REVIEW ARTICLE

Epizootiology, pathogenesis and immunoprophylactic trends to control tropical bubaline fasciolosis: an overview

R. L. Sharma · R. Godara · M. B. Thilagar

Abstract On the Indian sub-continent, nearly 5,000 years ago, the domestication of the riverine buffalo—the incredible Asian dairy animal was initiated. It plays a versatile role in socio-economic upliftment of its owners from the rural agricultural communities in Asian, African, South American and a few European countries. Comparatively, buffaloes are lesser evolved and susceptible to infectious diseases than cattle. However, poor body thermoregulation and wallowing nature predisposed them to snail borne infections, especially tropical fasciolosis—an incessant major constraint on buffalo production and improvement programmes. This review article is an insight into the global prevalence, varied epizootiological factors, offers possible explanation to pathophysiological clinical signs, deleterious effects of the tropical liver fluke, involving hepato-biliary system, haemopoitic system, endocrine glands and their secretions, oxidative stress, altered metabolism and significant fall in food conversion efficiency with unaffected digestibility of nutrients. Besides, the authors have briefly discussed and reviewed the developments and significance of successful immuno-diagnostic approaches for detecting and forecasting the disease during early pre-patency and feasibility of developing a cost effective immunoprotection strategies against tropical fasciolosis.

Introduction

Buffalo—the incredible Asian dairy animal, popularly known as "Black Diamond", for its versatile role in socio-economic upliftment of its owners from the rural agricultural communities. It is the largest high energy milk and lean meat producer in India (Gupta and Singh 2002). In the tropical and sub-tropical countries, including India, fasciolosis has been a major economic threat to the development of ruminants, especially buffaloes. High sensitivity to solar radiation and poorly evolved body thermoregulation in buffaloes are comforted by their wallowing nature in fresh water bodies that predisposes to snail borne metazoan infectious diseases, especially tropical fasciolosis mainly caused by *Fasciola gigantica*—an inhabitant of hepato-biliary system of domestic ruminants (Mehra et al. 1999; Garg et al. 2009; Edith et al. 2010a). In recent past, global losses incidental to fasciolosis were estimated over US $3,200 million per annum. This review is an insight into the latest accomplishments on epizootiology, pathophysiological impact and the feasibility of developing an effective vaccine against *F. gigantica*, without compromising with optimum growth and production in the parasitized buffaloes.

Epizootiology

Wild as well as domestic buffalo population is mainly confined to their native breeding sites at 28 countries in Asia, Africa and South America, located in tropical and

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sub-tropical regions of the globe. Of the 166.4 million global populations, 161.2 million (96.87%) are in Asian countries followed by 3.54 million (2.12%) in African, 1.4 million (0.84%) in South American and 0.26 million (0.15%) in European countries. India all alone contributes 93.13 million (55.96%) to global buffalo population. Amongst ten breeds of Indian buffaloes, Murrah buffaloes are highly susceptible to *F. gigantica* infection.

The prevalence of fasciolosis in buffaloes varies from 10.0 to 100% (Table 1). Amongst various epidemiological determinants, prevalence of disease mainly depends upon geo-climate of the region, nutritional status and exposure of buffaloes to *F. gigantica* contaminated biomass on grazing lands and in cultivated crop residue used as fodder, quantum and periodic intake of *F. gigantica* metacercariae (MC), above all scientific healthcare and management of the host.

Field investigations on snail vector of the disease revealed that fresh water aquatic lymnaeid snails act as intermediate host (IMH). These snails prefer low lying swampy areas with slow moving water, beside agricultural crops frequently irrigated with contaminated water, constituted the most preferred and suitable sites for shelter, propagation, breeding and burrowing of the snail population. Snails once infected with *F. gigantica*, normally do not accept other trematode infections, life-long remain positive for the infection, and have a shorter life span of 5–85 days than non-infected snails of 50–150 days. Ten to fourteen mm long *L. auricularia* snails, aged less than 14 weeks, were most susceptible to *F. gigantica* miracidial infection. These snails had smaller prepatent but longer period of patency, discharging *F. gigantica* cercariae.

