



History of Gumboro (infectious bursal disease) in Pakistan



Rai Shafqat Ali Khan^a, Sanaullah Sajid^{a,b,*}, Mudasser Habib^a, Waqas Ali^a, M. Salah-ud-Din Shah^a, Maliha Sarfraz^c

^a Pakistan Institute of Engineering and Applied Sciences, Islamabad, Pakistan

^b Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

^c Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

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ABSTRACT

Infectious Bursal Disease is the second important viral disease of poultry which affects the young growing pullets. The end fate appears in huge economic losses to poultry industry. Throughout the world, cheapest source of animal protein is chicken meat. It was initially reported in Europe; soon it spreads worldwide and causes drastic losses. In Pakistan, first of all this disease was reported in 1971. It is the first review to track the IBDV history in Pakistan. It provides comprehensive details of forty-six years researchers work in controlling this important disease. Different scientists worked to fill the gap areas to achieve the goal. Present review covers all the research aspects being explored in Pakistan since first report.

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1. Introduction

Poultry sector is second biggest organisation in Pakistan (Anjum et al., 1993a, 1993b; Siddique et al., 1987). Gumboro is economically important disease causing great losses to this emerging industry (Anjum et al., 1993a, 1993b; Tsai and Lu, 1993). IBD is continuously threat for the growing pullets. In 1962 this disease was discovered in town Gumboro (Cosgrove, 1962). Qureshi that Gumboro was first time diagnosed in 1971 in Pakistan (Qureshi, 1999). Since its first report it has not been given priority by the research workers. In next 10–15 years it appeared as havoc for poultrymen (Siddique et al., 1987). The Infectious bursal disease (IBD) is the second considerable threat among infectious poultry diseases (Alexander, 1996). In early ninety's this disease contributes in major losses to poultry farmers due to unawareness of controlling measures in Pakistan (Anjum et al., 1994). The causative agent of "Gumboro disease" is infectious bursal disease virus (IBDV) (Müller et al., 2003). It is an acute important disease of

young chickens of 3–5 weeks old (Yunus et al., 2008), which is caused by a dsRNA virus belonging to *Birnaviridae* (Ferrero et al., 2015).

2. Incidence, prevalence and epidemiology

In a short tenure the IBD spreads throughout the world. Limited resources and insufficient measures taken by the poultry breeders to control the viral diseases have declared the farming risky business. Poor vaccination programme added losses of farmers (Farooq et al., 2000). In early eighty's mortality in layers increased that might be due to viral infection as the mortality pattern repeated each time (Ahmad and Irfan, 1981). Increase incidence of poultry diseases in Pakistan change the scenario of poultry sector in future (Qureshi, 1981). In 1987, outbreak was quoted in Fayoumi birds in Government Poultry Farm, Peshawar during the months January–February in Pakistan. The morbidity rate was 70% while mortality rate raised up to 15% in affected ones (Khan et al., 1988b). Initially the diseased flocks were evaluated on basis of post mortem. Data analysis showed that the flock size, bird type and vaccination (strain and schedule) has significant effects in occurrence of disease (Farooq et al., 2000). During the year 1997 and 1998 data from 50 poultry farms was collected from Mirpur and Kotli districts of Kashmir. Concurrent occurrence of coccidiosis in a rearing flock had a significant effect ($P < 0.01$) on the prevalence of IBD. Average losses from IBD were $15.31 \pm 1.04\%$. Financial losses due to IBD per year were approximately

* Corresponding author at: Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

E-mail address: sanaullahsajid@gmail.com (S. Sajid).

