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Article 1

Prevalence of Echinococcosis in Buffaloes

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ABSTRACT

A study on the prevalence of echinococcosis was conducted by examining carcasses at a slaughter house over a period of nine month. It was observed that echinococcosis occurred with an over all prevalence of 34.5% in buffaloes with an infection rate of 40.9% in males & 44.1% in females. In adult buffaloes (above 2 years) the prevalence of hydatid disease was recoded as 43.6% against 11.1% of buffalo of below 2 years.

INTRODUCTION

The disease echinococcosis is caused by *the parasite Echinococcus granulosus*. The adult tapeworm is found in small intestine of dog. A wide range of mammals, including sheep, cattle, pig, buffalo, goat, camel, horse & man can act as the intermediate hosts (Khan et. al., 1990; Mandke & Sauzgi, 1991; Amrssi et. al., 1994 and Sharma & Roy 1998). The canine population accumulates infection by consuming cysts from the meat of the herbivorous intermediate hosts. In animals echinococcosis has been reported as one of the main health hazards all over the world, apart from the economic damage to livestock industry it poses a serious zoonotic threat.

MATERIALS & METHODS

A total of 200 buffaloes were examined for the presence or absence of hydatid cysts at zoo slaughter house of Bikaner city from August 1995 to April 1996 adopting the standard procedures described by Pandey(1970). The dressed carcasses were examined by visual palpation and incision of organs. The affected organs containing hydatid cysts were dispatched to laboratory as per standard procedures and maintained at 4°C till further examination. After removing the fluids & sediments, the cyst was cut open and the walls were scrapped. The membranous material was passed between two glass slides and examined microscopically. The cysts displaying laminated membranes, scoleces or hooklets were identified as hydatid.

RESULTS & DISCUSSION

The examination revealed an overall prevalence of hydatid cysts at 34.5% with infection rate of 11.1% in buffaloes below 2 years age & 43.6% in adults above 2 years of age. Among adults the prevalence was higher in females (44.1%) as compared to males (40.9%) (Table1). These findings fall in line with reports of Gill & Rao (1967), Abraham & Iyer (1980) and Hussain et al (1992). The increase in incidence of disease with advance in age of animal was observed during the present investigations and it may be attributed to a greater opportunity for infestation & development of cyst in animal rather than any influence of age. A higher incidence in the females may again be correlated to the higher age that females are slaughtered at owing to their utilization for reproductive purposes. In contrast males are slaughtered at a younger age. Similar argument has been recorded by Gemmel (1961).

Table 1: Showing prevalence of echinococcosis in buffaloes according to sex & age

Buffaloes	Overall	Male (a)	Female (b)	Adults (a+b)	Calves
Total no. of animals examined	200	44	102	146	54
Total no. of Positive animals	69	18	45	63	06
Prevalence (%)	34.5	40.9	44.1	43.6	11.1

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Article 2

A Survey of Plants Used for Wound Healings in Animals

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INTRODUCTION

A wound can be defined as a break in the continuity of the soft tissues like skin, mucous membranes, tissue surfaces etc. caused by physical, chemical or biological insult. Wound can also be called as a traumatic lesion.

Broadly, wounds are classified into two categories:

- 1) External wounds
- 2) Internal wounds

An external wound is one with a varying degree of damage to the tissue including skin e.g., incised wounds, lacerated wounds punctured wounds, penetrating wounds, perforating wounds gunshot wounds, abrasions, avulsions or evulsions.

An internal wound damages the underlying tissue to varying degree leaving the skin intact e.g., contusions, bruises, and hematomas.

Two basic objectives are the guiding principles for wound healing:

- A) The rapid and completed repair of the created defect and,
- B) The Prevention of bacterial invasion during the period the natural barriers are defective.

Although these principles appear to be separate goals they are impossible to attain separately in the clinical care of wounds. For practical purposes, the maneuvers employed to promote rapid wound healing are so intimately related to prevention of bacterial invasion, in fact so dependent on it, that a major portion of energy directed toward the optimal wounds healing is expended in the direction of prevention of bacterial infection.

WOUND HEALING: A BRIEF OVERVIEW

Proliferation of fibroblasts and capillary buds and the subsequent laying down of collagen to produce a scar is the usual consequence of most tissue damage. Connective tissue is a ubiquitous and efficient method but it necessitates a loss of specialized parenchyma function.

Connective tissue repair may be classified as:

- A) Healing by first intention
- B) Healing by second intention
- C) Healing by third intention

Healing by first intension or primary union is the goal surgeon has in mind with each surgical incision. It represents the results of primary suture and healing of an aseptic, properly incised and closed wound.

The formation of granulation tissue or healing by second intension occurs in those wounds that are allowed to heal without closure. It is in this situation that the biological processes defending against bacterial invasion are vital for survival. The size of wound is reduced by contracture of the underlying tissue. The time required for such a wound to heal will vary with the area involved and the mobility of the underlying tissue. As granulation tissue matures inflammatory cells decrease in number, fibroblasts lay down collagen and the capillaries become much less prominent.

Healing by tertiary intention is represented by the application of skin graft or by intentionally bringing together the granulating surface of an incised, previously unclosed wound after a period of several days. An example is the delayed primary closure and the delayed secondary closure.

In all forms of healing however, a series of various processes that involve re-epithelialization, scar formation and the completion of the inflammatory processes, the healing finally comes to an end with contact inhibition of the division process of the cells.

Since wound healing is a vital body response, in an ideally suited environment, the process may be hastened due to optimum biological repose of the body. In order to create similar situations applications of different medicaments are suggested.

The orient has a long history of using a number of plants for healing of wounds. The modus operandi though not clear in all the cases includes the antiseptic, astringent, immuno-modulatory and anti-inflammatory actions of these plant constituents.

The search for valuable pharmacological properties of plants has led to the emergence of several allopathic drugs. At present phyto-medicines and traditional remedies employed by various tribal, ethnic, nomadic and semi-nomadic societies in rural areas are being viewed with a great scientific interest. The knowledge gathered from such communities has played a significant role in the development of certain important remedies in India, China and other countries.

The development of anti-fatigue agent from *Ticopus zeylanicus* in Kerala (Pushpangadan et al 1995) and *Vicoa indica* (Rao et al 1960) used by some tribals in Bihar are two latest examples exhibiting renaissance of interest in the researchers of traditional plant based remedies. The WHO has also recommended to all member countries to actively promote native medicines and also to initiate steps to conserve and cultivate medicinal plants.

The patho-physiology and treatment strategies vary depending on the nature of wound e.g. in contusions wherein subcutaneous tissues is injured, the local application of astringents and administration of anti-inflammatory and analgesic agents could be beneficial, whereas administration of analgesic, antipyretic and antibiotic agents along with local application of antibiotics is essential to achieve good results in case of contaminated/septic wounds. Similarly in other wounds different strategies are applied keeping the situation in hand in view, for example in maggot wounds, in addition to the general therapy, an agent able to expel or kill the maggots has to be applied locally.

LIST OF COMMONLY USED PLANTS

A study was undertaken to establish the commonly used plants for treatment of wounds. The source of the data collected were the livestock farmers (home remedies), the village herbsmen/milkmen/herb-doctors etc, and also a review of literature related to pharmacognosy and phytomedicine available for the area.

Table 1. Plants Used in Treatment of Fresh Cut Wounds

Name of Plant		Part(s) Used and mode of usage
Botanical Name	Local Name (Region)	
<i>Agrimonia pilosa</i>	Belur (UPH) Agrimony (E)	Pounded whole plant is applied locally
<i>Anaphalis triplinervis</i>	Ekle ghans (N)	A paste made from flowers is applied locally
<i>Annona squamosa</i>	Mondal (Santhal) Jangali shareefa (UPH) Custard apple (E)	Leaves are bandaged and leaf juice is given with lime to cattle
<i>Azadirachta indica</i>	Neem (N)	Leaf decoction is used locally as antiseptic to wash cuts and wounds
<i>Betula alnoides</i>	Bhujpatra (H) Indian birch (E)	Bark paste is applied locally
<i>Baschniackia himalaica</i>	Ganelu (UPH)	Whole plant poultice is applied locally
<i>Chenopodium album</i>	Bathua (H)	Crushed leaves applied locally
<i>Circium veratum</i>	Kandaru (UPH)	Root paste is applied locally
<i>Circum longa</i>	Haldi (H) Indian saffron (E)	Rhizome paste is prepared using Brassica species oil and applied locally
<i>Eclipta prostrate</i>	Bharanjraj (H)	Leaf juice is squeezed locally for quick healing
<i>Eclipta alba</i>	Bhangra (H)	Pounded leaves with oil of kusum is applied
<i>Erythra variegata</i>	Badisa (APC)	Leaf juice is applied locally
<i>Euphorbiapilosa</i>	Chuplya (UPH)	Latex of plant is applied locally
<i>Jatropha gossypifolia</i>	Lal-gab-jara (Lodhas)	Piece of root is tied around the neck

Botanical Name	Local Name (Region)	Part(s) Used and mode of usage
<i>Lens culinaris</i>	Musuro (N)	Fried seeds are mixed with curds and given locally until recovery in cases of mechanical injuries to anus
<i>Oroxylum indicum</i>	Sonpatha (H)	Seeds fried in rape oil (<i>Brassica napus</i>) and made in to paste are applied locally on wounds and cracks
<i>Picorrhixa scrophulariiflora</i>	Kutki (N)	Root paste is applied locally as antiseptic for speedy healing
<i>Prunus amygdalus</i>	Badam (H)	Paste is made with equal amount of cow's butter with half the amount of salt and applied locally
<i>Solena heterophylla</i>	Chengor (santhal)	Leaf juice is applied locally
<i>Syzygium cumini</i>	Jamun (H)	Stem bark or fruit is given orally
<i>Tarrena asiatica</i>	Kommichetu (APR)	Leaf infusion is given orally in plough injuries
<i>Trachyspermun ammi</i>	Jwano (N) Ammi or Bishop's weed (E)	Seed paste is applied locally
<i>Tridox procumbens</i>	Balapaku (APC)	Fine leaf paste with a pinch of lime is applied locally
<i>Bergenia stracheyi</i>	Gatlis (L)	Root paste applied locally
<i>Codonopsis rotundifolia</i>	Kaerempo (L)	Root poultice is used to stop cutaneous eruptions
<i>Trapopogon dubis</i>	Budege (K)	Latex is applied on heel wounds
<i>Rumex orientalis</i>	Bedahabul (K)	Root and leaves on boils
<i>Indigofera heterantha</i>	Keiche (K)	Flower infusion on wound
<i>Euphorbia helioscopia</i>	Gurschel (K)	Latex applied on skin eruptions
<i>Datura stramonium</i>	Datur (L/K)	Leaf poultice on eruptions
<i>Bibersteinia emodi</i>	Drakspore (L)	Plant used to treat wound, cuts, ulcers
<i>Codonopsis ovale</i>	Ludut (L)	Leaf poultice applied on wounds

Table 2. Plants Used in Treatment of Horn Injuries

Name of Plant		Part(s) Used and mode of usage
Botanical Name	Local Name (Region)	
<i>Boschniackia himalaica</i>	Ganelu (UPH)	Whole plant poultice is applied locally
<i>Carissa opaca</i>	Karaunda (H)	Root paste applied locally

Table 3. Plants Used in Treatment of Septic Wounds

Name of Plant		Part(s) Used and mode of usage
Botanical Name	Local Name (Region)	
<i>Basella alba</i>	Poi-ara (Santhal)	Dried stem burnt in a sealed earthen pot until a fine powder is obtained which is made as a balm with oil of <i>Schleichera oleosa</i> and applied locally
<i>Cuscuta reflexa</i>	Amarbel (H)	Whole plant extract is used locally
<i>Eclipta alba</i>	Bhangra (H)	Pounded root is used with oil of kusum
<i>Lygodium flexuosum</i>	Durga-japhi (Santhal)	Root powder is applied locally
<i>Madhuca longifolia</i>	Mahua (H)	Seed oil is applied locally
<i>Trachsperrum ammi</i>	Jwano (N)	Seed paste is applied locally
<i>Woodfordia fruticosa</i>	Dhai (H) Fire flame bush (E)	Decoction of leaves is applied locally

Table 4. Plants Used in Treatment of Bruises and Contusions

Name of Plant		Part(s) Used and mode of usage
Botanical Name	Local Name (Region)	
<i>Asparagus adscendens</i>	Safed musli (H)	Infusion of rhizomes is given orally
<i>Solanum nigrum</i>	Makoy (H) Garden night shade (E)	Leaf juice is applied locally to provide quick healing after castration
<i>Solena heterophylla</i>	Chengor (Santhal)	Leaf juice is applied locally