Investigations on in vitro *F. gigantica* infection of *L. auricularia* revealed: (1) younger snails (1–4 weeks) were not suitable for in vitro infections and had higher mortality (Prasad 1989); (2) a higher intake of infection from the bubaline origin than caprine/ovine origin

F. gigantica miracidia (Dhar and Sharma 1986); (3) optimum temperature for in situ development of miracidia and metacercarial production was 25°C ± 0.5°C (Dhar and Sharma 1986); (4) laboratory infected snails had a lower metacercarial production than those collected from water bodies in the nature (Sharma et al. 1989); (5) positivity of snails was inversely proportional to availability of space for miracidial migration at the time of in vitro infection (Dhar and Sharma 1986); (6) cercarial production occurred in waves, with an in-between 1 and 12 days resting period(s) and floating cysts of *F. gigantica* were produced in the first few waves of cercarial production cycles (Dhar and Sharma 1986); (7) no development and emergence of *F. gigantica* cercariae from *L. auricularia* occurred below 12°C and above 33°C, however, a temperature of 20°C was optimum for the purpose (Sharma et al. 1989); (8) an infected snail, in optimum environmental conditions during autumn months was capable of producing sufficient number of *F. gigantica* MC, causing acute, clinical and fatal disease in ten buffalo yearlings. On an average 204 *F. gigantica* MC are produced per day by an infected snail (Sharma et al. 1989); (9) over 95% cercariae shedding by the infected snails were observed during night between 6 pm and 6 am next day (Gupta and Yadav 1994); (10) unlike *F. hepatica*, the escaping site of *F. gigantica* cercariae from developing rediae in *L. auricularia* was not confined to birth pore(s) in the rediae. The freely floating cercariae could emerge out from any site of the redial elastic, acellular but sticky tegument, leaving behind a self healing indentation (Yadav et al. 1997).

Pathogenesis

Fasciolosis in young buffaloes (<1 year old) occurs as acute and devastating to chronic and asymptomatic disease in immune carrier adults. Like other metazoan diseases, the

Table 1 Prevalence of *F. gigantica* infection in South Asian and African countries

Country	Prevalence (%)	References
India (Hills)	25–100	Sharma et al. (1989)
India (Plains)	13–60	Roy and Tandon (1992); Garg et al. (2009)
Nepal	34–100	Spithill et al. (1999)
Iran	27–91	Sahba et al. (1972)
China	10	Spithill et al. (1999)
Egypt	11–88	El-Azay and van Veen Schillhorn (1983)
Indonesia	25–90	Spithill et al. (1999)
Phillipines	34–100	Spithill et al. (1999)
Nigeria	65	van veen Schillhorn (1980)
West Africa	Upto 97	van veen Schillhorn (1980)
Vgarida	Upto 97	Okao (1984) and Fabiyi (1987)
Ethiopia	30–90	Fabiyi (1987)

severity and the course of the disease in buffaloes vary with the dose of MC ingested, in a primary single massive dose or perpetual and recurrent lower doses, over a shorter or a prolonged period. A primary infection dose of 800–1000 *F. gigantica* MC caused acute disease with pathognomic clinical manifestations synchronous with growth and maturation of the infection dose and was incidental to 20–33.3% mortality in Murrah buffalo yearlings (Yadav et al. 1999b; Edith et al. 2010c). The prepatent period of *F. gigantica* in riverine buffaloes is longer (92–97 days) than *F. hepatica* (50–70 days) in large ruminants. Likewise *F. gigantica* is more destructive than *F. hepatica*. Chronologically clinical signs comprised of dullness, weakness, besides onset of pathognomonic manifestations including apyrexic inappetance, anaemia, poor live weight gain, diarrhoea, and sub-mandibular and facial oedema, respectively from 5, 6, 8, 16 and 17 week post-infection (wpi).

