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REVIEW ARTICLE

The Application of Immunohistochemistry as a Research Technique in Veterinary Medical Schools in Nigeria

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SUMMARY

Immunohistochemistry involves the use of commercially prepared colour labelled antibodies to detect the presence and distribution of specific cellular proteins known as antigens. It is a diagnostic and research technique employed in medicine, but may have limited veterinary medical application in Nigeria. The present paper accessed the level of awareness and application of immunohistochemistry as a research technique among staff of the veterinary medical schools in Nigeria. As at the time of the study, ten veterinary schools were accredited by the Veterinary Council of Nigeria, thus used for the analysis. Respondents to a structured questionnaire from the veterinary schools included laboratory technologists and academic staff of the departments of veterinary anatomy, pathology and public health. Design of the questions progressed from mere awareness to theoretical and practical knowledge of the technique. Although 93.33 % of respondents who are academic staff were aware of the technique, and an equally high percentage of those aware of the technique have varying levels of theoretical knowledge, only 32.65 % of these respondents have practical experience of immunohistochemistry technique. Similarly, among the laboratory technologists who were aware of the technique, only 34.78 % have actively conducted it. Respondents of the veterinary anatomy specialty had the highest percentage of practical knowledge. Results also indicated that there was only one veterinary medical school with a functional immunohistochemistry laboratory in Nigeria. The paper recommended, among other things, the introduction of immunohistochemistry as a practical course in the curriculum of the veterinary schools in Nigeria.

Key words: Immunohistochemistry; Nigerian veterinary schools; NUC.

INTRODUCTION

Early histology and histopathology based research was based on staining tissue sections with conventional stains such as Haematoxylin and Eosin stain. This procedure reveals cells such as epithelial and connective tissue cells. A later technique, the method of histochemistry evolved, which depicts chemical composition of tissues such as the presence or absence of carbohydrates (Esteban, 2012). Thereafter, immunohistochemistry evolved as a method that visualizes the localization and distribution of specific antigen or cellular components in separated tissues, or tissue sections. Compared to other bio-techniques that are based on the antigen-antibody

reaction such as immunoprecipitation, immunohistochemistry provides *in-situ* information, without digesting the tissue, thus, providing a more convincing experimental result. Also, the advantage of immunohistochemistry method of antigen detection is that the tissue section can be examined with a standard light microscope unlike the immunofluorescence based detection which requires mercury lamps, dichroic mirrors and special filters for the excitation of the fluorochrome and visualization of the emitted fluorescence (Gershwin, 2008). Immunohistochemistry does not only describe the morphology of the cell but also defines its function based on the type of the cellular protein detected.

Immunohistochemistry is a diagnostic and research technique that combines anatomy, physiology, immunology and biochemistry. It is highly sensitive and specific and can detect a wide variety of antigens in multiple animal species. The technique is employed in disease diagnosis, drug development and biological research. By using commercially prepared colour labelled antibodies, immunohistochemistry is used to diagnose a cancer as benign or malignant and determine the stage of the tumour (Jiang *et al*., 2003). It is also used to identify cell type and origin of a metastasis (Duraiyan *et al*., 2012). Immunohistochemistry is used as a predictor for treatment outcome (Ramos-Vara and Miller, 2014). It is also employed in the diagnosis of infectious diseases, neurodegenerative disorders, and brain traumas (Duraiyan *et al*., 2012). The direct, rapid immunohistochemical test for rabies employs the principle of immunohistochemistry in veterinary public health (Banyard *et al*., 2013).

The principle of immunohistochemistry was first reported in 1942 by scientists who used antibodies to identify Pneumococcal antigens in infected tissue (Goswami *et al*., 2012). Since then, the technique has been progressively improved upon such that it has now become an important technique in diagnostic and research laboratories. The principle is based on the antigen-antibody reaction; specific primary antibody binds to specific antigen; the antibody-antigen complex is formed by incubation with a secondary enzyme-conjugated antibody; in the presence of a chromogen substrate, the enzyme catalyses the substrate to generate coloured deposits at the sites of antibody-antigen binding. The colour deposit indicates the presence of the antigen in the tissue (Gershwin, 2008).

