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Prevalence, Gross and Histopathological Study of Brain Disorders in Cattle-Kerala State, India

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Abstract: The present study, was undertaken to assess the prevalence and pathology of brain disorders in cattle. The data collected from the autopsy records (99 bovine carcasses) maintained at the Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India and 52 brain samples obtained from both the slaughtered cattle and autopsy cases formed the basis of the study. The only gross lesion evident was meningeal congestion, which accounted to 70% of the cases. Congestion and hemorrhages were found consistently in the slaughtered cattle. The other major lesions were revealed by histopathological examination of 10 different areas of the brain in each case. These included vascular changes, neuronal degeneration, inflammatory changes, gliosis, pigmentation and neoplasia. Two cases revealed cytoplasmic inclusions of which one was due to rabies and the other was attributed to unknown viral etiology. Based on the observations made here, it is evident that incidence of pathological disorders encountered is relatively high. Thus, the need and scope for investigation into the pathological disorders of bovine brain warrants further investigation.

Key words: Brain, rabies, autopsy, congestion, neuronal degeneration, inflammatery

INTRODUCTION

Brain is one of the vital organs of the body and remains in an exalted position protected within the cranial cavity cushioned by cerebrospinal fluid and protected from the external noxious agents by the blood brain barrier and perivascular space. It functions to dictate actions to all parts by accepting and coordinating input information from sensory receptors into the nervous system resulting in output information to muscles and glands.

Neuropathology has always tended to be set apart as an arcane specialist area to be entered by only a select few, as the nervous tissue is highly specialized and structurally complex (Summers *et al*., 1995). The main cell types of pathological significance in the nervous system are: neurons (Herrup and Yang, 2007) astrocytes, which act as specialized support cells, oligodendrocytes, which form myelin and microglia, which are resident cells of the monocyte/macrophage type (Nelson *et al*., 2003). The compact anatomy of the nervous system means that even mild lesions may produce severe functional disturbances.

There are some significant neurologic diseases in which dramatic clinical disturbances are not matched by equivalent morphological alterations in the nervous tissue. For instance toxins that interfere with synaptic function can have fatal consequences, yet leave neurons normal in appearance to routine examination. Botulism, tetanus and strychnine toxicities are well known examples (Nelson *et al*., 2003). Inflammation in the central nervous system is the area of neuropathology that is of much veterinary significance because it embraces many of the transmissible highly fatal infections of animals. Rabies is an important disease that is still endemic in many parts of the world. Eradication is difficult as the disease is established in multiple host systems. There is seldom anything even suggestive in the general autopsy unless examined microscopically (Burton *et al*., 2005).

Therefore, it is imperative that earnest efforts should be made to delineate the disease problems associated with the brain, so that a plan of action can be streamlined to prevent and control the brain disorders in cattle. This has been a neglected area and hence a study in this direction is relevant.

MATERIALS AND METHODS

The present study was conducted at the Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India to investigate the prevalence and pathology of brain disorders in cattle.

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Prevalence study: The prevalence of brain disorders in cattle during 1998-2002 were assessed by screening the autopsy records maintained at Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India.

Sample collection: Fifty two samples of bovine brain collected randomly from the cattle slaughtered at the meat technology unit, College of Veterinary and Animal Sciences, Mannuthy, India and the cattle slaughtered at the municipal slaughterhouse, Thrissur, India as well as from the carcasses brought for autopsy at the Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India were utilized for the study. Animals of all age groups varying from young to adult were included in the study population.

Gross examination: The whole brain was carefully removed from the cranial cavity. The brain was subjected to detailed gross examination for any changes like congestion, haemorrhage, abnormalities in shape and size, encephalomalacic changes and inflammatory conditions. The brains were fixed in 10% neutral buffered formalin. After 24 h of fixation, sections were sliced across all the brain tissue to ensure examination of wide range of neuroanatomical structures and to detect the presence of any cyst, tumor, fluids and abscesses. Several regions of the brain including the olfactory, middle and caudal parts of left and right cerebrum, cerebellum and midbrain through rostral colliculi and medulla through caudal cerebellar peduncles were further fixed in neutral buffered formalin.

Histopathology: Representative samples from the olfactory, middle and caudal parts of left and right cerebrum, cerebellum and midbrain through rostral colliculi and medulla through caudal cerebellar peduncles were processed by routine paraffin embedding techniques (Sheehan and Hrapchack, 1980). Sections were cut at 4 micron thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Gamble, 1996). Special stains like Toludine blue, Phosphotungstic Acid Hematoxylin (PTAH), Kluver-barrera method for myelin and nerve cells, Cresyl echt violet for nissl substance, Alizarin red for calcium deposits and Masignanni and Magferrari method for rabies inclusions (Luna, 1968) were employed whenever required to further elucidate the neuro-pathological changes. The stained sections were subjected to detailed examination under the light microscope and the lesions were classified.