Grossly the carcasses were emaciated and had icteric appearance, extensive gelatinization of subcutaneous fat, hepatomegaly (1.5 times) and liver had rounded margins. The establishment of adult flukes in biliary network was associated with enlarged, thickened and mineralized and prominent bile ducts beneath the liver capsule and distended gall bladder (Yadav et al. 1999b; Edith et al. 2010a). A hepatic load of 368 ± 110 adult flukes could cause death in buffalo calves, aged above 6 months by Day 140 post-infection (pi). Coprological egg counts >300/g was suggestive of acute fasciolosis, while its subsequent fall to 100–200/g was symbolic to onset of chronic phase of the disease and/or immune carrier status of the host (Yadav et al. 1999b).

Of late, sincere and intensive efforts were made to investigate, as to the deleterious effects of *F. gigantica* are factually confined to liver alone or it is sort of syndrome involving other systems, besides finding sagacious explanation(s) to sequential onset and duration of pathognomonic clinical signs of the disease stated above, through well planned in vivo experimental studies in buffalo yearlings under controlled and defined conditions. The significant and interesting findings are being briefly reproduced herein.

Apyrexic inappetance

In buffalo yearlings, a primary infection dose of 1000 *F. gigantica* MC with mean adult fluke count of 368 ± 110.3 induced apyrexic anorexia from 5th wpi onwards. The inappetance was more pronounced and marked during late prepatency (7–12 wpi), synchronous to the terminal stage of in situ fluke development. The feed conversion efficiency (unit intake per unit gain) of the diseased calves sharply declined from 9.6 to 33.4 in comparison to healthy controls. However, the digestibility of the nutrients remained unaffected in the infected host.

Obviously, the fall in feed conversion efficiency was incidental to fall in intake of feed (Mehra et al. 1999).

Anaemia

In large ruminants, including buffalo, anaemia and hypoalbuminaemia are being the most common and glaring manifestations, consistent of the fasciolosis (Kumar et al. 1982; Ganga et al. 2007; Edith et al. 2010b). The developing *F. gigantica* juveniles and the adults have been considered non-haematophagous and tissue feeding parasites yet cause severe anaemia in the host suffering from acute and/or chronic disease (Ganga et al. 2004b). Thus, pathogenesis of anaemia in fasciolosis has therefore been debatable. Various factors, such as haematophagia, haemorrhages during migratory phase and injurious nature of the fluke metabolites discharged into the host circulation significantly contribute to the development of anaemia (Ganga et al. 2004a). Buffalo calves, suffering from the acute course of the disease, with mean fluke load of 300 and above had a significant fall in erythrocytic indices during late prepatency caused normocytic normochromic anaemia (Yadav et al. 1999b; Ganga et al. 2007), whereas in the chronic phase, hypoalbumineamia is more marked than anaemia and an immune carrier host, on partial recovery from the acute course of the disease, continues to suffer from normocytic hypochromic anaemia (Edith et al. 2010b).

Endocrine gland dysfunction

It was strongly speculated that the fluke metabolites discharged into the circulation coupled with generalized oxygen starvation at tissue level, incidental to the severe and prolonged anaemia could influence normal functioning of the hypothalamus-pituitary axis and consequently, the altered physiological plasma levels of the tropic hormones leading to dysfunctioning of the target endocrine gland(s), especially thyroid and adrenal glands that influence and shift the entire metabolism in the host (Ganga et al. 2007; Edith et al. 2010a, c).

Thyroid gland

In the Murrah buffalo calves infected with 1000 *F. gigantica* MC, a progressive fall in the plasma levels of tri-iodothyronine (T_3) and thyroxine (T_4) were recorded from 6th and 8th wpi onwards, respectively. This was synchronous with in situ migration, growth and development of the juvenile flukes and their establishment in the hepato-biliary system as adult parasite, beside onset of loss in body weight and poor appetite during early prepatency (1–6 wpi). The physiological levels of the hormones in the host circulation recorded 54.7 and 16.21% fall of T_3 and T_4,

respectively during 13th wpi. The histopathological evidences of hypothyroidism in the diseased host were appearance of peripheral vacuoles and atrophy of the thyroid follicles and lymphocytic thyroiditis with diffuse mononuclear cell infiltration in inter and intracellular spaces, causing progressive destruction of thyroid hormone secretory cells and follicles. Even though the infected buffaloes did not show classical clinical signs of hypothyroidism, such as obesity and myxoedema yet lower concentrations of T_3 and T_4 in host circulation indicated a clear picture of non-clinical hypothyroidism (Ganga et al. 2003, 2007).