Table 5. Plants Used in Treatment of Maggot Wounds

Name of Plant		Part(s) Used and mode of usage
Botanical Name	Local Name (Region)	
Betula alnoides	Bhurjpatra (H)	Bark paste applied locally
Caltha palustris	Mamiri (H)	Root paste applied locally
Carissa opaca	Karaunda (H)	Root paste applied locally
Canthium parviflorum	Balasa (APR)	Decoction of leaves is poured locally
Caryopteris odorata	Karwi (UPH)	Leaf juice is applied locally
Filipendula vestita	Toser (UPH)	Leaf paste is applied locally
Micromeria biflora	Gorakhphan (UPH)	Whole plant paste is applied locally
Milletlia racemosa	Galuga (APR)	Root paste is applied locally
Morina longifolia	Biskandra (UPH)	Root paste is mixed with camphor and applied
Neolitsea pallens	Kaula (UPH)	Seed oil is applied locally
Schleichera oleosa	Kusum (H)	Seed paste is applied locally
	Honey tree (E)	
Tectona grandis	Sagwan (H)	Wood powder mixed with mustard oil is applied locally
	Teak tree (E)	
Prunus persica	Aaruu (H)	Juice of young leaves mixed with common salt and applied locally to expel maggots
	Peach tree (E)	
Syzygium cumini	Jamun (H)	Bark paste is applied locally to eliminate maggots
	Black plum (E)	
Filipendula spp.	Perennial herbaceous flowering plant	Leaf paste is applied locally on maggot wounds
Mimosa pudica	Lajjavanthi (H)	Leaves given as fodder to treat maggots
	Touch-me-not (E)	
Allium sativum	Lasun (H)	Bulb paste is applied locally
	Garlic (E)	
Crotalaria linifolia	Narrow-leaved crotalaria (E)	Whole plant is powdered with salt and applied externally

LIST OF ABBREVIATIONS USED

S	Sanskrit
H	Hindi
E	English
APC	Andhra Pradesh, Chitoor, District
APR	Andhra Pradesh, Rayalseema Bhamij, Birhore, Kondhs, Lodhas, Munda, Oranand, Santhal, Tribles in Bihar, Eastern India Orissa and West Bengal
Bhils	Tribe in Rajasthan
JKLM	Jammu , Kashmir, Ladakh and Morni hills of Haryana
Nepal	Central Nepal
Sikkim	Sikkim and Darjeeling Hills
UPH	Uttar Pradesh hills
L	Ladakh
K	Kashmir

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Article 3

Role of Anabolic Steroids in Meat Industry-Residual Effect and Safety Aspect

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INTRODUCTION

Animal growth is a complex interaction of genetic makeup, environment, nutritional and the hormonal influences. The quest for regulating the overall growth of farm animals has long evoked the interest of many a workers. With the advancement in scientific techniques and manipulation of the endocrine status of animals by administrative of exogenous compounds, this quest has seen a sort of a culmination through the use of anabolic steroids (testosterone, Estrogen and progesterone).

In 1984 A. A. Berthold, a German scientist, isolated for the first time the hormone testosterone from the male gonads and a few year later another hormone estrogen was isolated from the female gonads. Both these hormones are steroid in nature and exert genital effect (Development of secondary sexual characters) and extra genital/anabolic effect (retention of nitrogen, calcium and phosphorus). The nitrogen retaining effect of these hormones was reported for the first time by Kochakian and Murlin (1935) in a study on castrated dogs who were injected with androgen containing extract obtained from urine of a normal man.

Prolonged use of these hormones produced sexual dimorphism in food animal. This shortcoming led to search of a product with least genital and maximum anabolic effect and consequently in 1940 a Powerful protein sparing /synthesizing agent with least genital effect was developed and named as anabolic steroid. From time immemorial man had tried to exercise his control over the sex ratio and manifold theories & false beliefs had been practiced.

Anabolic steroids are derivatives of cholesterol and including

1. Natural Steroids: Mainly oestradiol, testosterone and progesterone.
2. Synthetic Artificial Steroids: include trenbolone, ethynyl estradiol, methyl testosterone, chlormadinone acetate, malengestrol acetate medroxy progesterone, nortertosterone (Nandrolone) etc.
3. Non-Steriod Compounds: these include diethyl stilbestrol, hexestrol, dienestrol, zeranol.

A significant improvement in feed conversion efficiency and body weight gain has been achieved in different food animals following administration of anabolic by various workers all over the globe (Table I). In Australia alone, anabolic steroids were used on 45% of nation's eligible cattle and claimed to provide a net benefits to the industry of over 60 million dollars a year (AVCA 1986). In spite of tremendous beneficial effects, the use of anabolic agents in food animals was not much appreciated by the European scientific community because the question of residues in edible tissues of food animals following treatment remained an important consideration in assessment of health risk to consumers.

The main objective of this article is to bring together major facts based on scientific evidences and opinions in an attempt to clarify some issues regarding residual problem of anabolic steroids and safety to consumers.

MODE OF ACTION

Anabolic steroids play a pivotal role in bone growth basal metabolism muscular development, calcium retention etc. (Guyton1976) They are believed to act directly at the cellular level in muscle tissue through specific receptors to regulate protein synthesis and degradation (Scott, 1978 and Peter, 1985) and indirectly by activating higher centers to stimulate release of anabolic hormones viz growth hormone, insulin (Wagner et al, 1978 and Peter, 1985) and prolactin (Heiztman 1979, Schan 1985).

Little doses of progesterone also activate higher centers to stimulate release of anabolic hormone. Moreover, continuous administration of progesterone in beef heifers prevents ovulation but allows follicular growth. The estrogen released from these follicles exerts anabolic effect which results in body weight gain (Hafez 1991).

The increase in secretion of growth hormone following the administration of anabolic steroids also results in increase in plasma glucose concentration. The combination of increased growth hormone and insulin in the muscle cell probably increase protein accretion (Trenkle 1970). The net effect of each anabolic is to improve the rate at which nitrogen is retained by animals inside the muscle cells i.e. production of more protein. Apparently the increase in N-retention occurs without altering the absorption or metabolisms in alimentary tract (Chan. et. al 1975). The Gross effect of these products are increase in rate of feed in take, daily weight gains, feed conversion efficiency and (proportion of) lean meat in carcass. Owing to increase in feed intake, the percentage increase in efficiency is approximately half that of the increase in growth rate (Sawyer and Barker 1988)

EFFICACY OF ANABOLIC STEROIDS

The Magnitude of growth response following administration of Anabolic agent is dependent upon various factors. Females tend to grow better with a compound having some androgenic properties. Entire males gain more with an estrogenic compound alone or in combination with androgen.

Where as castrated ones tend to grow faster in presence of smaller amountt of estrogenic activity.(Hieztman 1983, Shah and Shrivastava, 2002 and Lamming, 1986). Younger and lighter animals responds less than older and heavier ones (Wal et al , 1975 and Sammons 1980), yearlings are the most responsive class of cattle (Perry et al, 1970 and Hodge et al, 1983)

The increasing dose (the number of implants) does not increase growth promoting response because animals capacity to respond is limited and once this capacity is attained any subsequent response in progressively diminished. (Summons, 1980 and Roche & Davis, 1983)

Animals kept on ordinary glazing condition respond less than feedlots due to variability in level of nutrition, however the timing of implantation in relation to the animals cycle of pasture particularly the “flush” of feed is an important consideration which may affect magnitude and duration of response achieved (Sammon, 1980 and Hodge et al, 1983)

RESIDUE IN EDIBLE TISSUES

The anabolic agent’s residue in meat arises from three sources

1. Residue emanating from the animals own endocrine system.
2. Residue derived from natural anabolic agent administered to living animal.
3. Residue emanating from xenobiotic anabolic agents administered to living animal.

Meat may contain residue of drugs or their metabolites at very low concentration (PPM/PPB/PPT). The concentrations vary widely with the nature and origin of samples (animal species, tissue, biological fluid, excrete).

The concentration of anabolic agent remains highest in plasma following implantation and comes to basal level in 6 to 19 weeks. The residue concentrations tend to be highest in liver, kidney and subcutaneous fat and the least in muscles (Heitzman, 1977 and Macvanish & Galbrath 1988 and 1993).

SAFETY TO CONSUMERS

The natural steroids (testosterone, progesterone and estrogen) when used over a prolonged period at extremely high doses are believed to be carcinogenic particularly in animals/women having a hereditary susceptibility to mammary cancers. There is insufficient proof that trenbolone, zeranol and malengesterol acetate are not carcinogenic at such dose levels (Roe 1974). The question which arises is whether the residues in animal products are at a concentration which would be carcinogenic to the consumers. This is highly unlikely in the case of the naturally occurring steroids and extensive testing on xenobiotic agents revealed that these compounds are not mutagenic, carcinogenic or genotoxic (Lamming 1986).

Taking into consideration the human and animal endocrinology and metabolism of these hormones, firstly these hormones are present naturally in man and animals in concentrations which are manifold higher than the possible intake from eating meat of implanted animals. Each day a non-pregnant woman produces 5400 times and an adult man 13500 times the amount of estrogen found in 500g of steak from an implanted steer. Like wise one hen's egg (50-60gms) contains about 2800 times the amount of estrogen found in 200 gms of steak from an implanted steer (Gallrainth, 1981 and Rubens & Vermeular, 1983).

Therefore the hormone ingested in meat from a correctly implanted steer is negligible, when compared to concentration of hormones in different food stuffs (Table 2 and 3) due to endogenous hormone production. It is believed that such minute amounts of hormones consumed would not interact with endocrine mechanism. Mankind has been consuming meat and animal products from lactating, pregnant or entire animals for thousands of years and these are therefore natural constituent of food of animal origin and their consumption has been well tolerated.

These anabolic agents have low oral activity and are rapidly metabolized and excreted by entero-hepatic system (Hoffman, 1981; Karey et al, 1983 and Rico & Burgotsacaz, 1983). This even the negligible amounts consumed in meat are further diluted before entering the circulation.

Further safe guards may be provided by adopting following measures:

- a) The site of implantation and implanting of anabolic agent in non-edible part, ear etc which could be discarded.
- b) Designated withdrawal period.
- c) Exploring possibilities of altering postnatal growth by prenatal exposure of animal to anabolic steroids for example implanting pregnant ewes at 40-60 day of gestation with trenbolone (Dehenn et al, 1990).
- d) Instead of skin implanting which exhibit slow release and biodegradability, injectable anabolic agents be considered. Nandrabone injection has proved to be very successful in promoting growth in kids and calves (Shah and Shrivastava, 2002; Shaheen et al, 2002 and Dhurbajoti, 1994)
- e) Anabolic agents may preferable be tried in kids because goats produces more lean meat and lack tendency to deposit subcutaneous fat particularly over the lion region (Agnihotri 1993).
- f) The natural steroids exert 300 to 800 times more response than synthetic ones and are excreted more rapidly (Sawyer and Barker, 1988). Their use in low doses may thus nullify the residual effect in meat animals. A significant increase in body weight has been achieved by administrative of bovine follicle fluid (sources of natural estrogen) in kids (Shah and Shrivastava, 2002).