RESULTS

Prevalence study: During the 5 year's period (1998-2002), a total number of 99 bovine carcasses of different age groups were autopsied at the Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India. Out of 99 cases examined, 33 (33.3%) showed various brain lesions. The major pathology was of rabies (20.20%) and edema of the brain (3.03%). The other lesions recorded were congestion of varying degrees (7.07%) and sinus tumor (5.05%) (Table 1).

Field cases: Brain samples from 52 bovines were collected and examined. Thirty cases were collected from the autopsy room of Center of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, India. Ten cases were from the municipal slaughterhouse, Thrissur, India and 12 cases were from meat technology unit, College of Veterinary and Animal Sciences, Mannuthy, India (Table 2).

Gross pathology

Vascular changes: Generalized congestion (Fig. 1a), hemorrhage, edema and focal areas of discoloration were observed in most of the cases throughout the study.

Histopathology

Vascular changes

Congestion: Congestion was observed in different areas of the brain in most of the cases throughout the study.

Hemorrhage: The degree of hemorrhage varied widely. It was confined only to meninges in some cases, whereas it was very extensive involving all the different layers of the cerebrum, cerebellum and midbrain in 30% of cases (Fig. 1b). Sub-dural hemorrhages and hemosiderosis were also observed.

Table 1: Prevalence record of gross brain pathology in cattle for 5 years (1998-2000) maintained at the center for excellence in pathology, COVAS, Mannuthy, India

Total number of cases	99
Brain lesions	33.0%
Rabies	20.2%
Meningeal congestion	7.07%
Sinus tumor	5.05%
Brain edema	3.03%

Table 2: Sources of brain samples collected for the histopathological investigation during the study (2002)

Total number of brains examined	52
Autopsy cases COVAS, Mannuthy, India	30
Municipal slaughterhouse cases	10
Meat technology unit, COVAS, Mannuthy, India	12

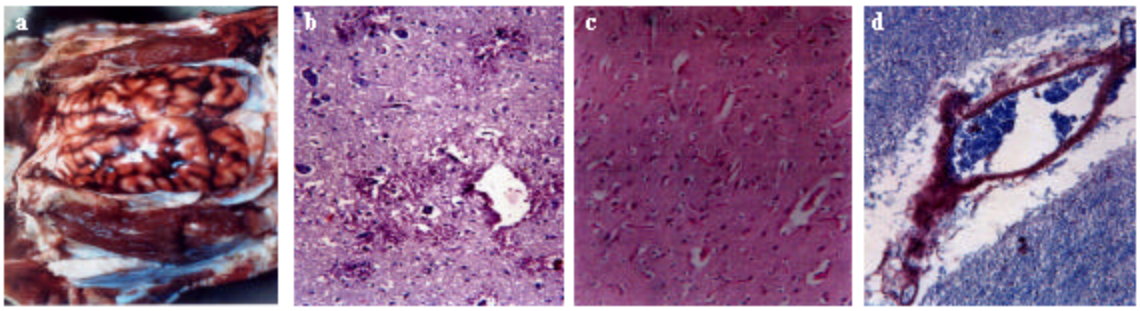

Fig. 1: Vascular changes (Gross and Histology). a): Congestion (Gross), b): Haemorrhage (H and E), c): Neovascularization (H and E), d): Bloodvessel thickening (PTAH)

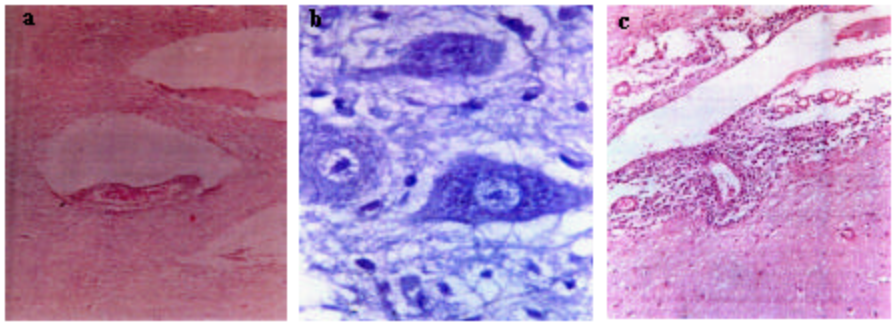

Fig. 2: Inflammation and degeneration. a): Cystic dilatation of capillaries (H and E), b): Chromatolysis (Cresyl Etch Violet), c): Meningo enchepalitis (H and E)

Edema: Edema appeared as several areas of spongy transformation uniformly distributed throughout the cerebral cortex. Edema was seen to be present both in white and gray matter of the cerebrum, cerebellum and the midbrain. It was exhibited as wide inter-fascicular spaces in the white matter.