Evidently, in the *F. gigantica* infected host, entire metabolism and mitochondrial oxygen consumption at cellular levels got affected due to hypothyroidism. Anabolic activities of the hormones in conjunction with the growth hormone and insulin, to synthesize proteins and excretion of nitrogen in urine were altered. At mitochondrial level, it possessed direct negative/depressing impact on oxygen consumption, thereby affecting the growth and differentiation, lipid, carbohydrate and protein metabolism.

Adrenal glands

The adrenals are the most sensitive and versatile steroid hormone producing endocrine glands. They spontaneously interact with the altered in situ environment/stimulus from a stressor, refluxed gluco-corticosteroids, especially cortisol into the circulation from the activated zona fasciculata, seemingly under the influence of Adrenocorticotrophic hormone (ACTH) from the hypothalamus-anterior pituitary-adrenal axis. The long-term effect of such stressors on growth, development, production and reproduction were monitored and quantified in different situations and were correlated with elevated blood levels of cortisol.

The Murrah buffalo yearlings, infected with 800/1000 *F. gigantica* MC, persistently had elevated hormone activity in the circulation indicated variable degree and magnitude of the adrenocortical response to the in situ distome population (Ganga et al. 2007; Edith et al. 2010c). The response was, however, variable in severity depending upon the clinical phase of the disease, in situ activities and location of the *F. gigantica* adolescercariae or adult fluke population. The highest concentration of the hormone in the infected animals was documented during early prepatency. Subsequent, progressive fall in the hormone activity in circulation synchronized well with the arrival and establishment of the causative organism in the hepato-biliary network, cessation of traumatic activity of the distome and partial resolution of the lesions in the hepatocytes and/or recovery of the animals from the disease stress. The histopathological evidences comprised of hypertrophy of cellular elements in zona fasciculata with a remarkable increase in its width, occupying major portion of the cortex

and altered cellular architecture in the cortex. It is therefore strongly speculated that the long-term secretion of cortisol (for 112 days) in the infected animals seems influenced the metabolism, suppressed immune response of the host and consequently resulted in characteristic clinical signs of bubaline fasciolosis.

Altered serum enzyme levels

The elevated serum enzyme concentrations in fasciolosis were exploited for early and semi quantitative diagnosis of the disease before the animal is coproscopically positive for the fluke eggs (Kumar et al. 1982; Swarup and Pachauri 1987; Edith et al. 2010a). The recent experimental findings on the liver specific enzyme concentrations, periodically refluxed by the damaged hepatic tissues in the circulation of *F. gigantica* infected buffalo yearlings, during the course of disease revealed that the elevated levels of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were suggestive of the hepatic tissue injury, whereas the elevated levels of alkaline phosphatase (AP) indicated establishment of adult flukes in the bile ducts.

The increasing patterns of the serum enzyme concentrations revealed two clearly demarcated stages. The first stage of massive invasion and traumatic hepatitis caused by the fluke juveniles during prepatency was coinciding with the onset and significant increase in the AST and ALT in the host circulation from 2nd wpi onwards. A remarkable increase (3.4 times) of ALT (140.0%) than corresponding increased serum AST concentration (40.8%) during prepatency in the infected buffalo yearlings signify traumatic hepatitis, whereas their moderate but persistently higher levels in the serum indicated parasitic hepatopathy/cirrhosis and onset of the regenerative process and/or partly resolution of traumatic lesions in the liver tissues during patency stage of the disease. The second phase (patency) in the infected buffaloes was synchronous with overall 107.9% increase in the serum AP concentrations. In contrast to AST and ALT, the elevated levels of AP are ascribed to decreased biliary excretion of the enzyme or obstruction of bile flow stimulate de novo synthesis of the hepatic AP and synthesized enzyme is refluxed into the host circulation. Over 28% increase in AP concentration indicates obstructive hepato-biliary lesions and less than 8% increased AP concentration was suggestive of chronic form of the disease or post-patency immune carrier status of the host (Edith et al. 2010a).