Tissue section for immunohistochemical technique can be preserved by freezing or using an aldehyde-containing fixative, such as 10 % neutral buffered formalin. When aldehyde-containing fixative is used to preserve tissue, methylene bridges are formed during the fixation, which cross-link proteins within the tissue sample thereby masking the antigenic sites (Gershwin, 2008). This extensive cross-linking can mask the protein epitope that specific antibody would normally recognize, thus the need for an antigen retrieval solution. The solution works by causing a reversal or unfolding of proteins within the tissue, by breaking the cross-links, leading to unmasking of the epitope, allowing the antibody to successfully bind. Both the Heat Induced Epitope Retrieval (HIER) and the Proteolytic Induced Epitope Retrieval (PIER) methods are routinely employed in immunohistochemistry. The former involves immersing the charged glass slide with tissue section into a HIER buffer, such as citrate or tris-EDTA buffer, and subjecting it to microwave heat for a given period of time at a specific pH. The later uses enzyme digestion instead of heat. However Kahveci *et al*. (2003) emphasised that microwave retrieval is easier to use and produces better result than the enzyme digestion method, and that the enzyme digestion method may destroy some epitopes and tissue morphology, thus should be employed only

when HIER is without the desired result. The application of antigen retrieval method in immunohistochemistry was discovered by Huang *et al*. (1976). It was then that formalin fixed, paraffin embedded tissue samples were successfully used in immunohistochemistry.

The commercially prepared antibody used in immunohistochemistry could be a polyclonal or monoclonal antibody. The former involves the mixture of different antibodies to a single antigen (Dalçık and Dalçık, 2012). Such an antibody is used for detection of a large complex antigen with multiple epitopes that elicit several antibody types. Conversely, a monoclonal antibody is specific for a single epitope, produced by a single clone, and commonly raised in mouse, rabbit and rat (Carpenter, 2014). Polyclonal antibodies are quicker and simpler to obtain than the monoclonal antibodies. The major disadvantage of the polyclonal antibody is the heterogeneous nature (Dalçık and Dalçık, 2012). The major advantage of a monoclonal antibody over the polyclonal antibody is its mono-specificity as it binds to a single epitope. Conversely, it has a lower sensitivity and more difficult to generate than polyclonal antibody. The binding of the commercially prepared antibody to the epitope of the antigen in the tissue section is localized under the light microscope by the activity of enzymes which serve as immunolabels. In direct Immunolocalization, the label is conjugated to the primary antibody. The common label used in direct Immunolocalization is fluorescein. In indirect immunolocalization, a secondary antibody is used as a bridge between the primary antibody and the label. Thus, the enzyme label is conjugated to the secondary antibody. The secondary antibody must be specific to the primary antibody, to which it binds. Enzyme labels used in secondary immunolocalization include horseradish peroxidase (PAP), avidin-biotin peroxidase complex (ABC) and Staph Protein A (Matos *et al*., 2010)