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Rs. 31701.38 ± 2345.36 from single farming unit. To prevent any stress or unsanitary conditions its better to maintain two weeks flock interval to avoid existing diseases (Farooq et al., 2003). It is recommended to improve vaccination programme and other managemental tools for better production (Abbas et al., 2015). The epidemiological studies poultry anomalies during 1998 (Farooq et al., 2002). In 2000–01 losses from IBD were reduced due to effective vaccination strategy and biosecurity measures in commercial sector. The net losses due to different factors were 50.4% in brooding and 31.3% during laying as compared to growing i-e 18.3%. Mortality was negatively correlated with peak lay. Losses of IBD were given in tabulated form mapped that IBD was present 60% as compared to all prevalent diseases (Farooq et al., 2002). (Table A).

Experts documented that IBD was one of the disease which was not affected by weather (Yunus et al., 2009). In broiler and layer flocks incidence of IBD was measured with respect of age and flock size. It is noted that IBD decreased with increase in flock size. This threat is at peak during third and fourth week of age (Khan., 1991; Sultana et al., 2008; Yunus et al., 2008; Sarfraz et al., 2017). (Figs. A.1 and A.2).

In the year 2007 the data was evaluated on the parameters of PM and previous history from fifty sheds. The prevalence of IBD in 7.75%. The overall mortality and morbidity rates were to be 6.38% and 1.35% accordingly. There was maximum mortality in fourth week 38% (Khan et al., 2009). IBD initiated in the north Islamabad area, severe form of disease appear in Karachi. It came

to notice that it is present in both vaccinated and non vaccinated flocks. It may cause 20% mortality in a flocks throughout the year in private and government poultry farms. The incidence of clinical Gumboro is higher in the commercial layers than broilers (Amin et al., 1991). Razia and co-workers investigated that 17.53% broiler sheds were affected from Gumboro whereas 13.45% of layer keepers suffered during the whole year. For future control strategies it was recommended to regulate surveillance and characterization of field strains (Sultana et al., 2008). Its interesting that occurring of disease in smaller flocks (<1000) was higher than larger flocks (>4000) but season had not major influence in disease spread (Farooq et al., 2000; Yunus et al., 2009; Zaheer et al., 2017). Anjum mentioned that if pullet vaccinated at age of 3 weeks its immunity lasts till 100 days. Booster should be given at age of 25 days. Severity of IBD was more in non-vaccinated flocks than in vaccinated flocks.

3. Transmission/spread

This disease prevails around the year however stress factors enhances its occurrence in birds. In winter months the sheds were kept air tight to maintain temperature so the virus load increases inwards when the fresh air moves through shed ventilation system it becomes the vital source of aerosol infection in nearby sheds (Sultana et al., 2008).

Table A
Impact of IBD losses at different ages of commercial flocks.

Disease	Brooding	Growing	Laying	Overall
Infectious Bursal disease	5.35 ± 1.89	3.44 ± 1.78	6.12 ± 2.02	4.97 ^a ± 2.91

^a Means with different subscripts are significantly different at $\alpha = 0.05$.

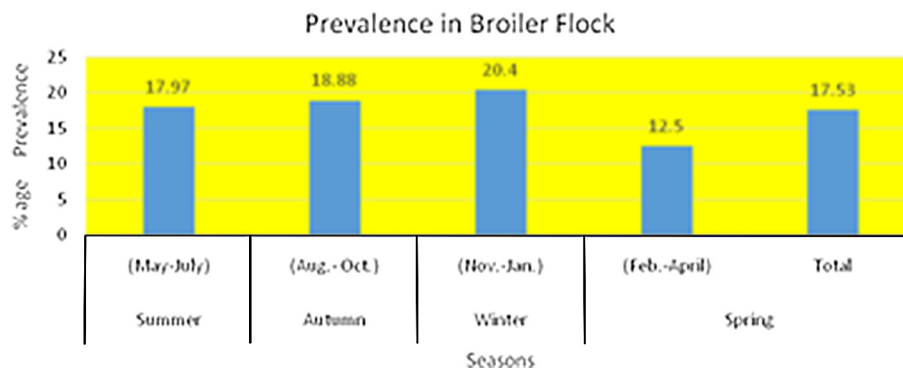


Figure A.1. Prevalence of Infectious Bursal Disease in Broiler Flocks throughout the year.