Neovascularisation: Sprouting capillaries engorged with blood could be observed both in slaughterhouse as well as in the autopsy cases (10%). They were seen widely distributed in the areas of cerebrum, cerebellum and the midbrain (Fig. 1c).

Blood vessel thickening: Thickening of the blood vessel wall was recorded in 3.85% of cases. These thickened vessels were distributed widely in different areas of the brain. The endothelial cells appeared to be swollen in a few cases. The thickening was demonstrated by special staining with Phosphotungstic Acid-Haemotoxylin (PTAH) method (Fig. 1d). Capillary damage was seen in 1.92% of the cases in the cortical and medullary regions of the brain.

Blood clot: Occlusion of blood vessel with clotted blood was observed in 7.70% of the cases. This was observed mostly in the slaughtered animals. Such clots were found to be present more towards the meninges in the intersulcal capillaries and even extending into the parenchyma.

Cystic dilatation of capillaries: Dilatation of capillaries was observed in 7.70% of the cases (Fig. 2a). Collapse of capillaries, perivascular space dilatation progressing to cavitation was observed and such cavities in certain areas were crowded with gemistocytic astrocytes.

Neuronal degeneration: Neuronal degeneration accounted to 38.46% of the total cases. The degenerative change varied from multifocal to diffuse involving the different cortical laminae and white matter. Diffuse neuronal degeneration could be seen in 70% of the cases, while the remaining 30% of the cases revealed focal areas of neuronal degeneration. Swelling of the cell body, dispersion of Nissl substance and peripheral displacement of the nucleus appeared in some. Cell body appeared shrunken and the cytoplasm stained highly eosinophilic in a few cases.

Satellitosis and neuronophagia: Satellitosis was exhibited as the crowding of the glial cells around the damaged or the necrosed neurons. It was mostly confined to the gray

matter. Grouping of the glial cells around the damaged neurons formed the glial nodules in a few cases. The cells involved were chiefly microglia and oligodendroglia. Satellitosis was observed in 21.5% of the cases. Few cases revealed neuronophagia.

Chromatolysis: Disappearance of neuronal nucleus and Nissl substance was observed in some cases. Granularity of the Nissl substance was not maintained and in completely chromatolysed regions, only a space remained with remnants of the neurons, which appeared blue on staining with cresyl etch violet (Fig. 2b). The motor neurons of the mid brain and to some extent in the neurons of the cerebral cortex appeared rounded with eosinophilic cytoplasm. This change was recorded in 5.77% of the cases.

Purkinje cell changes

Purkinje cell degeneration and loss: The degeneration of the purkinje cells could be detected in 7.70% of the cases (Table 3). The nuclear details had been lost and the cytoplasm appeared granular and eosinophilic. In few cases, the loss of these cells was encountered, which accounted to 3.85% of all the cases.

Necrosis: A few cases revealed the presence of necrotic neurons and the necrosis was at the cellular level and not manifested as liquifactive necrosis. It was observed in about 7.70% of the cases. The neurons of the cerebrum, cerebellum, midbrain, pons and the medulla oblongata revealed such changes. Many neurons particularly in certain areas of the brain appeared ballooned with virtually no cytoplasmic architecture. Central chromatolysis was prominent as revealed by Cresyl etch violet stain.

Inflammation

Meningitis: Abnormally large numbers of glial cells and mononuclear cells were found to be present in the space around the meningeal vessel walls in the leptomeninges. Meningitis accounted to 12% of cases (Table 3). It was observed chiefly in the cases brought for autopsy.

Meningo-encephalitis: Perivascular cuffing in both meninges and the brain parenchyma was observed in 12% of the cases. The cells observed varied from gemistocytic astrocytes, plasma cells and mononuclears. Varying degree of cuffs was noticed in different layers of the brain. The perivascular cuffing accounted for 9.62% of the total cases (Fig. 2c). They were mainly observed in the cases brought for autopsy and not in the slaughtered healthy animals.