Diagnosis

The diagnosis of fasciolosis is of prime importance for planning treatment and the eradication program from the

endemic area. Faecal examination is of little consequence in the host suffering from the acute course of the disease because the onset of adverse effects starts much before appearing of fluke eggs in faeces (Gupta and Yadav 1994; Dixit et al. 2008). Quantitative examination of faeces and assessing hepatic fluke load at necropsy are also not sensitive enough to precisely predict severity of the disease (Garg et al. 2009). However, it is possible to predict/suspect fasciolosis during early pre-patency (Day 40 pi onwards) by periodical monitoring the elevated levels of marker enzymes (AST and ALT) in the host circulation (Edith et al. 2010a).

Serological demonstration of anti-*Fasciola* specific antibodies, *F. gigantica* antigens and immune complexes in host circulation during prepatency appears to be a useful alternate option to coproscopic detection of fluke eggs (Yadav et al. 1999a; Dixit et al. 2008). Amongst various immunodiagnostic techniques used, ELISA is the most sensitive and acceptable technique but at times suffers from false positive reporting. Cross reactivity with other in situ metazoan infections is a major limitation and it prompted a search for specific immuno-dominant moieties in complex somatic and excretory antigens. The role of metabolites released during the development of adolescercariae in conferring acquired immunity in the host, immune evasion and diagnosis of the disease was thoroughly investigated (Dixit et al. 2008). Ultimately, Glutathione S-transferase (GST) and Cathepsin L cysteine proteinase (CS) molecules were identified as specific for the purpose. Cathepsin L cysteine proteinase was further purified, characterized and designated as FgCL-3 antigens. The FgCL-3 ELISA showed 100 and 97% sensitivity under experimental and field situations, respectively in the diagnosis of the disease. The infection could be detected as early as two wpi (Dixit et al. 2004; Raina et al. 2006). The FgCL-3 was also evaluated for its potential in the detection of early prepatent infection in experimentally infected bovine calves infected with *F. gigantica* infection. It could detect *F. gigantica* infection as early as 4th wpi, while using sensitive antibody detection assays with 100% sensitivity (Sriveny et al. 2006).

Control and immunoprotection

The control of fasciolosis has been a challenging task and routinely comprised of restrictive husbandry practices, proper disposal of animal excreta, drainage of water logged areas and the effectively harnessing measures to control the intermediate hosts and the specific integrated therapeutic management of the infected hosts. The elimination of snail IMH, using molluscicides or predators like ducks and frogs seems not feasible because of being costly and labour

intensive and ecological reasons. Benzimidazole (triclabendazole), Salicylanilides (closantel, oxyclozanide) and substituted phenol (nitroxynil) are the drugs of choice in use against *F. gigantica*, with an efficacy ranging from 20 to 100% depending upon the drug, dosage and administration modalities used (Gupta et al. 1989; Sanyal and Gupta 1996; Pal et al. 2003). Use of triclabendazole incorporated feed pellets and strategic application of the anthelmintic delivery device have also been claimed to reduce fluke burden (Sanyal 1998). In the endemic areas, epizootiology based specific therapeutic management of the disease in mid summer (May–June), in autumn (October), and in late winter (January), when animals are confined to indoor housing and are stall fed, have been advised and practiced. In the endemic areas, meteorological forecasting facilitates early warning of disease occurrence, so that control measures are timely applied, prior to shedding of cercariae by the infected snails and intake of MC by the host.