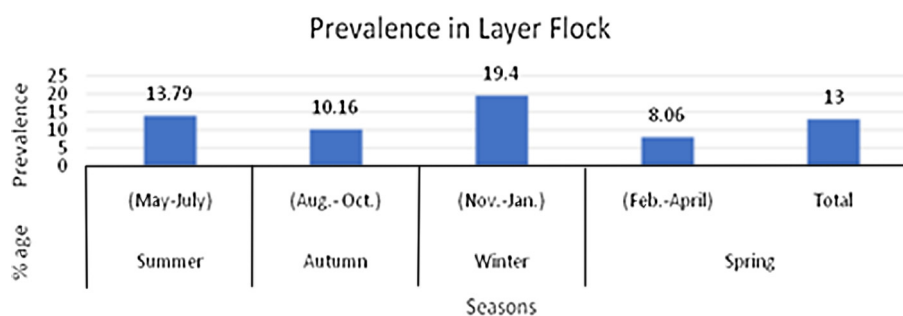


Figure A.2. Prevalence of Infectious Bursal Disease in Layer throughout the year.

4. Clinical and sub-clinical disease

IBD is present in clinical and subclinical forms. The recovered bird may serve as carrier. Affected ones were anorexic, dull and depressed in the initial stages. There were ruffled feathers stuck around the vent and mucoid diarrhea was found in majority of affected birds (Khan et al., 1988a). For the comparison of different IBD vaccines pathogenic lesions were evaluated in the three week old chickens on the basis of hemorrhages on the thigh and breast muscles, bursa weight to body weight ratio and virulence. The result showed that two could be classified as mild, two were moderate and two of the strains were for the susceptible host. These vaccines claimed that can be used in presence of maternal Ab which was not appreciated. High mortality was reported in broiler flocks in different regions of Pakistan. Severe immunosuppression was recorded in all the groups by analyzing the serum. Lack of antibodies to endemic pathogens was reflection of very high degree of immunosuppression in the broiler groups. The presence of Ab against IBDV showed that extreme immunosuppression might be due to IBD (Rehman, 2001). The co-relation between the mycotoxins and feed (commercial and home mix) was ensured in terms of disease occurrence. Toxin levels increased due to more moisture in feed, it result in immunosuppression (Yunus et al., 2009; Jamal et al., 2017).