Some cells contain endogenous peroxidase, whose activity must be inhibited for a successful immunohistochemical analysis of such cells. This is due to the fact that immunohistochemical analysis with peroxidase (PAP or ABC) conjugated secondary antibody results in high, non-specific background staining of cells that contain endogenous peroxidase (Ramos-Vara and Miller, 2014). This non-specific background can be significantly reduced by pre-treatment of the cells with hydrogen peroxide (H_2O_2) prior to incubation with the peroxidase conjugated secondary antibody (Ramos-Vara and Miller, 2014). If such pre-treatment aimed at inactivating the endogenous peroxidase is ignored, the endogenous peroxidase remains active and when reacted with the peroxidase conjugated secondary antibody, non-specific colouration will result, thus masking the expected specific staining. The pre-treatment of tissue with H_2O_2 results in the endogenous peroxidase catalysing the reduction of H_2O_2 to water (H_2O) and oxygen (O_2). This is an irreversible reaction that eliminates the endogenous peroxidase in the tissue. Such tissue can thereafter be incubated in a peroxidase conjugated antibody. According to Ramos-Vara and Miller (2014), the best way to know if a tissue contains endogenous peroxidase activity is to incubate the tissue section with a chromogen dye such as 3,3-diaminobenzene (DAB) substrate solution after rehydration. If it turned brown, the tissue contains endogenous peroxidase and a blocking step (i.e. pre-treating with H_2O_2) is needed. The principle behind the test is that the endogenous peroxidase will catalyse the oxidation of the DAB substrate and the oxidised product is a brown precipitate.

Adepoju (2008) reported the absence of immunohistochemical technique in most Nigerian laboratories. Furthermore, the

application of the technique in veterinary research may be limited in Nigeria. To this end, the present paper was designed to provide information on the level of appreciation of the research technique among laboratory technologists and academic staff in Nigerian veterinary schools. Results of the present study will be beneficial in decision making on ways to improve veterinary teaching and research, by the Nigerian regulatory agencies such as the National Universities Commission (NUC) and the Veterinary Council of Nigeria (VCN). Some probable reasons for the level of application of the technique as discovered in the present study were discussed and recommendations made to the appropriate authorities.

MATERIALS AND METHODS

As at the time of the study, veterinary faculties/colleges in 10 Nigerian universities were accredited by the Veterinary Council of Nigeria. These include Ahmadu Bello University Zaria, Michael Okpara University of Agriculture Umudike, University of Abuja, University of Agriculture Abeokuta, University of Agriculture Makurdi, University of Ibadan, University of Ilorin, University of Maiduguri, University of Nigeria Nsukka and Usman Danfodio University Sokoto. A close-ended questionnaire was distributed to laboratory technologists and academic staff of the departments of veterinary anatomy, pathology and public health in the 10 universities. There was no form of selection of respondents in any of the departments. Thus, all academic staff and laboratory technologists from the three departments who were willing to provide the needed information constituted the respondents.

The design of the questions progressed from mere awareness to theoretical and practical knowledge of immunohistochemistry as a research technique. Theoretical knowledge was graded from low to moderate and high knowledge, while practical knowledge ranged from observing the technique to actively performing it. Information on the frequency of active performance, ranging from once to twice, thrice and several times was obtained. Also, the level of awareness, theoretical and practical knowledge of the technique among the two categories of respondents was assessed according to the number of years of research experience. The respondents were grouped according to the years of research experience thus Group A: 0-5 years; Group B: 6-10 years; Group C: 11-15 years; Group D: 16-20 years; Group E: 21 years and above.

The respondents were asked if immunohistochemistry was taught as a course or topic in any course at undergraduate or postgraduate level. Information on the practice of the technique in the study departments or any other department in the veterinary school was obtained from the respondents. Lastly, the respondents were asked of their view on the introduction of immunohistochemistry as a research technique in their departments.

The data obtained was subjected to descriptive statistics using GraphPad Prisms version 4 for Windows 8. The values were expressed in absolute and relative terms and presented in tables and graphs.

RESULTS

Table I is a summary of the number of respondents from veterinary medical schools in Nigeria. A total of 135 respondents, including 105 academic staff and 30 laboratory technologists were obtained. Out of the 135 respondents, 57 were of the veterinary anatomy department, 42 were of the veterinary pathology and 36 were of the veterinary public health and preventive medicine departments.