An alternative approach aiming at early diagnosis of the disease and development of acquired resistance in the susceptible host, using different viable or non-viable antigens of the fluke has been an area of research interest on account of being an efficient, cost effective, environmentally friendly, and reliable long-term solution for the prevention and/or if possible eradication of the disease. Global approaches adopted by different workers have been briefly presented in Table 2. Protections conferred by live attenuated *F. gigantica* MC in the different experiments have been summarized in Table 3.

Thereafter, the vaccine targets against fasciolosis were vigorously pursued, using non-viable soluble purified parasite defined proteins molecules as antigens, including Fatty acid binding proteins, Glutathione-S-transferase, Leucine aminopeptidase, Paramyosin and Cathepsin L cysteine proteinases having the potency of inducing acquired protective response against the fluke in laboratory and large animal models (Tables 4 and 5). Amongst these, the parasite enzymes belonging to cysteine proteinase family secreted by the parasite to help it burrow through various host tissues or cleave the immunoglobulins of the host, have been most intensively studied and gave promising results when used as vaccine antigens (Dixit et al. 2008). Dalton et al. (1996) in cattle and Edith (2004) in buffalo documented a larger prepatent period (Day 142–143), low mean egg counts, significantly lesser mean wet fluke weight, and lesser mean length and width of the flukes recovered in experimentally immunized with ISAg and ESAg and challenged with the parasites. A reduction in size of adult flukes recovered from vaccinated cattle and buffaloes, suggested that immune responses in cattle and buffaloes vis-à-vis sheep may be different.

However, despite persistent efforts, a vaccine with adequate protection against fasciolosis has not yet been

Table 2 Various immunoprotective approaches against fasciolosis—an update

Period	Immunogen	Host	Protection (%), Max (Range)
1973–1989	Live attenuated mc	Goat, cattle, sheep	86 (29–86)
1972–1985	Crude antigen		
	Sonicated cultures	Sheep	79
	Freez driedSAg	Rats	41–55
	Immature fluke E SAg	Rabbits	Significant
1973–1981	Passive immunization (Immune Sera)	Rats	25–48
		Rabbits	
1990–1996	Defined antigens		
	GST	Cattle	18–69
	FABP	Cattle	55
	Cathepsin L	Sheep	69
		Cattle	42–69
	Cathepsin L + fluke haemoglobin	Cattle	52–72

Table 3 Immunoprotection against fasciolosis using live attenuated metacercariae (MC)

Parasite	Host	Immunogen	Radiation dose (GY)[a] %	Protection/findings (%)	References
F. hepatica	Sheep	1000 irradiated MC		82	Kendall et al. (1978)
		Gamma irradiated MC	30	71	Nansen (1975)
F. gigantica	Cattle	1000 irradiated MC	300	100	Bitakaramire (1973)
		200/2000 MC	200	83	Haroun et al. (1988)
	Sheep	6 doses of 100 MC	30	64	Dargie et al. (1974)
				Significant protection	Haroun et al. (1988)
	Goat	Double or triple dose of 400 MC at 15 days	35	83–86	Yadav and Gupta (1989)

[a] GY-Absorbed dose of radiation, 10 GY = 1 sm k rads

Table 4 Immunoprotection against fasciolosis using non-viable antigens

Parasite	Host	Immunogen	% Protection/findings	References
F. hepatica	Sheep	Sonicate culture	79	Hall and Lang (1978)
	Rabbit	SAg & ESAg	Significant fluke reduction	Dragneva (1972)
	Rat	In vitro culture material	No protection	Davies et al. (1979)
		Adult ESAg	No protection	Rajasekariah and Howell (1979)
		Immature ESAg	Significant protection	Rajasekariah and Howell (1979)
		Adult fluke SAg (freeze dried)	48–81	Oldham and Hughes (1982)
		SAg	41–55	Oldham (1983)
		SAg and ESAg	No protection	Pfister et al. (1985)

SAg Somatic antigen, *ESAg* Excretory-Secretory antigen

developed to the point of commercialization. This can largely be attributed to the fact that immune responses to immunogens are influenced by the route of administration, nature of antigens, delivery system and the adjuvants used in the trial. Native Cathepsin L could be purified from liver fluke ES products in sufficient quantities that allowed the detailed analysis of their biochemical and physio-chemical properties, and their evaluation as protective immunogen under defined experimental conditions. Ultimately, for field trials, recombinant Cathepsin L cysteine proteinase could be cloned and produced in large quantities. Immunoprotection properties of both native and recombinant Cathepsin L can be intensively studied, standardized and tested in the endemic areas for their effectiveness, before release of vaccine for field use.