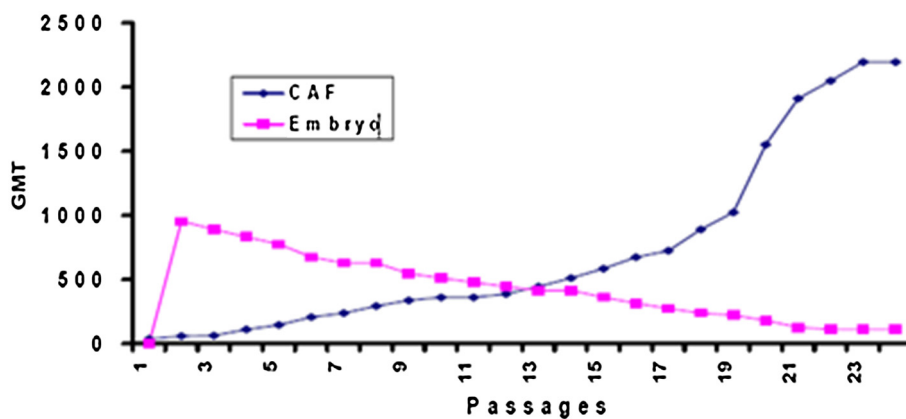
5. Adaptation of IBDV

The IBDV differs in infection and growth kinetics in cell culture and it depends on virulence of strain. IBDV was confirmed through AGPT and counter current immune electrophoresis (CCIE). The concentration of virus was checked by reversepassive haemagglutination (RPHA) test and egg infective dose fifty (EID50). During first three passages through chorio-allantoic membrane (CAM) Geometric mean titre (GMT) of the virus in CAF was 37–64 but titre of virus at 24th passage raised to 2195. On the other hand virus titre in the embryos was 1024–512 in first 10 passages, while the virus titre decreased to 111 up to 24th passage. Similarly, embryos were monitored for lesions and mortality. During first seven passages severe lesions were present on the CAM while moderate to mild haemorrhagic lesions were seen from 8th to 16th passages and no lesions were observed in 17–24th passages. The result of present study revealed that local IBDV was fully adapted and attenuated in embryonated chicken eggs. In early passages the

virus titre was low in CAF but boost with the passages. In initial passages the lesions were severe on CAM and embryos, but their severity decreased in later passages. The first passages of adapted and attenuated virus may be a good candidate for hot vaccine, the middle passages for the intermediate and last passages *i.e.*, 20–24th as mild vaccines, respectively (Ahmad et al., 2005) (Fig. A.3).

6. IBDV antigenicity/immunity

There is no alternative of vaccination in the prevention of IBD or Gumboro disease. Immune reflection was variable in each selected schedule depending upon level of maternal antibodies and time of vaccination. The affected birds showed depression of immune response (Khan et al., 1988b). For the evaluation of antigenic response of IBDV, a trail was conducted against various types of IBD vaccines purchased from market in broilers. The decline pattern of maternal antibodies was observed in non-vaccinated birds in comparison to different IBD vaccine protocols. It was recommended to adopt vaccination programme in preview of prevailing maternal antibodies in day old chicks. Single shot vaccination was compared with the two shot procedure of immunization, it was found that last one was proved better than the first programme (Ahmed et al., 2003; Shareef et al., 2017). It was investigated by selecting ten breeder flocks from the field for serum and egg yolk antibody titres against IBD. Geometric mean titre (GMT) ranged from 9 to 59 in serum and 20 to 127 in the eggs. The titres were reached at peak in 4 weeks post-vaccination while it fell down at 8 weeks. Antibodies (Ab) titres were higher in eggs rather than serum. To maintain protective levels for longer interval it is suggested to re-vaccinate breeder at age of 40–45 weeks for better maternal immunity. The level of Abs titre was not affected by age of the breeder in serum and the egg yolk of flock. The similar studies might be carried on Specific Pathogen Free (SPF) flocks in a controlled environment for valuable results (Ahmad and Siddique, 1998). The study was planned to observe the antigenic response of three commercial vaccinal strains [two intermediate plus (hot) vaccines (228-E and BUR-706) and one mild (Gumboral CT)] on bursa, spleen and thymus in birds. Present findings pointed that intermediate plus strains have good protective cover against infectious bursal disease. Some degenerative changes were noted in the immune organs but they recovered soon. There should be proper replacement with rationally designed subunit and/or



Title of the IBDV in chorio-allantoic fluid (CAF) and embryos in different passages of embryonated chicken eggs.

Figure A.3. Graph shows the titre of IBDV in chorio-allantoic fluid (CAF) vs embryos in different passages of embryonated chicken eggs.