Table 1

Product	Species	Response (Percentage Increase)		Reported by
		F.C.E.	Body weight	
exesterol	Steers	20	25	Gallbarth & Helen, 1978
lengesterol	Heifers	8	11	Hafez, 1991
eranol	Goats /Calves	10-15	25-55	Mac Gregor, 1984 and E. Katunguka et al, 1988
Trenbolone	Cattle, Sheep	10-12	15-25	Heitzman, 1978; Galbraith , 1980; Perry et al, 1990; Sulieman et al, 1992 and Galbraith & Berry, 1994
Testosterone	Rabbit, Lambs	10-12	15-20	Grigsby et al, 1976 and Gribsy, 1976
	Poultry	10-12	15-20	Suthama et al, 1989; Marry et al 1952 and Pope et al, 1950
Synovex-C	Calves	-	10	Gill et al, 1986; Sulieman et al, 1986 and Mader et al, 1994
Oestradiol 17 β	Calves, Goats, Steers	10-12	11-24	Shah & Shrivastava, 2002; Perry et al, 1991; Sawyer, 1987 and Wagner, 1984
Trenbolone acetate and estradiol	Calves, Lambs	15-20	20-70	Gallbarth & Helen, 1978; Coch et al, 1988, Sulieman et al, 1986 and Hodge, 1986
Estrogen & progesterone	Bulls	10-12	15-20	Gill, 1986; Lamnah, 1983 and Rochemarry et al, 1952
Nandrolone	Calves, Lambs	-	30-40	Galbraith & Berry, 1994; Shaheen et al, 2002; Greyling et al, 1993 and Shah & Shrivastava 2002

Table 2
Estimates of effective estrogen intake for various food portions

Food	Weight of serving (g)	Estrogen intake (ng)
Untreated steer meat	200	2.4-3.0
Estrogen implanted steer meat	200	1.0-4.0
Zeranol implanted steer meat	200	2.8
Cow Meat (Pregnant)	200	120
Heifer meat (Pregnant)	200	170
Hen's egg	50-60	1750
Cabbage	100	2400
Wheat germ	10	200
Soyabean	10 ml	20,000
Milk	200 ml	30

Adapted from Sawyer and Barker, 1988

Table 3
Residue level of anabolic steroids (ng/g) in tissues of treated and untreated cattle /steer

Compound	Animal	Muscle	Liver
Testosterone	Untreated		
	Bull	0.54	0.75
	Heifer	0.09	0.19
	Calf	0.02	0.04
	Treated		
	Calf	0.07	0.05
Oestradiol 17β	Untreated	0.4-0.9	
	Pregnant Cow	0.01	0.04
	Heifer	0.01	0.012
	Steer	0.01	0.1-1.5
	Male calf	0.02	
	Treated		
	Steer	0.02	
	Male calf	0.02	
Zeranol	Treated		
	Steer	0.1	0.3
Trenbolone	Untreated		
	Female sheep	0.18	0.09
	Treated		
Oestradiol	Female sheep	0.078	0.109
	Untreated		
	Female sheep	0.01	0.011
	Treated		
	Female sheep	0.012	0.011

Adapted from Macvanish and Gylbraith, 1993 and Sawyer & Barker, 1988

Gender Pre-Selection In Animals

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INTRODUCTION

From time immemorial man had tried to exercise his control over the sex ratio and manifold theories & false beliefs had been practiced. A popular theory that as right side of the uterus gives birth to male sex so woman desirous should lie on right side during sexual intercourse was proposed by Greek physicians as early as 5th century BC, another theory stated that right side of testis produce more male young ones & reverse for left. Aristotle explains sex of a child was determined by the partner who acted more vigorously. These false beliefs & practices disappeared with the advent of science as it became obvious that sex of a child or young one is dependant on sex chromosome (In female homogametic i.e. XX & heterogametic i.e. XY in males) and it is the presence or absence of Y chromosome that decides the sex. For last few decades sex or gender pre selection has been a dream project of animal breeders & scientists all over the world as it provides opportunity to improve management flexibility by helping farmers to get the required number of the desired sex without producing the other(Johnson et al, 1999).

BENEFITS OF SEX PRE SELECTION

1. Conservation of gene-pool of endangered & critically endangered species of animals.
2. It can aid us in pre-implantation genetic diagnosis.
3. It can help in increasing production of meat industry, more males & more fattening stock with increase in annual turnover in millions.
4. It can help in greater upliftment of dairy industry as female calves of excellent production traits can be produced.
5. It can help in elimination of lethal sex-linked traits e.g. ovarian or testicular hypoplasia.
6. It can help in avoiding inter-sexes in multiple births.
7. It helps in progeny testing.

TECHNIQUES FOR GENDER PREDETERMINATION

Theoretically, sex can be pre-determined if X & Y spermatozoa were separated before insemination & thus can be the ideal method of controlling sex ratio. Moreover, the ability to determine the sex of embryos prior to transfer in conjunction with embryo transfer techniques would be of great benefit to animal producers.

Sex pre selection for sake of convenience can be divide into:

- a) **Sexing of spermatozoa and**
- b) **Sexing of embryos**

a) **Sexing of Spermatozoa**

Method 1- Sexing based on DNA content:

The principle being that X and Y bearing spermatozoa carry different amounts of DNA. The female producing X- sperm contain 2.8 –7.5% more DNA than male producing sperm (Johnson & Lawrence, 1999). This method i.e. flow cytometry was actually described by Pinkel, et al in 1982 & adopted in domestic animals by Garner, et al 1983. Nowadays, high-speed flow cytometry is being used based on same lines. The wider the difference, the easier it is to sort with greater accuracy e.g. Boar carries about 3.6% >DNA in their X-sperm than Y-sperm. Bull Carries about 3.8% >DNA in their X-sperm than Y-sperm. Man Carries about 2.8% >DNA in their X-sperm than Y-sperm. A fluorescent dye is used which sticks to the DNA content, the dye binds to sperms based on how much DNA the X and Y chromosome in the sperms are carrying. The sperm cells are analyzed & sorted using a cell sorter. When laser beam illuminates the dye, the sperms gives off light proportional to its DNA content & it is separated into different tubes, depending upon the amount of light emitted. The X-sperms always glow brighter because of greater amount of DNA. With high speed flow cytometry 35-40 millions sperms can be sorted in an average of 8 hours day compared to 1-2 millions previously and with deep uterine insemination only about 3 lakh need to be placed to get pregnancy. In recent experiment, showing the effectiveness of the sperm sexing, 8 litters of pigs were born using sorted X chromosome sperm. 98% of the piglets born (i.e 43 out of 44) were females. Three control litters produced as the same time with unsexed sperms resulted in equal no. of the produced no. of male and female off-springs. Similarly insemination of heifers with freshly collected sperms already sexed with flow cytometer an accuracy approaching 90% male or females was observed. (Siedel, et al. 1999). A sperm FISH (Fluorescent In-situ hybridization) protocol can be used for validation of effectiveness of sperm separation procedure (Rens, et al. 2001) Therefore sexed sperms on demand over the next several years will provide with many options in seeking to improve efficiency of production & improve quality of products to enhance consumer acceptability (Johnson, 2000).

Limitation:

1. Expensive
2. Only a limited number of cows can be inseminated in a day

Method 2- Separation of spermatazoa on basis of presence of H-Y antigen:

The histocompatibility Y (H-Y) antigen was discovered in 1955 when it was observed that female mice rejected skin grafts from male syngenic mice (Eichwald & Silmsler, 1955) whereas skin grafts exchanged among other sex combination was accepted. Rejection in this case was due to H-Y antigen, present in cells of males (associated with Y Chromosomes), but not in females (Billingham & Silvers, 1960).

Among mammal it is present in males of cattle, sheep, goat, horses, pigs & human beings. (Wachtel, 1983). Koo, et al (1973) located the male specific (H-Y antigens as glycoproteins on plasma membrane of sperm by electron microscopy. If the expression of H-Y antigen is on the surface of these haploid cells is due to expression of Y chromosome, then this could be used to separate H-Y +ive spermatazoa carrying Y chromosome from H-Y negative spermatazoa.

Semen was layered on top of a sephadex column saturated with polyclonal antibody to H-Y & when the bound spermatazoa (H-Y) were used to inseminate mice 96% off-springs were males, (Bryant, 1980). Similarly Zavos in 1983 reported intra-vaginal administration of H-Y antisera to rabbits resulted in significant increase in number of female off -springs (74%) . But in 1983, Wachtel showed the presence of H-Y antigen in the membrane of both X & Y spermatazoa under usual circumstances. So sexing of spermatazoa on basis of using H-Y antigen is not effective.

Method 3- Separation on basis of difference in mass & motility:

- Sedimentation: Immobilized sperm on media, when inseminated produced 70% females with sperm that had sedimented the great distance. The determination of sex of calves by AI with spermatazoa separated by sedimentation was reported by Bhattacharya 1986.
- Albumin Gradient: - Spermatazoa sperms are layered on discontinuous albumin gradient derived from bovine serum, the X spermatazoa remains on top while as Y spermatazoa penetrate the albumin being faster (Zavos, 1985). This method actually enhances the motility but do not separate X and Y spermatazoa effectively as evidenced in rabbits (Zavous, 1985).
- Percoll's density gradient (Different velocity sedimentation in Percoll's Gradient. Percoll consists of colloidal silica particles coated with polyvinyl pyrrolldone. It can be used to separate the motile spermatazoa. Here semen is layered on top of percoll column and spermatazoa penetrate it. The penetration is a function of both mass & motility. Then centrifugation @ 200 rpm for 15 minutes is done minimizing the effect of motility & maximizing the effect of mass. The X-bearing spermatazoa being heavier are found at bottom and Y bearing being lighter remain at top.

Method 4- Separation on the basis of differences in surface charge:

- Free flow electrophoresis: Masuda H. (1981) subjected sperm to free flow electrophoresis. Fractions moving towards anode separated into 2 peaks, first peak being higher than second in motility was used to inseminate cow & 63.3% of calves born were female.
- Iso-electric focusing: Separation of sperms performed in columns with the fluid stabilize using density gradient, sperm layered on this solution migrate electrophoretically until reaching an iso-electric point. Iso-electric focusing of boar spermatozoa was reported by Moore, et al 1975.

b) Sexing of Embryos

Method 1-Sex Chromatin Identification:

In this method there is identification of a dark staining body lying adjacent to nuclear membrane. This body is known as barr body & occurs when there are 2 X Chromosomes & is seen in proportion of female cells. This method has been successfully used on trophoblast cells from 53-54 day old rabbit embryo at transfer (Gardner & Edwards, 1968).

Method 2- Sexing by chromosomal analysis:

It requires preparation of metaphase spreads from embryonic cells in mitosis.

1. Day 12 to day15 bovine & ovine embryos can be sexed before transfer by chromosome analysis on trophoblast biopsies. Betteridge, et al. in 1981 reported that 68% of day12 to day15 embryos can be sexed.
2. Chromosome analysis had been done on cells removed from day 6 to day 7 old bovine embryos at transfer with subsequent birth of sexed calves (Moustafa, et al. 1978 & Schneider & Hahn 1979). Small biopsies (10-20 cells) are collected by micro-manipulation technique for sexing.

Limitations:

1. Hare et al 1976 found that 2 experienced cytogeneticist/technicians take about 5 hours to process 12 to 15 embryo & since embryos of this age cant be stored for longer than four hours.
2. King (1984) mentioned that development of techniques for bisecting embryos on day 6 or 7 has raised the possibilities of sacrificing one half of embryos.
3. It is time consuming & affect the viability of embryos.

Therefore for these reasons it can't be used for commercial embryo sexing. However, King 1984 had concluded that these method are useful for confirming results of alternative means of sexing embryos avoiding risk of false diagnosis.

Method 3- Detection of HY-antigen:

Serological demonstration of the H-Y antigen is an alternative method for sexing embryos, because of the exclusive presence of H-Y antigen in males (Wachtel, et al. 1975) & its detection early in embryonic development (Krco & Goldberg, 1976), made possible to predict the sex of off-springs. Epstein, et al. 1980 exposed a large no. of 8 cell stage mouse embryo to H-Y antiserum & complement & observed that half of the embryos showed cell lysis, intact embryos not showing any cell lysis 92% karyotyped as females.

White, et al. 1982; Shelton & Goldberg, 1984 did similar experiments but instead of karyotyping, they transferred embryos to pseudo-pregnant recipients & obtained 82 & 86% females respectively. In another study White, et al. 1983 used monoclonal antibodies to detect H-Y antigen on morula & blastula stage using a cytotoxicity assay & an immuno-fluorescent assay.

Of those embryos classified as non-affected by cytotoxicity tests 81% were females & of those classified as non fluorescent 83% were females. With use of monoclonal antibodies accuracy has not gone beyond 87% (Wachtel, 1984).

In brief:

- 1) Donor cattle are super-ovulated & embryo obtained by flushing of uterus.
- 2) The embryos are washed & exposed to anti H-Y antibody conjugated with fluorescence iso-thiocyanate (FITC).
- 3) After incubation, embryos are washed again & evaluated for bound label under the fluorescent microscope.
- 4) Embryos are Karyotyped in order to confirm correct identification

Advantages over chromosomal analysis assay

1. Embryo can be sexed at day 6-12 days of gestation at which embryo transfer is possible.
2. The technique sets no limits on no. of embryos to be sexed & takes only 2 hours.
3. It can be easily applied by layman with no experience in immunological techniques.
4. The viability of the embryos is not affected by the manipulation (K.L, White, 1985).