Table 5 Immunoprotection against fasciolosis using purified immunogens

Parasite	Host	Protein	Vaccine protection	References
F. hepatica	Cattle	FABP	55%	Hillyer et al. (1987)
F. hepatica	Sheep	GST	57%	Sexton et al. (1990)
F. hepatica	Cattle		19–69%	Morrison et al. (1996)
F. gigantica	Cattle		18% (ns)	Estuningsih et al. (1997)
F. hepatica	Sheep	Cathepsin L	69% (FEC)	Wijffles et al. (1994)
F. hepatica	Cattle		42–69%	Dalton et al. (1996)
F. hepatica	Cattle	Haemoglobin	44%	Dalton et al. (1996)
F. hepatica	Cattle	Cathepsin L + Haemoglobin	52–78%	Dalton et al. (1996)
F. hepatica	Sheep	Cysteine proteinase	No difference in worm burden	Wijffles et al. (1994)
F. hepatica	Cattle	Defined	55% less fluke eggs	Hillyer (1984)
F. gigantica	Rats	32 kDa NEJ protein	90% protection	Florine et al. (2000)

NEJ Newly existed juvenile fluke, *FEC* Faecal egg counts

Future projections

A majority of metazoan parasite species exert pathogenic lesions and directly influence the functioning of the target organ that explains characteristic clinical signs of the disease. However, bubaline fasciolosis is an exception; it is a sort of syndrome, synchronously involving and collective influencing normal functioning of hepato-biliary system, haemopoitic system, hypothalamus-pituitary axis, and secretions of hormones from the target endocrine glands. Thereby affecting tissue level carbohydrate, protein and lipid metabolism and consequently feed conversion and live body weight gain efficiency. More planned in vivo investigations are therefore needed to elucidate pathophysiology of *F. gigantica* and precisely define the genesis of the deleterious effect of the fluke metabolites in circulation such as anaemia and hypoxemia, incidental to in situ *F. gigantica* population.

As early as 1883, it was believed that fasciolosis was preventable and its control ought to be integrated. The philosophy is applicable even today. Environmental changes associated with global warming and development of irrigation channels, construction of dams, rapid transport system, etc. created newer breeding sites for molluscan IMH and further complicated the problem. Consequently, semi arid and arid regions of western India, where distomes were hitherto non-existing, the snail borne diseases caused by flukes are emerging. Control of fasciolosis in the endemic areas, evidently requires strategic integrated well planned programmes, including generating epizootiological data from different agro climatic zones and identification of endemic areas, forecasting of the disease, immunoprophylaxis, and timely diagnosis of the disease during early prepatency and cost effective chemotherapy.

The containment of economic losses due to *F. gigantica* in buffaloes by immunological intervention seems to be an achievable objective. However, before entering into the developmental phase of liver fluke diagnostics/vaccine, the best and cheapest system will have to be evolved and considered. In this context, large scale production of recombinant infection specific immunodiagnostic/protective proteins, using the most suitable and acceptable vector(s), that mimic the required optimum level of specificity and sensitivity for diagnosing the disease during early prepatency and that confer required level of immunity in the susceptible host against *F. gigantica*, could facilitate achieving the ultimate objective and prove beneficial in longer run. Innovative immunization strategies ought to reduce in situ fluke population to a tolerable levels (<100 flukes), without a compromise with any sort of production losses. The effectiveness of the immunogen can be improved by searching a commercially viable adjuvant(s) and selecting the most appropriate route of delivery system such as oral vaccine or the use of live viral vector(s).

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