recombinant viral vector vaccines will be recommended (Azhar et al., 2012). The effect of three IBD vaccines were noted in the low titre of ND flock. 228-E, D-78 and Bursine 2 (IBD vaccines) were evaluated against other viral vaccine, it was noted that strain Bursine 2 live vaccine can be used in the layer flock which was already at risk. It was applied on the account of showing least immunopathological response in the immune organs (Ayyub et al., 2003). Recently, protein profile of local strains and imported vaccine (D-78) was compared and found that 60kd molecular weight protein band was common in all groups but it was absent in the vaccine D-78 strain. SDS-PAGE and Western blot techniques were used for studying structural polypeptides and protein analysis. It confirmed that antigenic heterogeneity was prevalent among the native strains and the imported vaccine strains of IBDV. It is observed that local isolates provide better protection due to more antigenic relatedness. It is the need of time to manufacture indigenous vaccines using locally available strains of IBDV for broader protection profile (Mannan et al., 2015; Rashid et al., 2017). Three immunoboosting products (levamisole, vitamin E and bursinex) from market were compared against IBD. Vitamin E played a leading role in response to others. Levamisole role was intermediate while bursinex did not show much protection against IBDV (Mushtaq et al., 2003). Recently, the trial evaluated hematology and immune responses against infectious bursal disease on two groups of broilers. One group serves as control while other challenged with mixed *Eimeria* species. Both the parameters were adversely altered (Akhtar et al., 2015; Sindhu et al., 2017). The immunosuppressive effects of IBD vaccine were checked against ND vaccination. The immunosuppressant behaviour of IBDV had adversely affect immunity produced against ND. In comparison, birds that received intermediate strain of IBD were found well immunoprotected (Rehman et al., 2014). In the experimental cages 20 commercial layers were reared. To enhance titre of IgY in eggs, multiple shots of oil based IBD vaccine were used. AGPT and ELISA tests were performed to determine the Abs level. Immune egg yolk having 64 AGPT units provide 80% recovery in diseased ones. It is noted that significant antibody titre was lost during the purification process from egg yolk. Hyperimmune yolk bearing titre about 6000 gives full protection in infected chickens however semi purified Ig did not give full protection to IBDV infected birds. However semi purified IgY and immune yolk having titre more than 4000 were relatively effective in prevention. It is disclosed that after killed vaccination protective antibody titre persist in yolk for two and half month (Farooq et al., 2012). It is recommended that 2–3 shots of vaccine in routine farming provides better outcome while it might be given more in breeder for maximum maternal antibodies in next progeny for early protection (Hasan et al., 1998).

7. Inactivation of IBDV

IBDV is very virulent and highly resistant to inactivation. The genetic alterations responsible for the attenuation of infectious bursal disease virus (IBDV) have been under research at the molecular level, although passage of the virus in cell culture ends in the loss of virulence. The study was conducted for the inactivation of infectious bursal disease virus (IBDV). It was isolated from the bursa of infected birds. Confirmation of IBDV was done by agar gel precipitation test. Binary Ethylenimine (BEI) was used at concentration of 0.001 and 0.002 mol/L. It showed completely inactivation of the virus after 36 h, whereas 0.1% and 0.2% formalin inactivated the virus after 24 h of incubation at 37 °C. These findings declared that BEI inactivated IBDV for further use as vaccine in chickens (Habib et al., 2006). IBDV is continuously present in poultry sheds as it is found resistant to inactivation by both heat and many disinfectants.

8. Histopathology

After postmortem samples were taken for investigations of pathological changes. The affected group showed typical changes from partial depletion of lymphocytes to cyst formation in the center of many follicles. In the medulla of follicles several small cysts were found. The bursae had post-necrotic follicular atrophy at age of 34 days (Khan et al., 1988a). In 2012, Azhar and co-workers noted histopathological changes in vaccinated birds challenged with vvIBDV. Two intermediate plus and one mild strain was used in different groups. Intermediate strains exerts adverse effects on the development of immune organs harboring B cells. In nutshell it is need of the hour to develop IBD vaccine with low virulence conferring excellent protection against the disease (Azhar et al., 2012). An experiment was designed to compare the commercial vaccine with the egg attenuated live vaccines. Different vaccine groups were given separate challenges and found that the egg attenuated vaccine having 24 passages proved best among all groups. In short its results were evaluated on histopathological lesion in different selected organs (kidney, muscles and thymus), better Abs titre and protection against IBD (Ahmad et al., 2014). Histologically, there was also proliferation of fibrous tissue in the interfollicular region (Khan et al., 1988b). In morbid tissue haemorrhages, lymphoid cell necrosis and depletion of lymphocyte were noted (Hasan et al., 1998).