TABLE I: Distribution of respondents from Nigerian Veterinary Schools

UNIVERSITY	STATUS	NUMBER OF RESPONDENTS		
		Veterinary Anatomy Department	Veterinary Pathology Department	Veterinary Public Health & Preventive Medicine Department
Ahmadu Bello University Zaria	AS	7	3	5
	LT	3	1	3
Michael Okpara University of Agriculture Umudike	AS	5	4	4
	LT	2	1	1
University of Abuja	AS	1	2	3
	LT	1	1	1
University of Agriculture Abeokuta	AS	5	5	3
	LT	0	1	1
University of Agriculture Makurdi	AS	7	3	2
	LT	1	0	2
University of Ibadan	AS	3	3	3
	LT	2	2	2
University of Ilorin	AS	2	2	1
	LT	1	1	0
University of Maiduguri	AS	4	5	1
	LT	0	1	0
University of Nigeria Nsukka	AS	8	5	3
	LT	1	0	1
Usman Danfodio University Sokoto	AS	4	2	0
	LT	0	0	0
Total Number of Respondents		**57**	**42**	**36**

AS: Academic Staff; LT: Laboratory Technologist/Scientists

Awareness of Immunohistochemistry Research Technique

A total of 98 academic staff, representing 93.33 % of respondents in the group were aware of immunohistochemistry research technique, while 7 academic staff, representing 6.67 % of respondents in the group were not aware of the technique. Similarly, a total of 23 laboratory technologists, representing 76.67 % of respondents in the group were aware of the research technique, while 7 laboratory technologists, representing 23.33 % of respondents in the group were not aware of the technique (Figure 1).

A total of 25 academic staff with 0 to 5 years of research experience, representing 92.59 % of respondents in group A were aware of immunohistochemistry research technique (Table II). As the duration of research experience advanced from group A to D, there was an increase in the number of academic staff aware of the technique, relative to those not aware, such that 100 % of academic staff in groups C and D were aware of the technique (Table II). A total of

TABLE II: Distribution of the awareness of immunohistochemical technique among Academic Staff and Laboratory Technologists in Nigerian veterinary schools according to duration of research experience

Duration of Research Experience (Years)		**Academic Staff** (n = 105)		**Laboratory Technologists** (n = 30)	
	Awareness of IHC	Absolute Number	Relative Number (%)	Absolute Number	Relative Number (%)
A (0-5)	+	25	92.59	1	100.00
	-	2	7.41	0	0.00
B (6-10)	+	38	92.68	7	77.78
	-	3	7.32	2	22.22
C (11-15)	+	6	100.00	5	83.33
	-	0	0.00	1	16.67
D (16-20)	+	5	100.00	2	66.67
	-	0	0.00	1	33.33
E (≥ 21)	+	22	95.65	4	80.00
	-	1	4.35	1	20.00
F (Unknown duration)	+	2	66.67	4	66.67
	-	1	33.33	2	33.33

+ represents respondents that were aware of the research technique
- represents respondents that were not aware of the research technique

22 academic staff with over 20 years of research experience, representing 95.65 % of respondents in group E were aware of immunohistochemistry research technique (Table II). Only 1 laboratory technologist, out of the 23 laboratory technologists who were aware of immunohistochemistry belonged to group A with 0-5 years of research experience (Table II). As the duration of research experience advanced from group A to C, there was an increase in the number of laboratory technologists aware of the technique, relative to those not aware (Table II). However, only 66.67 % of laboratory technologists in group D were aware of the technique, a value less than the 83.33 % for group C (Table II). A total of 4 laboratory technologists with over 20 years of research experience, representing 80 % of respondents in group E were aware of immunohistochemistry research technique (Table II).

Knowledge of Immunohistochemistry Research Technique

Theoretical Knowledge

The distribution of theoretical knowledge of immunohistochemistry research technique among academic staff and laboratory technologists from the study departments in Nigerian veterinary schools was represented in Figure 2. The graph represented a near-normal distribution for both the academic staff and laboratory technologists; respondents with moderate theoretical knowledge were represented by the highest percentage, while those with no and high theoretical knowledge were represented by lower percentages (Figure 2).

