

# **Cloning of Organisms**

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**Cloning of the first mammal, “Dolly” a lamb was accomplished in February 1997 by a group of embryologists led by Ian Wilmut and Keith Campbell at the Roslin Institute in Scotland.**

**Their reason for developing methods to clone farm animals was to provide identical transgenic animals that secrete pharmaceutical products, such as blood clotting factors or insulin, into their milk.**

**Thus, animals could be used as bioreactors to synthesize proteins that are difficult or expensive to synthesize in bacteria or test tubes.**

**The cloning method used -- “Nuclear Transfer” was first proposed in 1938 by embryologists Hans Speman.**

**Wimut and colleagues transplanted a nucleus from a mammary cell of a Finn Dorsett sheep into the enucleated egg of a Scottish blackface ewe.**

**The nucleus-egg combination was stimulated with electricity to fuse the two and to stimulate cell division.**

**The new cell divided and was placed in the uterus of a blackfaced ewe to develop.**

**Dolly was born months later.**

**Dolly was shown to be genetically identical to the Finn Dorsett mammary cells and not to the blackface ewe, which clearly demonstrated that she was a successful clone.**

**It took 276 attempts before the experiment was successful.**

**Dolly has since grown and reproduced several offspring of her own through normal sexual means.**

**Since Dolly, several university labs and companies have used various modifications of the nuclear transfer method to produce cloned mammals, including cows, pigs, monkeys, mice, cats.**

**A transgenic sheep has been produced (Polly) that bears the human gene for blood-clotting factor IX and secretes factor IX into her milk, paving the way for production of pharmaceuticals from cloned farm animals.**

**Besides benefits for drug production, cloning promises other advantages:**

- 1. Cloning might cure genetic diseases in farm animals.**

- 2. Provide animal models of human diseases for which there are presently no research models.**
- 3. Cloning might allow scientists to preserve and replicate endangered species.**

**In fact, January 8 2001, scientists at Advanced Cell Technology, Inc., announced the birth of the first clone of an endangered species, a baby bull gaur (a large wild ox from India and southeast Asia) named Noah.**

**Unfortunately, Noah died of an infection unrelated to the procedure.**

### **Risks of Cloning:**

- 1. *High Failure Rates:* Success rate ranges from 0.1% to 3% of the experiments.**

## ***2. Problems during later development:***

**Cloned animals that do survive tend to be much bigger at birth than their natural counterparts.**

**This is called the “Large Offspring Syndrome”.**

**Clones with LOS have abnormally large organs that can lead to problems with breathing and blood flow.**

## ***3. Abnormal Gene Expression Patterns.***

## ***4. Telomeric differences:***

**As cells divide, their chromosomes get shorter. The older the animal is, the shorter its telomeres will be. This is a natural part of aging.**

**Dolly’s chromosomes had shorter telomere lengths than normal sheep, meaning that Dolly’s cells were aging faster than cells from a normal sheep.**

*Antibiotic Resistance -- Stemming a biological Arms Race.*

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**New infections appear and old ones return for two reasons -- mutation and natural selection.**

**Infectious diseases that were once prevalent are resurging, including diphtheria, tuberculosis, denque, cholera, yellow fever, and polio.**

**Bacteria and viruses can mutate to produce a new toxin, infect new species, or exist in novel places.**

**Unfortunately, we cannot do much to prevent mutation -- it is a consequence of DNA replication.**

**We can however, intervene at the level of natural selection, countering environmental changes that permit disease-causing mutations to persist.**

**Human body constitutes a population of organisms, and as such they include different genetic strains.**

**Some bacterial strains inherit the ability to survive in the presence of a particular antibiotic.**

**Antibiotics at first work by killing those bacteria without resistance.**

**This leaves behind a small population of resistant bacteria that can now multiply without competition.**

**Antibiotic resistance is, in a sense, a biological arms race.**

**Antibiotic drugs do not cause mutations in bacteria; they select for preexisting resistant variants.**

**Bacteria with drug resistant mutations circumvent antibiotics in several ways.**

**Penicillin kills bacteria by tearing apart their cell walls. Resistant bacteria produce enzyme variants that dismantle penicillin or have altered cell walls that the drug cannot bind to.**

**Erythromycin, streptomycin, and tetracycline kill bacteria by attacking their ribosomes. Bacteria resistant to these drugs have altered ribosomes that the drugs cannot bind to.**

**Bacteria acquire antibiotic resistance in several ways:**

**--Their DNA may spontaneously mutate. This is how drug resistant tuberculosis arose.**

**--Bacteria may receive a resistant gene from another bacterium through transformation. This is how gonorrhea gained resistance.**

**--Bacteria can acquire resistance to several drugs at once by taking up a plasmid from another bacterium.**

**Plasmids move freely from one species of bacteria to another.**

**MISUSE OF ANTIBIOTICS**

**&**

**ANTIBACTERIAL SOAP**