Table 3: Characterization of Histopathological changes

Histopathological changes	(%)
Vacular changes	30
Meningeal congestion	
Hemorrhage	
Edema	
Blood vessel thickening	
Bloodvessel damage	
Blood clot	
Cystic dilation of capillaries	
Neuronal degeneration and necrosis	39
Satellitosis	
Neuronophagia	
Neuronal swelling	
Loss of neurons	
Chromatolysis	
Purkinje cell changes	7.7
Purkinje cell degeneration	
Purkinje cell loss	
Inflammation	12
Meningitis	
Meningo-encephalitis	
Inclusions	
Negribodies	1.92
Other inclusions	5.77
Spongiform changes	1.92
Demyelination	4
Cellular reactions	20
Dark neurons	
Binucleated neurons	
Gliosis	29
Glial nodules	
Sub ependymal accumulations	
Astrocytosis	
Gemistocytes	
Axonal swelling	
Gitter cells	
Cystic spaces	1.92
Autolysis	7.7
Mucocytes	1.92
Melanin Pigments	4
Sinus tumor	2

Inclusions

Negribodies: Negribodies could be detected in the Purkinje cells of the cerebellum (Fig. 3a) in 1.92% of the cases (Table 3). It was subjected to special staining as per Masignani Magferrari method and the Negribodies were demonstrated as round eosinophilic intracytoplasmic inclusions (Fig 3b). However, in these cases, the Negribodies could not be detected in the impression smear of the cerebellum, stained with Seller's stain. In 1.92% of the cases, the impression smear revealed the inclusions but histopathological sections did not reveal the inclusions. Negribodies could not be observed in the other regions of the brain both in the impression smear examination and also in histopathology.

Other inclusions: Inclusions in the neuronal perikaryon accounted to 5.77% of the total cases (Table 3). These inclusions were detected in the cytoplasm of almost all the neurons and the glial cells of the cerebrum, the cerebellum and also the Purkinje cells. These inclusions were not found to be Negri bodies on special staining and also were PAS negative.

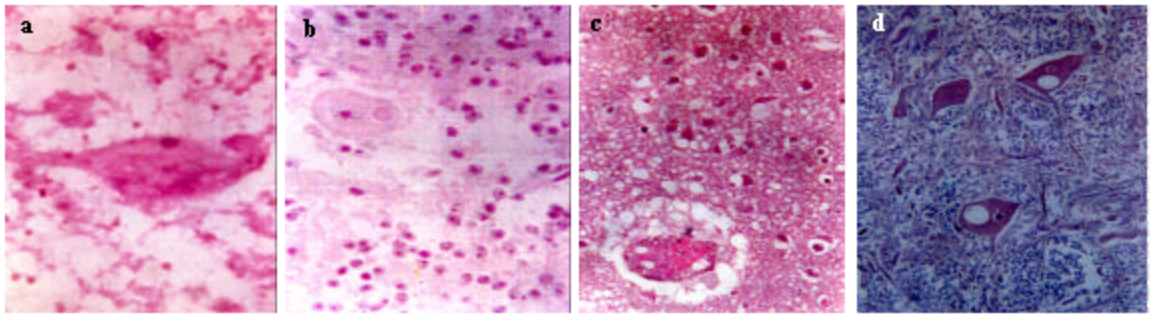

Fig. 3: Inclusion bodies and neuronal vacuolations. a): Cerebellum-negribody in the neuron (H and E), b): Negribody-(Masignani Magferrari), c): Spongiform changes (H and E), d): Intraneuronal vacuolations (H and E)

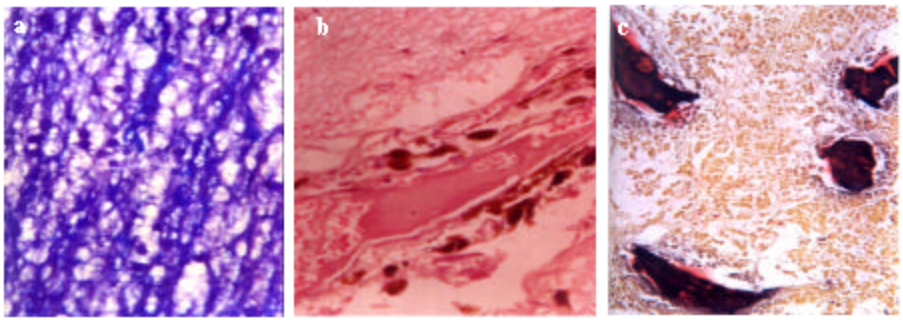

Fig. 4: Demyelination, pigmentation and calcification. a): Demyelination (Kluver Barrera Luxol fast blue), b): Melanin pigmentation (H and E), c): Calcification of spicules-sinus tumor (Alizarin red)

Spongiform changes: Spongiform changes in the brain were confined to the molecular layer of the cerebrum (Fig. 3c). Perineuronal vacuolations were present in the gray matter. Few neurons of the mid brain region especially the motor neurons revealed intra neuronal circumscribed vacuolations having no visible, stainable internal contents (Fig. 3d). Most of such cells revealed only one vacuole in the perikarya. The nucleus appeared displaced. Encephalomalacic changes were exhibited as vacuoles and spongy appearance in the white matter of the brain. Such changes were found around the capillaries too. Spongiform changes were evident in 1.92% of the cases (Table 3). In majority of the cases the spongiform change was confined to the white matter region.