But to eliminate use of fluorescent microscope Wachtel (1984) reported the development of immunoassay based on reaction of enzyme & substrate to generate colored product. This enzyme system allows visual scoring & can be made available in kit form for use at farm level is in trials.

Method 4- Polymerase chain reaction (PCR):

The PCR is a powerful technique that is widely used for amplification of specific DNA sequences in vitro using appropriate primers. PCR is a major breakthrough technology & is relatively rapid, sensitive & inexpensive procedure for amplification & cloning of the DNA of interest. It is also invaluable for genetic diagnosis, detection of mutation & genetic engineering. The general principle of the PCR starts from a pair of oligonucleotide primers that are designed so that a forward or sense primer directs the synthesis of DNA towards a reverse or anti sense primer & vice-versa.

During the PCR, Taq DNA polymerase, which is purified from bacterial *Thermus aquaticus*, is a heat stable enzyme & catalysis the synthesis of a non DNA strand that is complementary to a template DNA from the 5'-3' direction by primer extension reaction, resulting in the production of DNA region flanked by two primers. Because the Taq DNA polymerase is high temperature (95°C) stable, it is possible for target sequences to be amplified for many sequences by using excess primers in a commercial thermocycler apparatus. Recently, high quality Taq polymers such as recombinant polymerase Tth, long expand polymerase & high fidelity PCR polymerase has been developed. Due to their proof reading capability, these polymers are much advanced enzymes as compared with traditional Taq DNA polymerase

- a) Rapid sexing of pre-implantation of bovine embryo using consecutive & multiplex PCR with biopsied single blastomeres (Park, JH et al 2001) where satellite DNA sequences were selected for amplification of amplification of male & bovine specific DNA. The PCR was employed successfully using groups of 8, 4, 2 & 1 blastomeres dissociated from the embryos & sexing efficiency was 100.0, 96.3, 94.3 & 92.1% respectively and can be done within 2 hours. The multipurpose primer set have 100% specificity for sexing of cattle embryos (Limanskaya, et al 2000).
- b) Duplex PCR can also be used for sexing of embryos by employing cattle specific BRY – 1 primers for amplification of male specific fragments on Y-chromosome & the assay could be performed in < 4 hours (Sood, et al 2001).
- c) Reverse Transcriptase PCR (RT-PCR) can reveal bovine male enhanced antigen (Mea) gene from embryo from 8-cell stage with Y-specific probe (Kondoo, M, et al 1994).
- d) Sex diagnosis by detection of bovine foetal DNA from amniotic fluid by using PCR can be done successfully (Plippo, et al 1996). Besides, PCR has been useful in identifying gene-mutations (RYRL in pigs), Bovine Leukocyte Adhesion Deficiency (BLAD) & Uridine –5- Synthase deficiency (UMPS) in cattle & the advantage that implantation ability of the embryo is not impaired (Wayda, 1999).

Method 5- Ultrasonography:

Sex determination of bovine fetuses offers a tool for management of cattle husbandry with 80 – 90% accuracy. Viana, et al. 1994 found 96.3% accuracy in diagnosis between day 60th & 90th of pregnancy. While as Diaze, et al. 1995 found accuracy in diagnosis only up to 87.72% by transrectal ultrasonography on days 53 & 80 of pregnancy.

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Bovine Spongiform Encephalopathy and its Relation With Creutzfeldt Jakob Disease

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INTRODUCTION

Bovine spongiform encephalopathy (BSE) is a fatal brain disease of cattle. It has long been stated to have been first observed on a farm in Kent in April 1985, and to have been identified by Central Veterinary Laboratory (CVL), UK in November 1986 (Wells et al, 1987.) Bovine spongiform encephalopathy (BSE), widely known as "mad cow disease," is a chronic, degenerative disease affecting the central nervous system of cattle. World wide there have been more than 200,000 cases since the disease was first diagnosed in 1986 in Great Britain. BSE has had a substantial impact on the livestock industry in the United Kingdom. The disease has also been confirmed in native-born cattle in Belgium, Denmark, France, Germany, Ireland, Luxembourg, Liechtenstein, the Netherlands, Northern Ireland, Portugal, Spain and Switzerland. However, over 95% of all BSE cases have occurred in the United Kingdom. BSE is not known to exist in the United States. BSE belongs to the family of diseases known as the transmissible spongiform encephalopathies (TSE's). These diseases are caused by a transmissible agent which is yet to be fully characterized. Transmissible Spongiform Encephalopathies share the following common characteristics:

- a. prolonged incubation period of months or years;
- b. a progressive debilitating neurological illness which is always fatal;
- c. when examined by electron microscopy, detergent treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapie associated fibrils (SAF);
- d. pathological changes appear to be confined to the CNS and include vacuolation, and astroglycosis;
- e. (e) the transmissible agent elicits no detectable specific immune response in the host which has inhibited the development of a live animal diagnostic test.

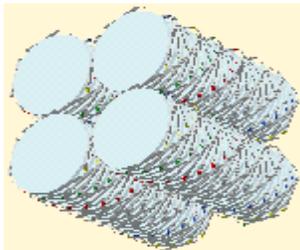
AETIOLOGY

The causative agent of BSE as well as other TSE's is yet to be fully characterized. Three main theories on the nature of the agent have been proposed

- 1) An unconventional virus.
- 2) A prion or an abnormal partially-proteinase K-resistant protein, devoid of nucleic acid, capable of causing a cell to produce more abnormal protein.
- 3) A virino or "incomplete" virus composed of naked nucleic acid protected by host proteins.

3-D Shape of Prion

Characteristics of the BSE agent. It,



- i. is smaller than most viral particles and is highly resistant to heat, ultraviolet light, ionizing radiation, and common disinfectants that normally inactivate viruses or bacteria;
- ii. causes no detectable immune or inflammatory response in the host; and
- iii. has not been observed microscopically.

According to the research conducted by Dr. Stanley B. Prusiner, for which he was awarded the Nobel Prize, Prions have been accepted as the most probable cause of BSE. Prions are responsible for transmissible and inherited disorders of protein conformation. They can also cause sporadic disease, in which neither transmission between individuals nor inheritance is evident. Moreover, there are hints that the prions causing the diseases explored thus far may not be the only ones. Prions made of rather different proteins may contribute to other neuro-degenerative diseases that are quite prevalent in humans. They might even participate in illnesses that attack muscles. The known prion diseases, all fatal, are sometimes referred to as spongiform encephalopathies. They are so named because they frequently cause the brain to become riddled with holes.

These illnesses, which can brew for years (or even for decades in humans) are widespread in animals. The most common form is scrapie, found in sheep and goats. Afflicted animals lose coordination and eventually become so incapacitated that they cannot stand. They also become irritable and, in some cases, develop an intense itch that leads them to scrape off their wool or hair (hence the name scrapie). The other prion diseases of animals go by such names as transmissible mink encephalopathy, chronic wasting disease of mule deer and elk, feline spongiform encephalopathy and bovine spongiform encephalopathy.

Gerald A. H. Wells and John W. Wilesmith of the Central Veterinary Laboratory in Weybridge, England, identified the condition in 1986, after it began striking cows in Great Britain, causing them to become uncoordinated and unusually apprehensive.

The human prion diseases are more obscure. Kuru has been seen only among the Fore Highlanders of Papua New Guinea. They call it the "laughing death." Vincent Zigas of the Australian Public Health Service and D. Carleton Gajdusek of the U.S. National Institutes of Health described it in 1957, noting that many highlanders became afflicted with a strange, fatal disease marked by loss of coordination (ataxia) and often later by dementia.

The affected individuals probably acquired Kuru through ritual cannibalism: the Fore tribe reportedly honored the dead by eating their brains. The practice has since stopped, and Kuru has virtually disappeared.

Creutzfeldt-Jakob disease, in contrast, occurs worldwide and usually becomes evident as dementia. Most of the time it appears sporadically, striking one person in a million, typically around age 60.

About 10 to 15 percent of cases are inherited, and a small number are, sadly, iatrogenic—spread inadvertently by the attempt to treat some other medical problem. Iatrogenic Creutzfeldt-Jakob disease has apparently been transmitted by corneal transplantation, implantation of dura mater or electrodes in the brain, use of contaminated surgical instruments, and injection of growth hormone derived from human pituitaries (before recombinant growth hormone became available).

The two remaining human disorders are Gerstmann-Straussler-Scheinker disease (which is manifest as ataxia and other signs of damage to the cerebellum) and fatal familial insomnia (in which dementia follows difficulty sleeping). Both these conditions are usually inherited and typically appear in midlife. Fatal familial insomnia was discovered only recently, by Elio Lugaresi and Rossella Medori of the University of Bologna and Pierluigi Gambetti of Case Western Reserve University.

A BRIEF HISTORY OF MAD COW DISEASE

Scrapie, a disease of sheep was investigated more as an oddity and for the interest that it caused specific groups of scientists, particularly in the UK, USA and to a small degree in Germany. Scrapie was thought of as a disease of sheep that did not infect humans, although its tissues were known to contain infection. When BSE arrived, it was immediately thought, because there were no other natural TSEs, to be derived from scrapie and for this to have been fed to cattle in the meal that they ate (to increase their milk yield). By the direction of the UK Ministry of Agriculture, Fisheries and Food (MAFF), a change had taken place in the way that this was made in the early 1980s and this, taking place at a similar time to the original infection of the cattle, was felt to be the answer. A small farm in Surrey reported more than one cow developing a strange neurological disease. The cattle were killed, the brains removed, and the animals destroyed.

When it was found that the cattle had a disease never reported before the farmer wanted to publish the data but was reportedly told not to by MAFF. When it is calculated, it seems that approximately 100 cattle had developed BSE symptoms before 1987 and many more would have been infected. It is now suggested that MAFF had been shown cattle with this disease before, and may have known about it in 1983, but did nothing.

1987; the publication of disease and Southwood:

Wells et al published the data showing that a cow had developed a spongiform encephalopathy. Little extra data was given. It was clear, however, that MAFF realized that this was no simple disease in that a committee was set up by them to advise on what action should be taken to avoid any risk to humans and cattle.

By this time, it was clear that the disease was appearing all over the country. Possibly it spread from the West Country to the other parts but, because of the speed of the spread this was not clear.

1988-the year of action that was too late:

Southwood, in the statement that was published stated that there would be minimal risk to humans as all infected cattle would be slaughtered. By not eating the animals with clinical illness there would be no problem and, as the disease was simply scrapie, and scrapie did not spread to humans, we should not expect BSE to spread to any other animals. Humans could continue to eat bovine brain and not worry about the consequences. The answer to BSE was to prevent all bovine material from entering the food that was fed to cattle. This was brought into action in July 1988. The feed manufacturers were warned that this was going to happen several months in advance. The reporting of cases of BSE to MAFF was made obligatory and half the value of a non-sick animal was given in compensation.;

1989; the year of the specified offals ban:

The scientific community was surprised by the relative inaction recommended by Southwood. The committee that was set up by Southwood, known as the Spongiform Encephalopathy Advisory Committee, immediately recommended that specific offals (brain, spleen, thymus, tonsil, gut) should be discarded and that all clinically ill cattle should be destroyed by incineration or burial. By this time BSE had been transmitted to mice in the laboratory and apparently to various zoo animals through the eating of the same feed. Compensation for farmers reporting cases of BSE was only half the value of the cow.

1990; the year of the media hype:

The CJD surveillance unit was set up in Edinburgh, UK to find out if BSE was giving rise to extra cases of CJD. The British Parliamentary Agriculture Committee follows a media scare on the risks from BSE. John Gummer, at the time the UK Minister for Agriculture, tries to give his daughter a beef burger in front of the cameras outside parliament (she refused). By this time the numbers of cases were reaching 300 per week. Compensation was stepped up to the full value of the cow and numbers continued to rise. The German Government decided that it would not accept British beef as food in their country because of the risk that it potentially had to their population. Gummer was furious and demanded that less strict laws be taken through the EC Agriculture Committee. The amount of compensation payable to farmers for a case of BSE was increased. Lacey demanded that all infected herds should be slaughtered and that restocking should take place from abroad. Roger Eddy made it clear that he may have seen cases of BSE before the epidemic and suggested that scrapie may not have been its source at all. Gummer made it absolutely clear to the British National Consumer Council that beef was safe and said that there was no risk whatever. A domestic cat develops what was later confirmed as BSE. An American had inoculated scrapie into a cow and it developed a SE, but under the microscope it was not the same as BSE.