A total of 7 academic staff, representing 7.14 % of those who were aware of the immunohistochemistry research technique (98 respondents) lack any theoretical knowledge (Figure 2). Also, 18 academic

staff, representing 18.37 % of those who were aware of the technique claimed to have high theoretical knowledge. This value was lower than that of the academic staff of low theoretical knowledge (27.55 %) and the academic staff of moderate theoretical knowledge (46.94 %).

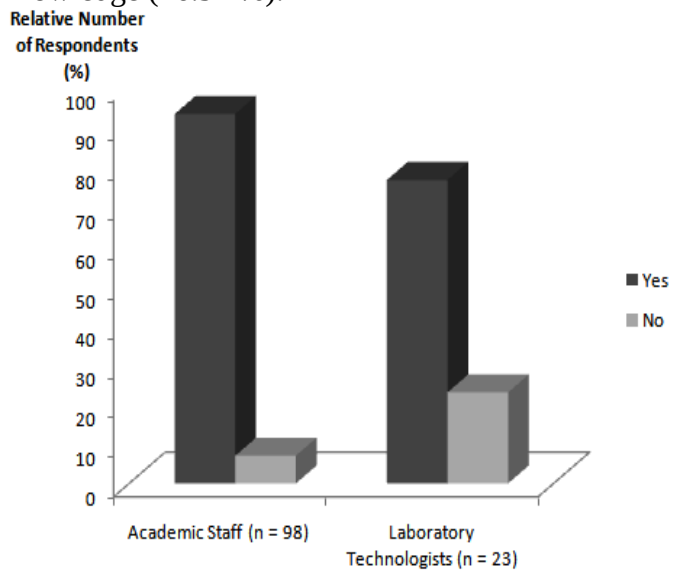

Figure 1: Distribution of the awareness of immunohistochemical technique among laboratory technologists and academic staff in Nigerian veterinary schools

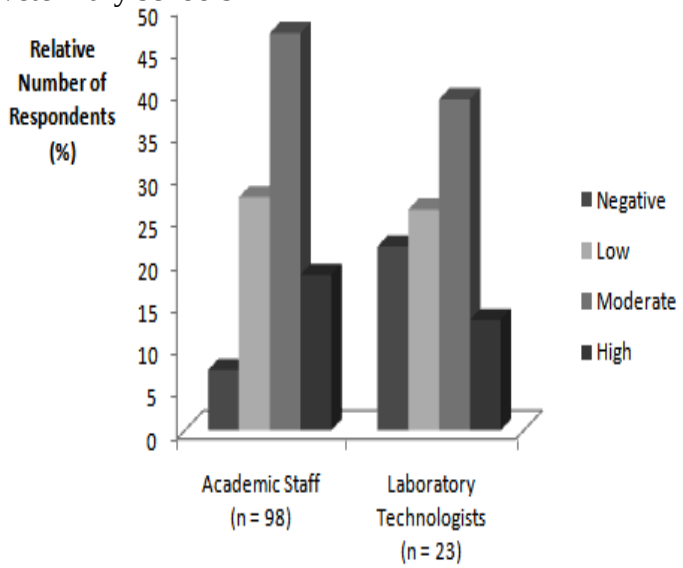

Figure 2: Distribution of theoretical knowledge of immunohistochemical technique among academic staff and laboratory technologists in Nigerian veterinary schools

A total of 5 laboratory technologists, representing 21.74 % of laboratory technologists who were aware of the research technique (23 respondents) lack any theoretical knowledge. Also, 13.04 % claimed to have high theoretical knowledge, while those of low and moderate theoretical knowledge were 26.09 % and 39.13 %, respectively (Figure 2).

Practical Knowledge

A total of 47 academic staff, representing 47.96 % of those aware of immunohistochemistry, claimed to have observed the procedure, while the remaining 52.04 % have never witnessed the procedure being conducted anywhere. Among the laboratory technologists that were aware of the technique, 10 respondents, representing 43.48 %, claimed to have observed the performance of the technique and 56.52 % have not witnessed the procedure. While some of these respondents who have observed the procedure have actively conducted it themselves, others who have observed it claimed never to have practiced the technique.