9. Vaccines

IBD is one of the economically major diseases that affects growing chickens throughout world. To prevent IBD strict biosecurity programmes along with efficient vaccination strategies have been in practice. Commercial vaccines with egg attenuated live vaccines had been compared, final results declared that live vaccines provide wider protective profile (Ahmad et al., 2014). A new concept of ovo route vaccination was given. Different strains of IBDV were practiced hot strain proved significantly better than others (hatched chicks). It concludes that *in ovo* vaccination was effective and highly safe in protecting the growing pullets against IBD. Higher immune titre was recorded which enabled maximum safety against exposure to virus (Riaz et al., 2004). Different adjuvants were tried for best selection. Serum was raised against IBD in rabbits from field isolates. Three different preparations in rabbits were used. Adjuvanted with incomplete Freund's adjuvant provides better immune effect (Hussain et al., 2004).

10. Cell culture

For better understanding of relation between host and IBDV interaction its better to observe growth pattern on cell culture. For provision of immunoprotected candidate for local live attenuated vaccine will be provided by cultivation in embryonated eggs. Adoption was successfully achieved and no pathological lesions were found in natural hosts. Protection level was much better in limited vaccination study as compared to commercial vaccine (Anjum et al., 2010). Different beneficial micro organisms were used as pro-biotics and found that it has positive co-relation to water and feed intake which leads to weight gain and increase in antibody titres. It was found that group "G" had maximum IBD titre at age of 15, 30 and 45 days. Antibiotic growth promoters were found to develop antibiotic so these probiotic (beneficial microbes) were considered highly value (Ashraf et al., 2005). A local wild type (vvIBDV) was successfully adapted for growth on chicken embryofibroblast (CEF) cell culture after thirteenth passage with concurrent loss of pathological imprints in specific pathogen free (SPF) chicks. Adapted virus showed 1:1024 titre at

fifteenth passage with complete loss of pathological lesions (Khan et al., 2007). Successful attempt was made for production of cost effective cultured base vaccine from local strains on chicken embryo fibroblast cells. It is observed that maximum level of immunogenicity was efficiently adapted to grow local IBDV in Vero-cell line within limited passages. The live vaccine prepared from this cell line produced high immunogenicity as compared to other commercially available live cell culture vaccines (Rasool and Hussain, 2006). In this study it pointed out that ISCOM-based IBDV vaccine manufactured from local vvIBDV attenuated Vero cell is more friendly in terms of VN-antibody titres as compared to commercial vaccines. In commercial layers and breeders this type of vaccine will provide prolong immune response (Rasool, 2008).

11. Post mortem

Post-mortem examination showed haemorrhages in the thigh and breast muscles. The lesions in the bursa ranges from flakes of pus to haemorrhages (Khan et al., 1988b). In addition to above said kidneys were also found swollen along with deposition of urates (Khan et al., 2009). It was noted that 20.05% haemorrhages in the bursa and 18.85% were found in thigh muscles. Bursal atrophy was found in 61% where as 11% showed haemorrhages in breast muscles. 89% affected with nephritis and only 4% pictured necrotic liver (Sultana et al., 2008).