Various schools ban beef in meals. The centre for agricultural research in Reading demanded that MAFF let professional independent researchers carry out the research into BSE as the results MAFF was releasing led to hysteria.

Kiethley News shows that the number of BSE cases was building up so fast that the various parts of the animal could not be incinerated and had to be buried on a local tip. Beef consumption in the UK dropped to the lowest level since 1962. It becomes clear that many of the cases of CJD were never reported. 65% of doctors 'changed their habit of eating beef' due to BSE. All offal banned from export to the EC. A marmoset monkey inoculated with BSE dies.

1991; the year of refusals:

UK experts were sent to convince them that BSE was not a risk. Harash Narang was reportedly told to stop carrying on his research into BSE and its risk to humans. A case appeared in a cow that was born after the feed ban and they were sure could not have been fed infective material. Fears arose that BSE would also infect the rest of Europe because we had exported infected animals there. The UK would just be ahead of the rest. In the past the knackers would pay 30 for a cow but after 1990 they may actually charge 40 to take it away. The PHLS refused to allow Narang to continue his research. Health and Safety executives bring in directives on how to handle BSE infected carcasses as they might be a risk to the people involved. Watkins the reporter from Today showed that people that had received growth hormone inoculations were still acting as blood donors. It appears that some genetics of a human makes them more likely to develop CJD and have a shorter incubation period. A statutory order from MAFF prevented any use of the specified offals; for a while they had been used for the feed of other animals and as fertiliser. The 'mad calf' syndrome; a calf born to a cow with BSE develops the disease.

1992; the year of the zoo cats:

A cheetah and the puma died of a TSE now thought to be BSE in the food that they had eaten. It was not clear, however how this could have been through eating brain, as they were never fed this. Fatal familial insomnia is found to be a SE and due to a genetic change.

How now mad cow? An editorial in the British Medical Journal saying that we simply did not have enough knowledge to pronounce BSE as safe.

1993; 800 cases per week:

The number of cases was still rising with approximately 800 cases reported in each week. The vets were now being told that many of the cases that they accepted were not actually infected when the animal's brains were looked at under the microscope for evidence of disease; little evidence was ever presented for this and the rate for negatives seemed to remain at approximately 15%. Changes were made in the way that cattle could be sold. The vets that had been at the auctions were decreased in hours and a computer system was organised so that the ear tags on a cow could be used to find out if it was from an infected herd or not. Dealler publishes the data showing that, even using underestimation methods, that the risk to humans was unacceptably high for medical ethics to accept. Farmers were often no longer being asked at the auction if their cattle were from an infected herd and they were receiving better prices from the buyers as a result. Two dairy farmers with BSE in their herds, Mark Duncan and Peter Warhurst, were found to have died of CJD. MAFF claim that there was no infectivity in any tissue outside the central nervous system. A group of chemicals was found that prevented the growth of the infective agent of scrapie in the test tube. The mice without the prion protein gene were grown and found not to be open to infection with scrapie.

1994; the year of Victoria Rimmer:

Victoria Rimmer, the 16 year old from North Wales was claimed to be dying of CJD and for this to be due to having eaten BSE-infected cattle. Cattle meat was being exported for sale in Europe without evidence that it did not come from a BSE-free herd. Claims were made that pressure was being put on the vets to sign certificates without evidence. The computer system that had been set up was now found to be ineffective. It could only take information from the abattoirs and could not supply information as to whether a cow that was being slaughtered was from an infected herd or not. London Zoo revealed that it was planning to remove the top foot of soil from the Kudu enclosure and was destroying any faecal matter from the animals; meanwhile it was being denied that the soil of farms could become infected and that cattle could become endemically infected. The large number of cattle with BSE that had been born after the feed ban suggested that endemic infection, or vertical infection from the mother, could be taking place. MAFF denied this risk. Infectivity found in the gut of a 6 month old veal calf that had been fed BSE when very young. All gut and thymus from calves could not then be eaten. Animal protesters attempted to stop the export of calves for veal production but little information was passed to European countries about the risk from BSE. No calculations were released about the amount of these tissues that had already been eaten.

The start of the Spongiform Encephalopathy Research Campaign: The Germans were unimpressed by 'safe beef pledge' from UK. The EC now made it essential that any meat on the bone being exported could only come from herds unaffected with BSE in the previous 6 years. Gillian Shephard had thought of this as a success and came back and told the newspapers. They quickly realised it was a defeat. A farmer suggests that organophosphorus insecticides may be important in the cause of BSE. CJD reported as being in a similar prevalence in many European countries. It is admitted that, of 156 cases of CJD since 1990, 22 are believed to have given blood at some stage. CJD was found in a butcher from Whitby. Waldergrave takes over as Minister at MAFF. It was shown that abattoirs were attempting to export beef that was from infected herds and that the computer, supposedly carrying the information about all the cattle, was not permitted to give that information out for data control reasons.

1995; the year that underreporting of cases became clear and further farmers died:

It became clear that 1.8 million infected cattle would be eaten from UK farms by the year 2001 and that most of these had already been eaten. Underreporting of cases in 1992 and 1993 was shown to reach 60%. A further farmer died of CJD and a second was dying of what seemed to be the disease. Both were from BSE affected farms. Two teenagers (including Stephen Churchill) developed CJD. Only 4 teenagers had been reported with CJD at any time throughout the world. It now became clear that the feed ban that took place in 1988 was too late. In fact, around 90% of the dairy cattle in the UK turned out being in an infected herd and, due to the apparently limited in-herd rate it seemed that the disease was, by 1988 running out of cattle to involve. If the ban had been in 1987 the number of affected cattle would have been less than half.

1996; The year that it became clear that BSE may well have infected humans:

March 1996 speeches by Stephen Dorrell (UK Minister for Health) and Hogg (UK Minister for Agriculture Fisheries and Food) admitted that 10 people with a new form of CJD, a variant form, CJD2, had appeared and 8 had died.

They must assume that the disease was due to BSE being present in food before November 1989, when the offals ban was introduced. This was followed by a melt down of the credibility of MAFF and the newspapers and TV were determined to make this happen.

The European Union quickly banned the export of cattle from the UK and all bovine products and the viewpoint of the Spongiform encephalopathy advisory Committee (SEAC) was that the actions taken so far was probably all that was needed as long as the directions were really carried out. It was soon made clear that these directions were not. The renderers had been permitting tissue from infected cattle to reach further cattle food, the farmers had been permitting cattle with infection to reach human food also. It was admitted on the TV that certain tissues from a cow should not be accepted as being safe unless de-boned in specific places and overseen.

The farmers could not take the effect from the EU and demanded that cattle over 30 months not be eaten but slaughtered as if they had BSE when they went to slaughter at the end of their milking lives. This was followed by an uproar in the press and demands from the EU that this was carried out. Hogg backed down and put forward plans concerning major slaughter policies to the EU. They said they were completely inadequate and demanded a greater action. Hogg put up the slaughter from 40,000 to eventually 147,000 but even that was not good enough for some members as they saw no way to get rid of all infected cattle. Mr. Major was determined to complain at the way that the EU had put a ban on the UK beef and refused to allow further EU major action unless this ban was removed after several weeks of the UK preventing EC action, and just before a conference the EC they would permit the decrease in the ban and Major declared that he had won. In fact the EC hardly offered anything and any cut in the ban depended on further demands of the EC at later dates. It became clear after 19th of July SEAC meeting that there had been vertical transmission of BSE between dam and calf. The disease was not likely to leave the UK for many years and, even though MAFF said that 2005 would be good, anyone looking at the figures could see that these were very hopeful. Shortly afterwards it was announced that sheep could easily become infected from BSE and, as we had infected plenty of cattle, there is no reason why sheep could not already be infected. The French quickly put a brain and spinal cord offals ban on all sheep for human consumption and the same may appear in the UK.

The prevention of information being released was made clear in a major Nature article in September and this was followed by calls for the release of the information. MAFF agreed, but it has still not happened to any great degree (by Dec 1996). Indications that new variant CJD really was derived from BSE came when Collinge's group in London showed them to be of the same glycoform (i.e. had similar sugar chains attached).

The European Commission had by this time been shown to have deliberately played down BSE and its potential problems all the way through and that there had been a sort of agreement to silence among the Agriculture Commission. The European Parliament found this intolerable and set up a separate committee to look into the matter. This decided that the silence from the EC was intolerable and that many of the official groups were guilty of inaction. Eventually the Governments and the research groups stirred themselves to produce specific research committees and timetables.

The dreadful misleading of the population and parliament and the confusion of the farmers by MAFF action, even though they had seen their action as been for the good of farming in the UK, all became much clearer to the population.

Even though beef infection had almost been accepted by the UK population, who did not stop to the same degree as those in Europe eating it, it was clear that to get rid of the disease was going to be difficult and may be expected to take many years. By the end of the year it seems that the UK Government have admitted that little beef will be exported for many years and that they will have to carry out the cull of animals that was temporarily dropped by MAFF. Also, the highly expensive actions (mentioned at 3.2 billion pounds by Tony Blair in December 1996) did not take into account the realisation that huge amounts of research were going to have to be done.

1997; The year in which BSE was involved in bringing down the Government, and human blood was realized to be a risk:

The continuous fight by Mr Hogg for the EC to withdraw its ruling that no UK beef should be exported was unsuccessful, and visits to the UK by various foreign groups simply made things worse as they spoke to the farmers who must have seemed simply misled to the visitors. Hogg would not withdraw his demand, asked Pattison to go to Brussels to fight for him, and continued to pay the farmers really quite large amounts of money, realising that after the election, if the levels of compensation continued it would be in billions of pounds.

The Tory Government was crushed in the election, which showed the greatest Labour majority ever and a new Prime Minister, Tony Blair, who was willing to make peace with the European neighbors. The new Agriculture Minister, Dr. Jack Cunningham, was apparently much more understanding of the problems than his predecessors and immediately stated that he did not expect the EC to back down while a risk was present and the only way out was to remove the risk, initially by permitting Northern Ireland beef (the area with the fewest cases and good computer regulation of cattle), and then allowing this to spread to other areas as they improved. He announced that the costs would be over 3.5 billion pounds and gradually, as the year went on he admitted that the figure may well turn out to be much greater.

The scientific publication by Moira Bruce that BSE, when inoculated into mice produced the same disease as nvCJD was the nail in the coffin for the continuing groups that claimed that the association between the two 'was not proved' (and therefore should not be assumed). Dolly the sheep appeared as a sheep made from a single breast cell (named after Dolly Parson, the singer with the large breasts) and it was realised that the making of sheep that could not develop BSE or scrapie was at last possible. Research money arrived for this.

The major researchers for MAFF in the field; Tony Austen and Mike Richards left them and the research money started to flow into the medical side as the ban on Public Health involvement in the subject was 'lifted'. The realisation that blood transfusion could represent a risk hit the press but was overrun by other subjects. This became a major problem for the Government behind the scenes, as there appeared to be no way in which UK blood could be looked on as being totally safe. Eventually the blood products manufacturers realised that this was indeed true and looked for any way in which they could keep going and the foreign suppliers moved in.

The Haemophilia society and the Directors of the Haemophilia Centres (the doctors that proscribe the drugs) made it clear that they did not want UK factor 8 etc. It was announced that various other tissues would be included in the list of Specified Bovine Materials: bone, the dorsal root ganglia, and the lung (the lung part hardly seemed to reach the press).

This was handled well by the new Government and the media ended up asking why they had banned them at all as the 'risk was so low'. The announcement that there was to be a public judicial inquiry into BSE and nvCJD up until 1996 was announced by Jack Cunningham but the scientific advisors to Lord Justice Phillips are not yet named. They are likely to be some of the most important people in the inquiry, as they will direct him towards certain aspects of the epidemic. The aim is to find out why it all took place, and why such poor action was taken. The inquiry has to report before the end of 1998. During the year about 12 further cases of nvCJD appeared, compensation reached approximately 1 billion pounds, research budgetting was in some quarters put as 'unlimited', and nvCJD was admitted to be BSE by the UK Government.

The developments since 1997 are well known and need not necessarily be included under history.