Demyelination: There was demyelination in between the nerve fibers in the white matter which was demonstrated by special staining with Kluver Barrera luxol fast blue method (Fig. 4a). Such change accounted to 4% of the cases (Table 3). The demyelination was evident in the white matter of both cerebrum and cerebellum and also in the midbrain areas.

Cellular reactions

Dark neurons: Dark basophilic neurons were found to be present mainly in the slaughterhouse cases. The neurons appeared elongated, condensed and shrunken. It accounted to 20% of the cases (Table 3).

Binucleated neurons: Binucleated neurons accounted to 1.92% of the total cases. This was seen amidst the normal neurons of the cerebral cortex.

Gliosis and glial nodules: Abnormally large number of glial cell population could be detected in a few cases. Wide spread diffuse gliosis with occasional associated neuronal degeneration throughout the brain particularly in the cerebral cortex occurred in some of the cases. Mostly, gliosis was a diffuse change all throughout the brain. Among the glial cell population, the microglia and the oligodendroglia were found to be in larger proportion when compared to the few astrocytes. Gliosis accounted to 29% of the cases (Table 3). Some cases showed the presence of glial nodules.

Astrocytosis: Astrocytosis was chiefly found in the gray matter of the brain. Such changes accounted to 5.77% of the cases.

Gemistocytes: Gemistocytes were found to be present amidst the inflammatory cells around the blood vessels along with other glial cells in the cerebral cortex as well as in the white matter region of the cerebellum. These accounted to 9.62% of the cases.

Gitter cells: Gitter cells were seen around the blood vessel in the perivascular cuffs. These cells accounted to 3.85% of the cases.

Cystic spaces: Abnormally large spaces were found to be present in the white matter of cerebrum and cerebellum in a few cases. This change was not seen in the gray matter. Such changes accounted to 1.92% of the total cases (Table 3).

Mucocytes: Mucocytes (Buscaino bodies) appeared as irregular pale blue gray amorphous bodies in the cerebral cortex region and were PAS positive and metachromatic. It was noticed in 1.92% of the cases (Table 3).

Melanin pigments: Unusually, high deposits of melanin appearing as dark brown granules crowding the cells were seen in the intersulcal areas and the leptomeninges (Fig. 4b). This was observed in 3.85% of the cases (Table 3).

Sinus tumor: Sinus tumor (2% of cases) (Table 3) was found invading the brain after lysing the ethmoid bone and pressing the brain. The meninges appeared very much thickened and the area of the brain appeared depressed. Histologically, thickening of the meninges was observed. The fibers appeared thickened and sclerotic. Calcification of spicules of the bone was observed in the dura and the pia-arachnoid which was confirmed by staining with Alizarin Red (Fig. 4c). Squamous cells were seen as sheets in the pia-arachnoid. Extensive haemorrhage along with siderophages was seen in the affected area. Invasion and infiltration of the cells to the gray matter was limited.

DISCUSSION

Analysis of the data collected from the autopsy record revealed 33% occurrence of various brain disorders in cattle during the period starting from 1998-end of 2002 (5 years). On the other hand, the present investigation during the period from January 2001-October 2002 revealed 72% prevalence of brain disorders. This significant increase in the disorders of the brain could be attributed to the small sample size, short duration of the period of the study as well as lack of examination of brain during routine autopsies in the previous years. There was marked difference in the prevalence of brain lesions among the animals brought for autopsy and those observed at slaughter. The higher incidence of lesions was noticed in the samples brought for autopsy, which invariably had suffered some disease and indicated the underlying systemic involvement. Unfortunately, the whole carcasses were not available for detailed systematic examination to correlate the neuro-pathological lesions to the systemic, which limited the scope for systematic correlation of the lesions encountered in histopathological investigations of the brain. Therefore, these observations necessitate the insistence of complete examination of the carcass during routine autopsy in all cases showing some form of nervous disorder. The disorders were limited in slaughtered animals, as they were apparently healthy at the time of slaughter. There was no significant difference between the sexes in the prevalence of brain disorders.

The various brain lesions were categorized based on the gross and histopathological lesions. It is worthy to note, that not all animals reported to have shown nervous signs before death brought for autopsy revealed histopathological lesions. At the same time some of those having no nervous signs revealed considerable lesions in the brain. Samples from the slaughter house revealed chiefly the vascular changes like meningeal congestion and hemorrhage that could be due to the stunning before slaughter.

Histopathologically, the lesions varied from congestion, hemorrhage, degeneration and necrosis to inflammation, which could be associated with hypoxic injuries, infection and toxicities. It is worthy to mention that some of the cases that showed histological lesions of degeneration and inflammation had no gross lesions in the brain.