The frequency of conducting the research technique among the two groups in the study departments was represented in Figure 3. A total of 66 academic staff, representing 67.35 % of those aware of the research technique have not conducted the technique. Among the remaining 32.65 % that have actively conducted the technique, 3.06 % did it once, 6.12 % did it twice, 3.06 % did it thrice and 20.41 % did it several times. Similarly, a total of 15 laboratory technologists, representing 65.22 % of those aware of the research technique have never conducted the technique. While 17.39 % have conducted the

technique once, 4.35 % have conducted it twice and 13.04 % have conducted it several times. None of the laboratory technologists have conducted the technique for only 3 times.

The distribution of practical knowledge of immunohistochemistry research technique according to duration of research experience among the academic staff and laboratory technologists who are aware of the technique is represented in Table 3. Only 3.70 % of the academic staff in group A who were aware of the research technique, have conducted it, while 96.30 % have never conducted the technique. As the duration of research experience increased from group A to D, there was a reduction in the number of academic staff who have not conducted the research technique, from 96.30 % to 40.00 %. Thus, more staff were exposed to the practical knowledge as duration of research experience progressed from 5 to 20 years (Table III). However, 60.87 % of academic staff with over 20 years of research experience (group E) have not conducted the research technique. Only 1 out of the 23 laboratory technologists who were aware of immunohistochemistry research technique belonged to group A which had 0-5 years of research experience, and has conducted the technique severally (Table III). Converse to the result of the academic staff, there was an increase in the number of laboratory technologists who have never conducted the technique as the duration of research experience progressed from group A to D. This was such that 100 % of laboratory technologists with 16-20 years research experience have never conducted the technique. Also, 80.00 % of laboratory technologists with over 20 years of research experience (group E) have not conducted the research technique.

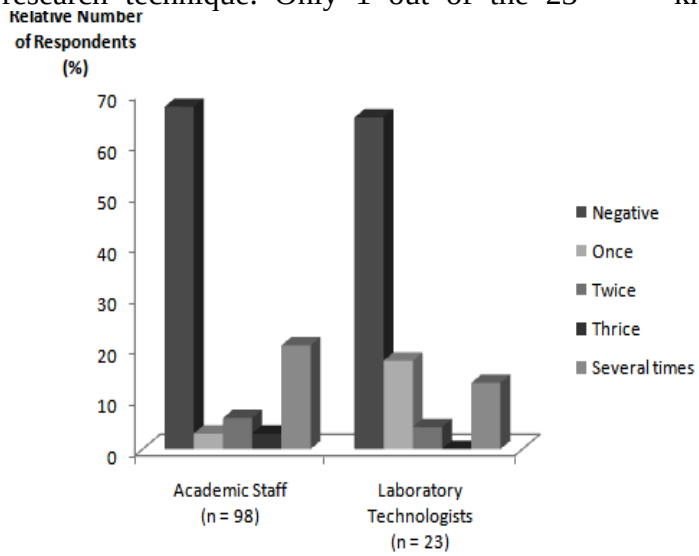

Figure 3: Distribution of practical knowledge of immunohistochemical technique among academic staff and laboratory technologists in Nigerian veterinary schools

The distribution of practical knowledge of immunohistochemical technique among staff in Nigerian veterinary schools according to research specialty was represented in Figure 4. Respondents (both academic staff and laboratory technologists) in the Veterinary Anatomy specialty have the highest percentage of practical knowledge of immunohistochemistry. This was followed by respondents of the Veterinary Pathology specialty. Respondents of the Veterinary Public Health and Preventive Medicine specialty have not actively participated in conducting the technique as those of the other two specialties.