ORIGIN AND EPIDEMIOLOGY

Various hypothesis have come up to explain origin of BSE, some of the popular ones include:

- A. **BSE derived from scrapie**
- B. **BSE is a rare sporadic disease of cattle**
- C. **BSE derived from CJD**
- D. **Organophosphorus insecticides involved**
- E. **Knackers yard greaves involved in transmission**
- F. **Bacterial toxicosis causes BSE**
- G. **BSE is a lysosomal storage disease**
- H. **Horizontal BSE transmission through the eye**
- I. **A list of feed changes that may be involved**
- J. **BSE is an autoimmune disease**
- K. **BSE transmission is via a campylobacter-like organism**
- L. **Alkaloidal glycosidase inhibitors cause PrPc structure change**
- M. **Smarden spill caused toxic induction of initial BSE outbreak**
- N. **Thioldisulphide interchange chemistry caused PrP configuration changes**
- O. **Bovine pituitary hormone use caused initial spread of disease**
- P. **Oligonucleotide involvement in transmission agent**
- Q. **BSE cases seen are vertically transmitted but a much higher proportion of herd are asymptomatic**
- R. **A range of trace metals may be involved in BSE symptoms and pathology.**
- S. **BSE controlled by prostaglandin**
- T. **BSE is the clinical sign seen when a chromosomal virus is expressed**
- U. **Maddocks and Dealler hypothesis: MBM increases in 1980
Infection taking place on farms almost entirely withing the first month:
faecal or meal contamination**

An attempt is made to put forward the arguments for and against each of the hypothesis.

BSE derived from scrapie and was present in bovine feed as a result of changed in the manufacture processes for MBM.

Pro:

1. Cattle could be infected with scrapie by experiment.
2. Epidemiology did not start as a single case and followed by multiple peaks.

Anti:

1. BSE was not the same strain of TSE as any scrapie investigated previously.
2. When scrapie inoculated into cows they got a different form of illness.
3. No fall in incubation period was seen.
4. BSE did not have the same range of infectivity as scrapie.
5. No apparent increase in scrapie during the period despite large amounts of MBM fed to sheep.
6. Areas where BSE started were not particularly sheep areas (could be explained by movement of sheep carcasses to renderers).
7. No clear alteration in infectivity of MBM using solvent extraction methods.
8. No alteration in infectivity expected in MBM due to continuous rather than batch rendering.
9. Epidemiology and clinical nature of disease suggests that all BSE derived originally from a single point source i.e. not from many different strains of scrapie.

BSE existed as a rare disease in cattle and the epidemic was due simply to this becoming established in the MBM feed chain.

Pro:

1. No fall in incubation period was seen early in the epidemic
2. This would not require the first case to have taken place in 1981 but could have been earlier.
3. No necessity for rendering processes to be involved (no evidence that they they were).
4. Areas where BSE started were not particularly sheep areas (could be explained by movement of sheep carcasses to renderers).
5. BSE has a specific range of infectivity (separate from scrapie apparently).
6. No increase in sheep disease was necessarily going to be seen.
7. Outbreak of TME in Stetsonville possibly associated with a TSE in a cow.
8. Reports from veterinary surgeons that they feel they had seen cases of BSE before the epidemic simply as rare conditions in cattle.
9. Work by Collinge and by Bruce indicates that BSE is not similar to scrapie of the strains that they have available.
10. Epidemiology and clinical nature of disease suggests that all BSE derived originally from a single point source. i.e. not from many different strains of scrapie.

Anti:

1. The index BSE case would be expected to give rise to BSE cases that were most infective between 3-6 years later and those would be expected to give rise to a second peak 3-6 years after that (each peak broader but 10-100 times higher (Dealler, Donnelly). As such it might be expected to see a specific shape to the epidemic curve. No such shape was seen. This is a quite difficult problem to avoid.
2. Unclear why other countries using similar agricultural methods did not get a similar outbreak of disease.

3. If it came from a single animal with disease then this index case may have been in the early 1970s or 1960s and could not have been associated with rendering methods. No evidence for this.

BSE was derived from CJD

Pro:

1. Antibodies were raised for the radio immuno assays for various human pituitary hormones by inoculating the hormones into animals. The animals would have been destroyed at the end of their useful lifespan. The most impure hormones used for inoculation would have been in the 1960s.
2. Body destruction following murder has been suggested to have been by rendering.
3. The spread of human body ashes on land.
4. No requirement for the initial inoculation of cattle to have taken place in 1980. As such it would not be likely to see a series of peaks before the main epidemic rises.
5. Poor evidence that BSE derived from scrapie and no certainty that BSE appeared in UK as a sporadic infection

Anti:

1. Work by Collinge and by Bruce showed BSE not to be similar to CJD of strains that are known but rather to be similar to nvCJD, which was unknown prior to BSE. (but some aspects of strain may change when transferred from one species to another)

Organophosphorus pesticide causing BSE

Pro:

1. Similar symptoms to OP toxicity
2. Changes in OPs during epidemic period
3. Same OPs used in specific countries with BSE
4. Warble fly treatment at a specific part of the year and this could explain why BSE is more common in cattle born at specific times.
5. Low rate of BSE in cattle of organic farms that did not use OP.

Anti:

1. Epidemiology of BSE does not fit the usage of OP
2. OPs used in specific groups whereas as a toxicity it would be expected not to have a peak at 4-6 yrs.
3. Cattle claimed not to have had any OP exposure develop BSE
4. OPs were in use before BSE appeared.
5. No specific OP changes took place in 1988 when the peak was reached in BSE.

Knackers yards also melt down tissue and sell greaves, often with a high fat content to renderers. This has taken place for many years and the output from knacker's yards does not seem to have been fully investigated. A particular proponent of this was a member of the industry. He looked at the changes in the way in which various knacker melters lost revenue as a result of the solvent extractors and he explained why a high titre of infectivity would be derived directly from these sources than through renderers.

Bacterial toxicosis

This is a complex hypothesis put forward by Tom Stockdale and involves the carriage of bacterial toxins to brain by a glycoprotein receptor corresponding to PrPc. This would result from the presence in the gut of abnormal proteins (e.g. fish).

BSE disease associated with a lysosomal storage type disease

This is the complex hypothesis put forward by Kevin O'Donnell from Edinburgh University. In this he notes that the PrP^{sc} form of the prion protein builds up partly because it cannot be broken down by lysosomes. He suggests that sporadic cases and familial cases may actually not be due to PrP alterations but possibly because the lysosomes fail to destroy them and they then build up as a crystalloid structure. The narrow genetic difference among dairy cattle in the UK might be involved in this.

Horizontal Transmission

It has been suggested that insects may have permitted the transmission of prion infection horizontally from the eye of one animal to the eye of another. (Vince Lisa, 284 Birchwood Rd, Medford NY. 11763, USA). The spread by mosquitoes has also been suggested but probably inadequate amounts of blood spread are present (Dean Goins, N. Carolina).

Feed Changes

Other changes in animal feed took place over the same period in 1980-81. The large increase in the growing of rape seed plants and the change in the color of the countryside as a result. Rape contains a high selenium content apparently and this has been suggested as a neuro toxin that might affect a prion infection. Rape oil is extracted and the remaining material is used for meal. It would also lead to a sudden drop in selenium in diet when other diets were introduced. It is this sudden decrease in the availability of selenium that is considered to be a possible cause by Tom Stockdale. The bringing in of UDP feeding, which involved feeding cattle with much large amounts of protein than the rumen could handle and hence led to the appearance in the jejunum of intact protein. It was decided that the overload of rumen bacterial action would lead to much greater amounts of amino acids being available for milk production. The calculation for the protein content for the feed was difficult and really only done by feed manufacturers, who used this to produce much higher protein feeds and promise greater milk output from a single cow. This has been fully explained by Dr. Maddocks. The use of black polythene plastic bags for holding offal for rendering. These were often simply thrown into the rendering plant mix and the offal not removed. This was simply because in the rendering system, the material was cut into small fragments for a continuous process and the bags disappeared. It was suggested that the bags, being of polythene would act as a hydrophobic surface and hence protect prions.

BSE in cattle may have arisen from a rare disease which affected the brain and spinal cord of horses

This disease is referenced in old books as one of a group of ailments known as staggers. About 50 years ago, the disease was 'lost', possibly because its incidence decreased, and all references to staggers are now assumed to relate to alkaloidal poisoning, most commonly by ragwort species (*Senecio jacobea* and *Senecio vulgaris*) in the UK countryside. There is no cure for staggers caused by alkaloidal poisoning. The symptoms occur when liver damage has reached a critical and irreversible level. However, the 'lost' disease also referred to as staggers was considered to be curable, and one cure was minute doses of ragwort.

Giving more ragwort to an animal already suffering from ragwort poisoning could only hasten its death, but it is just possible that the specific alkaloids in ragwort could have a beneficial effect in BSE in very small doses. It is possible that infected horsemeat was introduced into cattle feed during the 1980s, and that nvCJD may be transmitted directly from horses to humans, through vectors such as ticks or faeces. From press reports, at least some nvCJD victims had links with horses.

Molybdenum toxicity

Possibly associated with the lysosomal storage type syndrome of Kevin O Donnell.

Importation of tropical bones

The value of the pound at the beginning of the 1980s was extremely high and the importation of protein for animal food was felt to be worthwhile. It has been claimed (Independent 1997) that the disease was in fact prevalent in one of the animals from which the bones were from e.g. gazelles.

Shortage of copper in bovine diet

The change on bovine diet due to alterations in the type of source. This may explain why the disease started when it did in 1980 as it was then that the diet changes took place. Also there have been many indications that copper deficiency gives rise to a spongiform encephalopathy although no sign has been present as to why this is present. The suggestion is that possibly either copper deficiency sparked the epidemic by making animals sensitive to a transferred disease or it is more involved in the ongoing disease process.

Auto-immune reaction causing BSE brain damage

This is partly described by Axelrad in Medical Hypotheses (March 1998) and by Alan Ebringer in various publications.

Pro:

The appearance of the brain similar to auto immune disease Chronic process Antibodies to certain bacteria (acinetobacters) higher in cattle with BSE than in cattle without BSE. These bugs produce proteins that cross react antigenically with PrP Minor fall after feed ban.

Anti:

Epidemiology does not fit with autoimmune disease. Acinetobacters are not new and are present in all countries of the world. Peak at age 4-6 yrs and incubation period relatively steady. But no reason why this should be so for auto-antibody disease. Transmissibility of disease by inoculation Fall in the disease when feed ban introduced. Some farms that do not use certain feeds or OPs do not also have BSE but they would be expected to have acinetobacters similar to other farms. No antibodies to PrP found in blood of animals with BSE or any TSE. Inflammation at the plaque site is present but is not in an auto immune fashion. No reason why a prion form to a PrP protein should be involved in any way No experimental data concerning the inoculation of acinetobacters and the presence of antibodies being associated with the development of a TSE that is then transmissible. TSE can be transmitted from one animal to another by inoculation of purified material that could not contain bacteria. No response to antibiotics: even in animals given heavy doses from long before symptoms appear. Animals that cannot produce any antibodies whatever still develop TSE when inoculated with material.

Animals that cannot produce any antibodies or T-cells still can develop TSEs. Alterations using chemicals of the immune system affect the incubation period only marginally or the development of disease (e.g. steroids, anti-cancer drugs). Disease is modified by amphotericin B, which is not thought to alter the immune system at all. Infection can be demonstrated in vitro in which no immune system is present whatever.

Transmission of the organism in a campylobacter-like organism among young cattle

This initially seems implausible but the work done by Done, who indicated that an organism that could be cultured on agar could be shown to be resistant to all the factors that seemed to be similar to prions. i.e. heat, light, radiation etc. He also showed that it could be transmitted. His research has been little noticed except by one group that suggest that the BSE agent was transmitted in the same way as bovine gut flora in advance of the rumen bacterial flora (Austen, T) or as a campylobacter-like organism between animals (Roland Heynkes). The research into it seems to have been refused by MAFF. This sort of hypothesis can explain the epidemiology of the disease quite well and the reasons found that cattle born within a few days of each other seem to share the same risk of BSE on a particular farm. It should be brought in at this point that the amazing work by Bastian concerning the possibility that there is an agent for TSEs that are spiroplasma has been largely ignored in the literature of BSE. Ed Gehrman has put forward this repeatedly on the net but little has got into the literature.

Alteration of PrP glycosylations by alkaloidal glycosidase inhibitors

These are small, sugar-like compounds that prevent the breakdown of carbohydrate chains that are on the surface of proteins. The effect they have mainly is to cause all the chains to be the same. i.e. when the AGIs are not present, the PrP made by one cell will carry different carbohydrate chains from the PrP made by another. When the AGI is added the carbohydrates become the same. Now, one of the things that is required for the transmission of PrPsc infection may well be that the carbohydrate chains are identical on the infecting PrPsc and on the PrPc that is changing into its form. This can be made certain of by AGIs. Remember, at the beginning of the 1980s there was a huge increase in the production of foods from potatoes, which contain large amounts of AGI (Nash et al) and remember that animals were fed with the potato fractions that were not needed. Also remember that the sweet potato also contains a lot of them and that the Kuru affected tribe in New Guinea would have eaten large amounts. (Further work taking place currently at the University of London.)