Sprouting of capillaries engorged with blood could be seen in the cerebrum, cerebellum and areas of the midbrain in a few cases. Schulman (1968) regarded these vascular changes as simply reactions to the intense parenchymal degeneration. Sprouting of capillaries in the cerebral cortex and prominence of them was described in Polioencephalomalacia (PEM) by Lonkar and Prasad (1994). However, in these cases no lesions suggestive of PEM could be observed. Meningeal and meningeal sulcal vessels appeared engorged with blood and thickened in a few cases which indicated primary injury to the blood vessel wall. Truceman and Clague (1978) found thickening of arterial walls in salt poisoning in cattle. Capillary bed dilatation, proliferation of endothelial cells along with sprouting and formation of new vessels, swollen astrocytes, hemorrhage and formation of cavities and

were observed in few cases. These changes were previously reported to be due to altered capillary permeability (Schulman, 1968). Perivascular space dilatation progressing to cavitation and crowding of gemistocytes were found in a few cases. This space formed may be due to hypoxia and edema. Similar findings were reported by Nair *et al.* (1985) in *Ficus tsiela* Roxb poisoning in calves, *Trypanosoma brucei* rhodesiense infection in cattle (Wellde *et al.*, 1989) and in PEM (Lonkar and Prasad, 1994).

Degenerative and necrotic changes observed in the neurons were diffuse and seen both in autopsy cases and the slaughtered animals. In some cases, the neurons appeared condensed and dark indicating hypoxic injury. Neuronal degeneration and necrosis were common findings observed in various conditions such as salt poisoning, BSE, PEM, thiamine deficiency and bovine herpes virus type V infection (Truceman and Clague, 1978; Jeffery, 1992; Lonkar and Prasad 1994; Shibahara *et al.*, 1995; Perez *et al.*, 2002). Abnormally large spaces were found in the white matter of the cerebrum and cerebellum in a few cases, which could be due to the necrosis and liquefaction process. The area adjacent to this was normal and free of cellular accumulations. In contrast to many extraneural tissues, areas of CNS necrosis and liquefaction do not heal by vascular proliferation and fibroplasia and so a cyst or cavity remains (Summers *et al.*, 1995). Degeneration and loss of purkinje cells could be observed in a few cases. The cells were condensed, homogenous, angular and eosinophilic. Hager (1968), observed these types of changes in hypoxia and subacute ischemic cell damage. Neeraja *et al.* (1999) observed similar changes in the brain of calves with monocrotophos (nuvacron) toxicity.

Focal to diffuse neuronal degenerative and necrotic changes were seen in various segments of the brain in which the cell body appeared shrunken and the cytoplasm was highly eosinophilic with pyknotic nucleus. Similar changes were reported in bovine cranial zygomycosis, BSE, Aujesky's disease and ischemic injury, rabies, idiopathic brain stem neuronal degeneration and thiamine deficiency (Hill *et al.*, 1992; Jeffery, 1992; Summers *et al.*, 1995; Singh *et al.*, 1992; Stewart, 1997; Shibahara *et al.*, 1995). Satellitosis and neuronophagia were also observed in many cases. Summers *et al.* (1995) suggested it to be an inflammatory reaction to any type of injury to the neuron. Neuronophagic nodules were observed in PEM, rabies, nuvacron toxicity, Akabane virus infection and bovine herpes virus V infection (Lonkar and Prasad, 1994; Singh *et al.*, 1992; Neeraja *et al.*, 1999; Lee *et al.*, 2002; Perez *et al.*, 2002).

Loss of neurons and dark neurons were found in the cerebral cortex in a few cases. The loss of neurons could be due to the aging process. Lee *et al.* (2002) observed this in Akabene virus infection. Dark neurons chiefly found in the pyramidal cell layer of the cerebral cortex could be due to ischemic change and immediate removal of perfused brain from the skull (Summers *et al.*, 1995). Also, Lonkar and Prasad (1994) reported dark neurons in PEM. The dark neurons in the pyramidal layer of the cerebral cortex in a sinus tumor case indicated hypoxic change, as there was compression of the brain by the growth obliterating the capillaries. Few cases revealed binucleated neurons. An amitotic nuclear division during the process of cell division may produce such neurons where in the cytoplasmic cleavage causes the binucleations (Lumsden, 1968).