Teaching of Immunohistochemistry

Respondents from 3 veterinary schools accepted that immunohistochemistry is taught at postgraduate level, while 1 out of the 3 veterinary schools also introduces the technique at undergraduate level. Two out of the 3 veterinary schools teach the technique theoretically only, while 1 veterinary school teaches the

TABLE III: Distribution of practical knowledge of immunohistochemical technique among Academic Staff and Laboratory Technologists in Nigerian Veterinary Schools according to duration of research experience

		Academic Staff		Laboratory Technologists	
Duration of Research Experience (Years)	**Practical Knowledge of IHC**	**Absolute Number**	**Relative Number (%)**	**Absolute Number**	**Relative Number (%)**
A (0-5)	-	26	96.30	0	0.00
	+	0	0.00	0	0.00
	++	0	0.00	0	0.00
	+++	1	3.70	0	0.00
	>+++	0	0.00	1	100.00
B (6-10)	-	29	70.73	7	77.78
	+	0	0.00	1	11.11
	++	1	2.44	0	0.00
	+++	0	0.00	0	0.00
	>+++	11	26.83	1	11.11
C (11-15)	-	3	50.00	5	83.33
	+	1	16.67	0	0.00
	++	0	0.00	0	0.00
	+++	0	0.00	0	0.00
	>+++	2	33.33	1	16.67
D (16-20)	-	2	40.00	3	100.00
	+	1	20.00	0	0.00
	++	1	20.00	0	0.00
	+++	0	0.00	0	0.00
	>+++	1	20.00	0	0.00
E (≥ 21)	-	14	60.87	4	80.00
	+	1	4.35	1	20.00
	++	2	8.70	0	0.00
	+++	2	8.70	0	0.00
	>+++	4	17.39	0	0.00
F (Unknown duration)	-	3	100.00	5	83.33
	+	0	0.00	0	0.00
	++	0	0.00	1	16.67
	+++	0	0.00	0	0.00
	>+++	0	0.00	0	0.00

- represents respondents that have never practiced the research technique
+ represents respondents that have practiced the research technique once
++ represents respondents that have practiced the research technique twice
+++ represents respondents that have practiced the research technique thrice
>+++ represents respondents that have practiced the research technique several times