Smarden spill

The manufacture of methyl bromide and fluoracetemide from the Rentokil factory at Smarden in Kent gave rise to chemical release, and groundwater contamination reaching peak in 1963. Cattle that died as a result of chemical poisoning were sent to knackers and renderers in the 1960s. The BSE epidemic first appeared 5 miles from this site.

Chemical interactions that cause alteration in PrP shape

(Abstract from paper: Barry Willis, THIOL-DISULFIDE INTERCHANGE THE KEY TO CONFORMATIONAL CHANGE OF PRION PROTEIN?)

Prion protein is involved in a group of neuro-degenerative diseases. The conversion of the soluble cellular prion protein into the pathogenic insoluble isoform is accompanied by a decrease in α -helical structure and an increase in the β -pleated sheet content which aggregate into amyloid fibrils. The conversion of α -helices of prion to the β -pleated sheets is most likely to result from the reduction of stability of the α -helices relative to the β -pleated sheet. Such a mechanism of stability reduction exists due to the presence of the disulfide bond between two α -helical components of the cellular prion protein and their ability to undergo thiol-disulfide interchange.

Alterations in the use of bovine pituitary hormones in various countries

This was particularly of significance in countries outside the UK and the bovine growth hormone (BGH) was not licensed for use there. However it is not at all clear that it was not imported and used illegally and, as a farmer's cattle were valued for breeding according to the quantity of milk that they produced (BGH causes milk production) it would be reasonable to consider that it did take place here. Knowing that HGH caused a spread of CJD from rare human cases it is not unreasonable to consider that BGH might do so in cattle. This hypothesis would require the BGH being infected with small amounts of BSE and also that there was another change in the UK that caused the epidemic to spread, whereas it did not do so in other countries. BGH use took place after the second war and rose markedly during the 1970s (but not in the UK legally). There are now good indications that bovine pituitary hormones were used for both GH and as a method for embryonic transfer and implantation in the UK herd. The way in which BSE seemed to start in the South East of the UK but cases appeared suddenly throughout the UK is odd and the easy transfer of pituitary hormones around the country could explain this. The sudden rise in the cases of BSE long after the use of MBM started was strange and was the fact that the numbers rose so rapidly whereas if BSE was derived from a single sporadic BSE case we would have expected a different growth pattern of disease. The subject is fully discussed in the Phillips Inquiry and the explanations are fairly convincing as the method of transfer of the original cases to get the numbers up to a level to cause the epidemic. Farmers have been fairly clear that they did use these hormones but it is unlikely that they will make this public.

Small DNA chain associated enters host genome and is protected by protein when multiplied

According to the hypothesis the causer of the disease is oligonucleoprotein, that is situated in the genome of the host. In fact, the causer is a nucleosome which is constructed of oligonucleotide and histones. It differs from nucleosome by the succession of nucleotides and this very difference is the cause of the pathology. That infectious oligonucleoprotein integrates into the construction of the host genome. After being integrated into the host genome, it makes changes in the construction of the gene and if it is a unique gene, then the primary construction of the corresponding protein changes. The gene mutation caused by the integration of the infectious oligonucleoprotein is a reason for an anomaly constructed enzyme production. The mutation of the given gene may occur spontaneously too as well as the mutation may be inherited. The causer of the disease is not inactivated by DNase because DNase ruins DNP/P-protein/ up to oligonucleoprotein, which is resistant. According to the hypothesis the causer is the stated above oligonucleoprotein, which is resistant to the DNase inactivating influence and vice versa.

After the influence of DNase the causer separates itself from the genome and may produce infection. The DNase cannot ruin the oligonucleoprotein, because the molecules of the histones protect it. But the causer may be inactivated by PROTEase, because the PROTEase destroys histones and they don't protect anymore the oligonucleotide of the destruction. Furthermore, prions appear to remain infectious even after being exposed to treatments that destroy nucleic acids. Besides, the fact that in PH=10 condition the causer is inactivated, it also agrees with the hypothesis. As the histones are alkaline proteins, then in PH=10 condition they are deproteinated/they lose H⁺ ion/,they lose the positive charge, resulting the lost of ionic contact between negative charges of phosphates of nucleotid and positive charges of the histones. That is histones separate themselves from oligonucleotides. The hypothesis also gives explanation to the question how the multiplations of the causer takes place. Antibodies don't produce to oligonucleoproteins, because last one have small sizes. (This hypothesis requires further information to justify various interactions with PrP and to explain the many scientific findings with the disease)

High proportion of BSE cases vertically transmitted

Some reason was needed to work out why cattle seemed to become infected before 18months of age. Statistically it seems that they are either infected between 6 months and 18 months or, to a large degree before or shortly after birth. As there seems to be little reason why 6-18 months should be the reason at all, it has been considered that almost all the cases that we see are in fact infected from their mother. For some reason the proportion of cattle in any herd that develop BSE rises quickly to around 15% but uncommonly gets any further. Because of this the epidemic in case numbers that is seen is not so much due to an increase in cases in herds but an increase in the number of herds that are affected. The question arises as to why only 15% should be affected and this is unclear; why not 100%? Surely cattle would be more likely to be exposed to BSE in their milking life when they were fed larger quantities of MBM, but it is not these cattle that seem to develop any disease but rather ones exposed early in their life. The hypothesis suggests that when a herd is infected, in fact it is a very large proportion that do. However they would develop symptoms (if at all) late in their life (perhaps aged 17) i.e. long after slaughter. As such the proportion that we see developing symptoms are the offspring of those mothers and that the VT rate is 15%. The epidemiology for this is not unreasonable but involved Ro factors dropping long before the feed ban was introduced. Fergusson's work at Oxford suggests that the Ro did in fact drop in association with the feed ban although the refusal of MAFF to permit any other group but this one to work on the data makes this difficult to argue.

A range of trace metals may be involved in BSE symptoms and pathology

This was put forward by Dr. Jane Karlsson and goes through how it is not really surprising that certain symptoms are involved in UK animals and perhaps may be particularly susceptible to BSE in that neurotoxic heavy metals and interactions between them are repeatedly discussed as being of importance in the bovine diet of the UK.

BSE is associated with prostaglandin activity

This follows the ideas of Arne N Gjorgov, who published an article 'Prevention and eradication of the BSE: A solicited response and proposal' *Macedonian Vet Rev.* 1996;25:97-101.

It follows the fatal neurological condition of pseudopregnant mice following sterile mating whereas none of the controls that received prostaglandins developed the condition. The hypothesis is that this kind of disease may be transferred through a mating system in the mice and prevented by prostaglandins. It is suggested that prostaglandins may be a prophylactic factor in BSE.

BSE is seen when a chromosomal virus is expressed

Another view is that outbreaks of actual disease occur when an otherwise silent C-DNA resulting from initial accidental retrotransfection of RNA semi-virus is activated into re-transcription. The question to think is why so many cows and people do NOT get BSE/CJD once infected and whether this happy state can be prolonged throughout life. Part of this hypothesis therefore is that "environmental" affronts whether by atmosphere (insecticides) or food may activate "disease" from benign latency. Queniborough is the next village to Syston where Asmer Flower Seeds greenhouses were overcrowded with hanging insecticide impregnated yellow terrors. How many at Queniborough worked at Syston? Was there or is there any intensive horticultural glasshouse nursery near the Doncaster street where the two new occurrences come from. Did such nurseries if they exist also used toxic hanging strips at high intensity? This idea of course also relates to sheep dips etc. The hypothesis explores specific reasons why this should be so and how the PrP becomes changed as a result. Some of this relates to the ability to immunise an animal with one scrapie strain against another. The idea will explain a number of things such as the rapid spread of BSE in the UK but the low percentage of cattle that actually die per herd.

Maddocks and Dealler hypothesis

By 1994 it had become clear that it was difficult for the initial scrapie hypothesis to be accepted and that the 'sporadic BSE' hypothesis was more reasonable. However, by 1995 the findings by Taylor et al that the changes in the rendering processes could not be clearly seen as the cause of the epidemic and the other factors in the discussion above made it clear that a further examination was required. Without the rendering changes being involved, the index BSE case would have had to have taken place in the early 1970s or late 1960s. Because of the problems concerning the source of BSE from a single case a further hypothesis is considered as below in conjunction with Dr. A. Maddocks: "BSE is derived from a random disease in cattle, it is not adequately destroyed by MBM rendering and it is fed to cattle. This has taken place for many years but will only be in adequate doses when UDP feeding techniques (UnDegraded Protein) are used (which were introduced progressively as of 1980). It is the offspring of these cattle that develop the symptomatic disease having become vertically infected." This hypothesis would not require repeated peaks after an index case, it would not require the animal to have been fed any infective meal and it would not expect large numbers to become symptomatic from a single source. Also, it would expect to produce a drop in R_0 early in the epidemic, without large numbers being apparently infected (although they really were). It also explains the relatively slow (after correcting for under reporting) fall in BSE cases in cattle born after the feed ban. The reason that the hypothesis was put forward was because it answered the problems listed in 'discussion' above. The only clear problems with the hypothesis are that it cannot explain why few cases of BSE have been diagnosed in Europe* and that several cattle (about 20) would have been infected with BSE at the point at which UDP was introduced (not a single cow).

A statistical model has been made of this hypothesis but not published at this time. *NB sheep exported from the UK did develop scrapie in New Zealand and Australia and were genetically the same range as the ones that developed the disease in the UK. However the no further sheep, including the offspring of the infected sheep developed the disease. This suggests that an environmental factor is involved and the possibility must be considered that a similar factor is also involved in BSE in Europe.

Infection taking place on farms almost entirely within the first month: faecal or meal contamination

Logic: Rapid drop in cases of BSE born in the short period before the 1988 feed ban shows infection to be taking place within first 6 months and mostly within the first month of life. (not under argument now) Age distribution of cattle with clinical BSE did not change as the epidemic progressed: therefore they must be getting infected with a similar dose throughout the epidemic. Extremely low amounts likely to be found in the MBM anyway of prions. Cattle gut mucosa will absorb large molecules easily after birth but this effect disappears within the first few months. It is thought that the effect is to permit the uptake into the bloodstream of mother's antibodies from colostrum. The mucosal resistance to this builds up and is thought to be caused by the change in diet after around 1-4 weeks of life on the farm. This would be expected to cause the dose size of BSE reaching the blood of the animal to decrease markedly as the calf gets past the first few days of life and hence the incubation period would be expected to increase. Therefore it is not surprising to find a wide range of age distribution in the cattle with clinical BSE. Cattle developing BSE when born on farms where no MBM was used but brought from other farms and a large proportion of the calves were not fed any MBM but were suckled until relatively old (particularly in beef farms). Cattle born within a month of each other share the chance of developing BSE to some degree. This could be perhaps because of feed (feed deliveries were often a month apart), or because of contamination that was washed away at a later date. These findings suggest that some factor is not exposed to all the cattle born at roughly the same time (e.g. common mixed colostrum) but affect a small proportion. The suggestion is that the infection is either present in environmental contamination to which the calves are exposed and is washed away, or to feed contamination that will only infect cattle if young enough to get past their gut mucosa. As a result of this a wide age distribution would be expected for clinical cases and no change in age distribution expected as a result of the feed ban.

CATEGORISATION OF POTENTIAL INFECTIVITY OF DIFFERENT ORGANS IN BSE AFFECTED ANIMALS

Categorising the potential infectivity of different organs in BSE-infected animals. The assessment of the infectivity is based in part on scrapie titres, on the finding of high infectivity in the brain of BSE-affected cattle, on the differential impact of BSE-infective organs on the infection of mice to intracerebral inoculation and on the presumed CJD infectivity of human dura mater and human pituitary gland based on transplant data and the effects of human growth hormone infection. For practical reasons relating to slaughterhouse contamination, some tissues are categorised at a higher level than warranted by their intrinsic infectivity.

a) Organs with high infectivity

- I. Bovine brain, eyes, bovine spinal cord and bovine dorsal root ganglia, dura mater, pituitary, skull and bovine vertebral column , lungs
- II. Ovine/caprine brain, eyes and spinal cord, dorsal root ganglia and vertebral columns; ovine and caprine spleens, lungs.

b) Medium infectivity

- I. Total intestine from duodenum to rectum, tonsils
- II. Bovine and caprine spleen, placenta, uterus, fetal tissue, adrenal, cerebrospinal fluid, lymph nodes

c) Low infectivity

- I. Liver, pancreas, thymus, bone marrow, other bones, nasal mucosa, peripheral nerves

d) No detected infectivity

- I. .Skeletal muscle, heart, kidney, colostrum, milk, discrete adipose tissues, salivary gland, saliva, thyroid, mammary gland, ovary, testis, seminal testis, cartilaginous tissue, connective tissue, skin, hair, blood clot, serum, urine, bile, faeces.