Perivascular cuffing within the brain was observed in a few cases that indicated inflammation. The cuffs contained gemistocytic astrocytes, plasma cells and mononuclears. In few cases, which were confirmed as rabies on the basis of the finding of intracytoplasmic inclusions, no cuffing with infiltratory cells was observed. Cuffing of varying degree was observed in a variety of conditions like neosporosis, rift valley fever, clostridium chauvoei infection, borna disease, PEM, rabies, chlamydiosis, Akabane virus infection and bovine herpes virus V infection (Dubey *et al.*, 1992; Rippy *et al.*, 1992; Singh *et al.*, 1992; Caplazi *et al.*, 1994; Lonkar and Prasad, 1994; Foley and Zachary 1995; Piercy *et al.*, 1999; Lee *et al.*, 2002; Perez *et al.*, 2002). Abnormally large number of glial cells and mononuclears were present in the space around the menengial vessels in some cases, which could be due to the inflammation of the meninges. Liggitt and Demartini (1980), reported lymphocytic vasculitis as a pathognomonic lesion in malignant catarrhal fever. Multifocal meningitis in rift valley fever, meningoencephalitis in neurovirulent bovine herpes virus type 1.3 infections and lymphocytic meningo-encephalitis in rabies were observed by Rippy *et al.* (1992), Belknap *et al.* (1994) and Foley and Zachary (1995). A nonsuppurative meningo-encephalitis in cattle was observed by Theil *et al.* (1998), which was also observed by Gardiner *et al.* (1989) and Piercy *et al.* (1999) in *Trypanosoma vivax* and *Chlamydia psittaci* infection, respectively.

Gliosis was seen in many cases, which was more evident in the white matter region. It could be due to the inflammatory reaction to any injury of the brain. The etiology of these changes could not be classified as the study was confined to histopathological examination alone. Therefore, for better understanding it is essential to

undertake cultural examination of the brain tissue. Gliosis was reported previously in *Haemophilus somnus* infection, *Clostridium chauvoei* infection, neuro virulent bovine herpes virus type 1.3 infection, PEM, Neosporosis, monocrotophos toxicity, *Clamidia psittaci* infection in a 14 weeks old calf and Akabane virus in adult cows (Yamasaki *et al*., 1991; Singh *et al*., 1992; Belknap *et al*., 1994; Lonkar and Prasad, 1994; Dannatt *et al*., 1995, Neeraja *et al*., 1999; Piercy *et al*., 1999; Lee *et al*., 2002). Glial nodules were observed in few cases. Similar findings were reported by McGee *et al*. (1992) and Singh *et al*. (1992) in eastern encephalomyelitis in adult cow and rabies cattle.

Intracytoplasmic eosinophilic negribodies were found in the purkinje cells of the cerebellum. Burnes *et al*. (1997) reported vampire bat transmitted rabies in cattle and described non-suppurative encephalitis with intracytoplasmic inclusion bodies. However, in this case no encephalitis was observed. A few cases revealed inclusions of varying sizes in the neuronal cytoplasm in the cerebral cortex, which were not found to be rabies inclusions on special staining with Masignanni Magferrari method. They also appeared PAS negative proving that these inclusions were not developed in the process of aging and indicated that the animal had contracted the viral infection and subsequently it might have recovered. Summers *et al*. (1995) found light acidophilic viral inclusions near by astroglia. Eosinophilic cytoplasmic inclusions were found in the degenerating neurons in the equine motor neuron disease (Cummings *et al*., 1993). However, in this case, the inclusions were found in almost all the glial cells and the neurons. Faintly eosinophilic and homogenous intracytoplasmic inclusions of shape varying from round to oval or crescent were found in an encephalopathy with argyrophilic inclusions in a Holstein-Friesian cow (Nakamura *et al*., 2000).

Demyelination is the destruction of a normally formed myelin sheath leaving the axon naked but otherwise unscathed. It was observed in a few cases and was confirmed by special staining with luxol fast blue technique. Raine and Cross (1989), observed these changes in multiple sclerosis and autoimmune encephalomyelitis. Summers *et al*. (1995) observed demyelination as a characteristic lesion in hepatic encephalopathy, amino-acidopathy maple syrup urine disease; intoxication caused by rodenticide bromethalin and hexachlorophene and in globoid cell leuckodystrophy in which condition a substrate accumulates which is toxic to the oligodendrocytes. The demyelination was found in chela leaf poisoning in calves, *Ficus ingensvar* ingens and *Ficus chordate* sub sp. *salicifolia* poisoning and Wilson's disease like lesion in calf (Nair *et al*., 1985; Myburgh *et al*., 1994; Wada *et al*., 1995). However, it was not possible to associate the demyelination in any of these conditions except in one which was found associated with theileriosis.