12. Diagnosis

Initial diagnosis can usually be made based on flock history, clinical signs and PM examinations. However, definitive diagnosis can only be made by the specific detection and/or isolation and characterization of IBDV. The disease was diagnosed on the basis of clinical signs and postmortem findings first time in Pakistan (Khan et al., 1988a). The level of Abs were calculated. Serum antibodies against infectious bursal disease were detected by IHA (Indirect Hemagglutination) test. It was found that maximum positive sample were between 6 and 8 weeks of age and minimum Abs were present in group below 3 weeks of age. For the characterization of Pakistan isolates, a reverse transcriptase polymerase chain reaction/restriction fragment length polymorphism (RT-PCR/RFLP) technique was used. SspI restriction enzyme was used. By this study it was confirmed that vvIBDV strains were existed in commercial set up. RT-PCR/RFLP is a very useful and rapid method for characterization and identification of existing and evolving strains of IBDV (Zahoor et al., 2011). In this study three immunodetective techniques were compared to assess the best one. For the IBDV detection reverse transcription polymerase chain reaction (RT-PCR), reverse passive haemagglutination assay (RPHA) and agar gel precipitation test (AGPT) were compared by screening suspected virus in bursa of Fabricius, liver, kidneys, spleen and thymus of chicken. It is noted that RPHA were less time consuming and sensitive ones as compared to AGPT. In field conditions RPHA has given economical results in contrast to other techniques. RT-PCR was found more sensitive than other local available techniques (Mahmood and Siddique, 2006; Ali et al., 2017). A strip was developed in Dot-ELISA for quick diagnosis of multiple pathogens in a single kit during field inspections. This diagnostic tool was successful with little false results but its highly specific, very sensitive and less time consuming for trained field workers (Alam et al., 2012). The performance of DOT-ELISA was weighed with agar gel precipitation test (AGPT). The DOT-ELISA showed better sensitivity than diagnostic AGPT. The other edge of DOT-ELISA was that it has ability to detect IBDV at early stage of infection so it was best low cost screening test (Sultana et al., 1999).

13. Therapy

The pathogenic viruses spreads infections in the targeted populations. There is no effective remedy for the viral diseases but by adopting control measures and supportive therapy, the losses can be reduced. Vaccination may not be successful 100% for raising immunity. The multivitamin therapy combined with vaccination showed boosting effects in protection. IBD strain D-78 caused low stress with good protective titre in the birds (Khan et al., 2003). The comparative immunostimulatory studies of two available market products i-e Livol (herbal supplement) and immunotone (selenium and vit E) by ELISA were noted. Livol showed more encouraging results than immunotone to minimize the adverse effect of IBDV vaccine. Different immunogenic products were tried to select the better one available in commercial markets (Arshad et al., 2005; Qayyum et al., 2012; Saleem and Mahmood, 2013; Zahid et al., 2015). It promoted immune status against IBD and result in better feed conversion ratio (FCR) (Mahmood et al., 2014). It was observed that hyperimmunized yolk controlled IBD infection in rural and hybrid birds (Malik et al., 2006). Medicinal plants extracts were used in designed experiments. Immunological and hematological profile of the birds showed that there was significant improve in antibodies titres against IBD. Findings depicts that oral administration enhances immunological profiles (Mushtaq et al., 2012; Muhammad et al., 2017). In this study high molecular mass glycoproteins (HMMGs) derived from sugar cane (*Saccharum officinarum*) juice were tried. It was found that it had immunostimulatory response of both cellular and humoral aspects on growth rates in commercial broiler. HMMGs were extracted from sugar cane juice by size exclusion chromatography (Awais et al., 2013; Raziq et al., 2012).

14. Conclusion

In Pakistan during last forty-three years most of work was done on incidence, prevalence and epidemiology of IBD. In initial stages disease was traced on the basis of clinical signs and post mortem lesions. During intermediate period histopathology confirms the changing culprit. The other diagnostic tools were IHA, AGPT, different kinds of ELISA, CCIE, egg infective dose fifty (EID)50, RPHA, and RT-PCR/RFLP. The second largest aspect was use of immunostimulants to combat immunosuppression. Immunoboosters may be natural or synthetic in nature. The sophisticated work of virology required huge funding so this aspect was not touched up to the required levels. Although different characters of IBDV were studied in cell culture and embryonated eggs. For preparation of local IBD vaccine trials were proved quite successful. But final products were not commercialized due to many reasons. Pakistan is still spending huge revenue for importing IBD vaccine. Imported vaccines are not providing more than 80% protection. The developed countries are working on novel aspects along with handsome knowledge of continuously circulating local strains. Production of successful IBD vaccine based upon native viruses is the only cost effective tool to save major losses from this immunosuppressive disease.

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