PATHOGENESIS

The "infectious" prion protein is felt to be an abnormally folded version of a protein that is normally found in every animal specie, called PrP. In other words, it is a single protein chain that is just put together in an unusual way. The abnormally folded or knotted version of the prion protein has some unusual and remarkable characteristics. First, it is extremely stable, surviving conditions that would destroy most proteins. Second, it seems to have the ability to cause other normally folded prion proteins to adopt the abnormal folding pattern. Imagine that some abnormally folded prion protein got into your brain. Since it appears to be able to cause the normal protein to become abnormal, i.e. through propagation of an abnormal structure, you can imagine that over time you would start to have an accumulation of the abnormal protein in your brain.

How could that abnormally folded protein get into your brain to start with? Well, you could come in contact with the brain of a person or animal that has an accumulation of abnormal protein in their brain. Once it found its way to your brain (?by eating it?), the slow process of accumulation could begin. Otherwise, some PrP in your brain may just spontaneously fold abnormally, i.e. by itself. In fact, there are some human mutations that can cause this abnormal transition to happen with increased frequency, leading to inherited forms of CJD that do not require exposure to an infected person. In the end, there is a lot of evidence to support the notion that the infectious prion agents act by the transmission of an abnormal protein structure, but this is not yet formally proven. Even if you accept this hypothesis, it still falls short of explaining how prion proteins actually cause neurological disease!

In other words, why does an accumulation of abnormally folded prion protein cause neuro-degeneration. There is no clear answer to this question yet. It does not appear to be due to loss of function of the normally folded protein. Instead, it seems more likely that the abnormal protein is in some way toxic, causing neurons to die or make ineffective and inappropriate connections.

The one thing that is certain is that the brain eventually becomes highly disorganized in its activity, and to degenerate physically, which signals the beginning of the end. Evidently, the scrapie protein propagates itself by contacting normal PrP molecules and somehow causing them to unfold and flip from their usual conformation to the scrapie shape. This change initiates a cascade in which newly converted molecules change the shape of other normal PrP molecules, and so on. These events apparently occur on a membrane in the cell interior. The differences between cellular and scrapie forms of PrP must thus be conformational since other possibilities seem unlikely. For instance, it has long been known that the infectious form often has the same amino acid sequence as the normal type. Of course, molecules that start off being identical can later be chemically modified in ways that alter their activity. But intensive investigations by Neil Stahl and Michael A. Baldwin in USA have turned up no differences of this kind.

Electron Micrographs of brains carrying TSE's

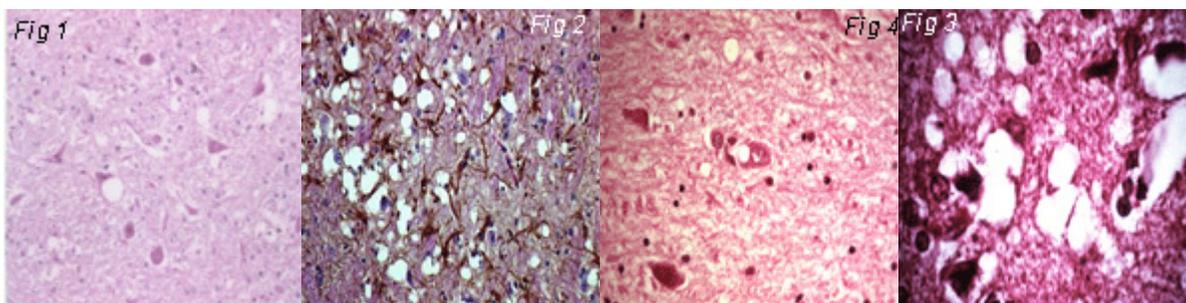


Fig 1: BSE (Cow)

Fig 2: Scrapie (Sheep)

Fig 3: Kuru (Man)

Fig 4: CJD (Man)

CLINICAL SIGNS

The affected animals may display changes in temperament, such as nervousness or aggression; abnormal posture; in-coordination and difficulty in rising; decreased milk production; or loss of body condition despite continued appetite. There is no treatment, and affected cattle die. The incubation period ranges from 2 to 8 years. Following the onset of clinical signs, the animal's condition deteriorates until it dies or is destroyed. This usually takes from 2 weeks to 6 months. Most cases in Great Britain have occurred in dairy cows between 3 and 6 years of age.



Cow in Clinical Stage of BSE

DIAGNOSIS

There is no test to detect the disease in a live animal. Microscopic examination of brain tissue at necropsy is the primary laboratory method used to confirm a diagnosis of BSE. There are also several techniques used to detect the partially-proteinase resistant form of the prion (PrPres) protein. These techniques are immuno-histochemistry and immuno-blotting.

RELATION BETWEEN BSE AND CJD

CJD is a slow degenerative human disease of the central nervous system with obvious dysfunction, progressive dementia, and vacuolar degeneration of the brain. CJD occurs sporadically worldwide at a rate of 1 case per 1 million people per year.

There is a strong epidemiologic and laboratory evidence for a causal association between new variant CJD and BSE. The absence of confirmed cases of new variant CJD in other geographic areas free of BSE supports a causal association. In addition, the interval between the most likely period for the initial exposure of the population to potentially BSE contaminated food (1984-1986) and onset of initial new variant CJD cases (1994-1996) is consistent with known incubation periods for CJD.

An experimental study reported in June 1996 showed that three cynomolgus macaque monkeys inoculated with brain tissue obtained from cattle with BSE had clinical and neuropathological features strikingly similar to new variant CJD (Nature 1996;381:743-4).

A study published in 1996 indicated that a Western blot analysis of infecting prions obtained from 10 new variant CJD patients and BSE-infected animals had similar molecular characteristics that were distinct from prions obtained from patients with other types of CJD (Nature 1996;383:685-90). Most recently, interim results of an ongoing experimental study involving inoculation of a panel of inbred mice with the agents causing BSE and new variant CJD substantially increased the strength of the scientific evidence for a causal association between new variant CJD and BSE (Nature 1997;389:498-501).

The incidence of classical CJD in the United States (about 1 case per 1 million population per year) is similar to the incidence found in the rest of the world, which includes Australia and New Zealand--countries that have NOT reported either scrapie or BSE. CJD, which was first diagnosed in the 1920's, occurs with roughly the same frequency in Britain as in other countries at the present time. On March 20, 1996, the UK's Spongiform Encephalopathy Advisory Committee (SEAC) announced the identification of 10 cases of a new variant form of CJD (vCJD). All of the patients developed onset of illness in 1994 or 1995.

The following features describe how these 10 cases differed from the sporadic form of CJD: The affected individuals were much younger than the classical CJD patient. Typically, CJD patients are over 63 years old. The average patient age for the onset of variant CJD is 28 (range of 14 to 52) years. The course of the disease in the vCJD averaged 13 months. Classical CJD cases average 6 month duration. In the variant cases, electroencephalographic (EEG) electrical activity in the brain was not typical of sporadic CJD. Although brain pathology was recognizable as CJD, the pattern was different from sporadic CJD, with large aggregates of prion protein plaques.

Epidemiological and case studies have not revealed a common risk factor among the cases of vCJD. According to the SEAC, all victims were reported to have eaten beef or beef products in the last 10 years, but none had knowingly eaten brain material. One of the affected individuals had been a vegetarian since 1991. The SEAC concluded that although there was no direct scientific evidence of a link between BSE and vCJD, based on current data and in the absence of any credible alternative, the most likely explanation at that time was that the cases were linked to exposure to BSE before the introduction of control measures, in particular, the specified bovine offal (SBO) ban in 1989. Research reported in later 1996 and 1997 has found evidence to further support a causal association between vCJD and BSE.

Two significant studies published in the October 2, 1997 edition of Nature lead the SEAC to conclude that BSE agent is highly likely to be the cause of vCJD. Dr. Moira Bruce and colleagues at the Institute for Animal Health in Edinburgh, Scotland inoculated 3 panels of inbred mice and one panel of crossbred mice with BSE, vCJD and sporadic CJD. Interim results indicate that mice inoculated with BSE showed the same pattern of incubation time, clinical signs and brain lesions as mice inoculated with tissues from patients with vCJD. This provides evidence that BSE and vCJD have the same signature or are the same "strain". In addition, sporadic CJD and known scrapie strains were not similar to vCJD or BSE. Results from another study published by Dr. John Collinge and colleagues of Imperial College School of Medicine, London, UK strongly support Bruce's results. Collinge's paper reports findings of BSE transmission to transgenic mice expressing only human PrP.

Another paper by Collinge et. al. in the October 24, 1996 edition of Nature also provides data to support the association between vCJD and BSE. More recently, studies using transgenic animals expressing the bovine PrP have supported the view that BSE infected cattle are responsible for vCJD. These mice not only propagated the BSE infectious agent in the absence of a species barrier, but also were highly susceptible to vCJD and natural sheep scrapie. Furthermore, the transgenic mice inoculated with either vCJD or BSE had indistinguishable disease characteristics.

TREATMENT AND PREVENTION

Since the nature of the pathogenic organism/material is obscure no currently developed treatment can be effective in this disease. It's only when research has thrown enough light on the aetio-pathogenesis of the disease that a rational treatment can be worked out. Till then the major push will be towards control and eradication of the disease. Some measures taken to control disease in many countries are as under: In July 1988, the UK banned the use of ruminant proteins in the preparation of animal feed. The use in the food chain of bovine offals considered to pose a potential risk to humans was also banned in the UK in 1989. The list of banned bovine offals was revised and expanded on several occasions as new information became available. In other countries, including those in Europe, measures taken, the date of implementation and the extent of enforcement vary from country to country. Starting in 1996, bans prevented the sale of food and food products containing beef from the UK to other countries. Other products (e.g. tallow, gelatin) derived from bovine tissues were also prohibited from sale from the UK to other countries.

However, in 1999 the EU lifted the ban for meat fulfilling specific requirements; for example, de-boned beef from animals from farms where there have been no cases of BSE and where the animals are less than 30 months of age at slaughter. Cattle are continuously monitored for BSE and BSE is decreasing in the UK. The number of reports of BSE in the UK began to decline in 1992 and has continuously declined year by year since then. New monitoring programmes using newly developed tests for the diagnosis of BSE in dead cattle have been introduced in Switzerland and France, and may be expected throughout the EU.

WHO CONCLUSIONS AND RECOMMENDATIONS TO REDUCE EXPOSURE TO THE BSE AGENT

All countries must prohibit the use of ruminant tissues in ruminant feed and must exclude tissues that are likely to contain the BSE agent from any animal or human food chain. BSE eradication was recommended during a WHO consultation held in December 1999. All countries are encouraged to conduct risk assessments to determine if they are at risk for BSE in sheep and goats. It is advised that any tissue which may come from deer or elk with Chronic Wasting Disease (CWD, a transmissible spongiform disease of North American mule deer and elk) is not used in animal or human food; however, at this time there is no evidence to suggest that CWD in deer and elk can be transmitted to humans. No infectivity has yet been detected in skeletal muscle tissue. Reassurance can be provided by removal of visible nervous and lymphatic tissue from meat (skeletal muscle). Milk and milk products are considered safe. Tallow and gelatin are considered safe if prepared by a manufacturing process which has been shown experimentally to inactivate the transmissible agent. Human and veterinary vaccines prepared from bovine materials may carry the risk of transmission of animal TSE agents. The pharmaceutical industry should ideally avoid the use of bovine materials and materials from other animal species in which TSEs naturally occur. If absolutely necessary, bovine materials should be obtained from countries which have a surveillance system for BSE in place and which report either zero or only sporadic cases of BSE. These precautions apply to the manufacture of cosmetics as well.

Editors note: Due to this being an extensive and long paper the references (covering 7-8 pages) have been omitted. Yet if any reader especially requires them, please send your request and we will dispatch them to you.

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