Status spongiosis with multiple vacuolations were seen in the gray matter and white matter. One case revealed large single intra neuronal cytoplasmic vacuolation in the midbrain area. The vacuole had no stainable material inside and had a regular circumscribed appearance. It is also worth mentioning that vacuolation of neurons occur at all levels of the brain but it is pronounced in certain cell types of neurons or in certain well defined nuclear groups (Summers *et al*., 1995). However, the area was not particular nuclear region. Multiple intra neuronal cytoplasmic vacuolation in the midbrain region is suggestive of BSE. Pathak *et al*. (2001) described profuse vacuolation in both gray and the white matter and attributed to BSE. Summers *et al*. (1995) described fine neuronal vacuolations in the ischemic neurons due to mitochondrial swelling and also suggested intramyelinic edema as a reason for the spongiosis. Severe spongiform change in the thalamus and cortex was observed in rabies by Foley and Zachary (1995). Also, vacuolated neurons were observed in bβ mannosidosis by O'Toole *et al*. (1993). Few cases revealed encephalomalacic changes, which exhibited as vacuoles and spongy appearance in the white matter of the brain. Yamasaki *et al*. (1991), observed foci of malacia and perivascular microgliosis throughout the CNS in *Haemophilus somnus* infection. But in this case, there was no perivascular microgliosis. Focal area of malacia in Sarcocytis infection in a steer was described by Dubey *et al*. (1992), while Jeffery (1992), described focal symmetric encephalomalacia in BSE. Also, Sagar *et al*. (1990) described linear area of cavitation and cerebrocortical necrosis in calves in PEM. Diffuse irregular vacuolations were observed in the brain parenchyma in a few cases. The neurons and the glial cells appeared much eosinophilic and were surrounded by vacuoles. This may be attributed to the activation of lysosomal hydrolytic enzymes, which increase the cell permeability and thus, make it appear swollen and acidophilic (Hager, 1968). Granular cell degeneration was seen in the cerebellum. Netsky (1968) also suggested that this could be an autolytic change.

Gitter cells were observed in a few cases around the blood vessels in the brain parenchyma. These are large rotund cells with eccentric nucleus and a pale foamy

cytoplasm without cell processes found within the perivascular space and the adventitia of the vessels. They are found in the brain wounds, allergic encephalitis, cerebral wallerian degeneration and infaction of the brain (Luse, 1968). This finding was in agreement with Truceman and Clague (1978), who observed these in salt poisoning in cattle. Gemistocytes could be observed in few cases, around the blood vessels amidst the inflammatory cells. The common astrogliotic response to cell injury is manifested by the development of visible cytoplasm in the cell, which typically takes the form of a broad polygonal shape with the nucleus at the margin. Such large reactive astrocytes are deemed gemistocytes (Summers *et al*., 1995), where cytoplasm is eosinophilic. Astrocytosis was seen in a few cases, which is an inflammatory response to any CNS injury. Clark *et al*. (1995) and Piercy *et al*. (1999), recorded astrocytosis in *Chlamydia psittaci* infection in a 14 weeks old calf and encephalopathy caused by scrapie agent in cattle, respectively.

Brownish yellow granular pigmentation was observed in and around the walls of the meningeal blood vessels in a few cases. Cozzi *et al*. (1988), stated that neuromelanin, which closely resembled the lipofuschin (Merighi and Peirone, 1985; Bianchi and Merighi, 1986) accumulated as fine brownish granules in the cytoplasm of certain neurons. However, it was not the case in the present study.

In the present research, it was proved that immersion fixation was equally good as perfusion fixation in routine examination of brain as the neurons and the other structures were found highly preserved and no artifactual changes could be observed in these cases.

CONCLUSION

The brain disorders in cattle were not less frequent. A high incidence of brain lesions could be recorded in autopsy cases than in samples collected from the slaughter house cattle, which were apparently healthy. Some of the conditions encountered in the study were detrimental and resulted in the death of the animal. The findings suggest that many of the sudden death cases of unknown etiology in field conditions could probably be the result of underlying brain disorders. Lesions of BSE could not be observed in the present study. The export of livestock and livestock products from India primarily depends on establishing a sound system for monitoring and diagnosis of all neurological disorders occurring in all species of animals and to differentiate it from BSE. A proper reporting of BSE will depend upon a systematic examination of the brain stem segments. Hence, this systematic histopathological examination of the brains though extended to only a limited number of animals proved that the animals were free of BSE. Considerable efforts should be made to conduct examination of large number of animals from different localities and the results of histopathology should be correlated with other molecular diagnostic techniques employed to detect BSE. Additionally, the cases that were negative for rabies inclusion bodies in the direct impression smear revealed negribodies in the histological sections. Thus, this study proved that diagnosis of rabies by impression smear examination alone is not sufficient and needs examination by FAT or immunoperoxidase techniques. Therefore, it is imperative that earnest efforts should be made to delineate the disease problems associated with brain, so that a plan of action can be implemented to prevent and control the brain disorders in the cattle which had been a neglected field, has therefore great relevance.

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