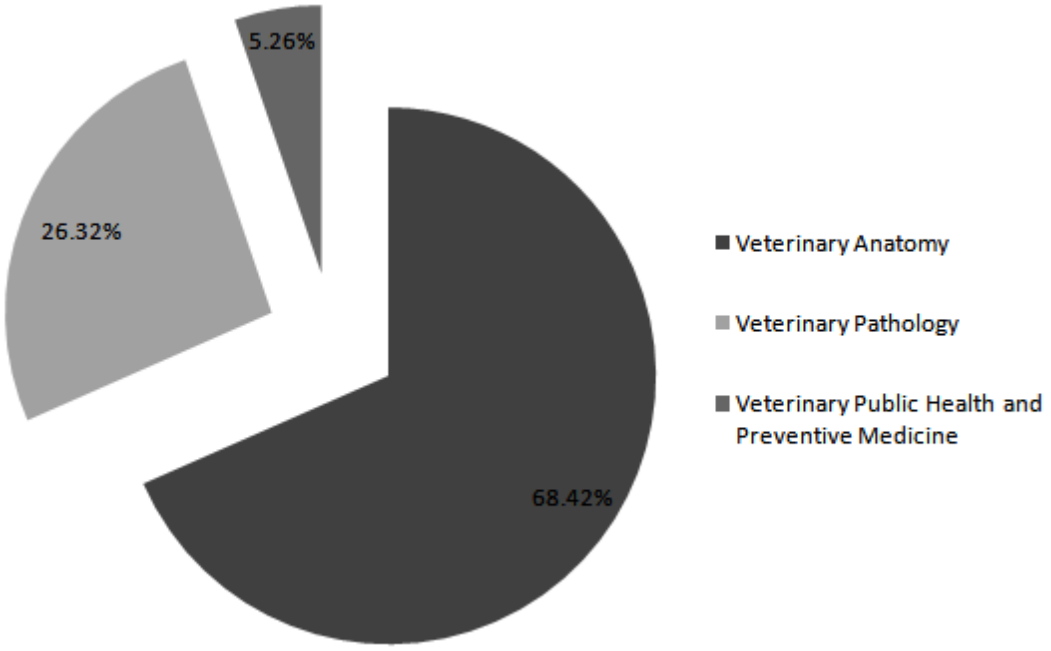

Figure 4: Distribution of practical knowledge of immunohistochemical technique among staff in Nigerian veterinary schools according to research specialty

technique both theoretically and practically, as the school has a functional immunohistochemistry laboratory. The remaining veterinary schools do not teach the technique at either undergraduate or postgraduate level.

Practical Demonstration of Immunohistochemical Technique

Respondents from one veterinary school unanimously reported the existence of an immunohistochemistry laboratory in their veterinary anatomy department. These respondents reported that the technique is practically demonstrated in their veterinary school. Respondents from the other veterinary schools do not conduct the technique in any of the three study departments, as they lack an immunohistochemistry laboratory.

DISCUSSION

Veterinary research is currently on the increase with the present technological advances. Immunohistochemistry has been established as a solid and reliable methodology for both routine diagnostic and research activities in veterinary medicine Ramos-Vara, 2005). The present paper has attempted to discuss the principle of immunohistochemistry, and assess its application among academic staff and laboratory technologists in Nigerian veterinary schools. The choice of respondents employed in the present study was based on the fact that the study departments (Veterinary Anatomy, Veterinary Pathology and Veterinary Public Health) are expected to use immunohistochemistry as a research tool. Thus, academic staff and laboratory technologists of the three sampled departments served as the sample for the population of academic staff and laboratory technologists in Nigerian veterinary schools directly linked to the use of the technique as a research tool.

The respondents who were not aware of the research technique definitely had no theoretical or practical knowledge. This category of respondents constituted 6.67 % and 23.33 % of all academic staff and

laboratory technologists, respectively. Although the values are not alarming, still, it is worrisome that in a fast developing research oriented veterinary world, some academic staff and laboratory technologists in Nigerian veterinary schools, especially those in the departments directly involved in the use of the technique, have never heard or read of such research technique. Some respondents who were aware of the technique but lack any form of theoretical knowledge constituted 7.14 % and 21.74 % of academic staff and laboratory technologists, respectively. These respondents were opportune to have heard of the technique mentioned by colleagues, maybe in a discussion forum or come across it in articles, but have not been exposed to any knowledge of the technique. Again, this is of immense concern.

Result of the present study indicated that the percentage of veterinary laboratory technologists and scientists who are not aware of the technique (23.33 %) was quite higher than that of the academic staff (6.67 %). Similarly, only 34.78 % of the laboratory technologists who are aware of the technique have practical experience, while 65.22 % have never been involved in the practical. It is pertinent to note that immunohistochemistry is a laboratory technique, expected to be conducted by laboratory technologists and scientists. Most of the respondents with practical knowledge also testified to the fact that they were trained by laboratory technologists and scientists who conduct the technique in the laboratory. Thus, it was expected that almost all the veterinary laboratory technologists will be aware of the technique and a higher percentage should have good command of practical knowledge. Rather, only 34.78 % have practical knowledge. This implies that the application of immunohistochemistry research technique among veterinary surgeons in veterinary medical schools in Nigeria is at its barest minimum. The higher theoretical knowledge relative to practical knowledge implies that veterinary surgeons and laboratory technologists in Nigeria devote more attention to knowing the technique by reading or listening to experts' exposition, with little efforts on the application.

There was no much difference between the number of academic staff of groups A (0-5 years of research experience) and E (≥ 21 years of research experience) who were aware of immunohistochemistry research technique. However, a higher number of academic staff in group A lacked any practical experience, compared to those in group E (96.30 % vs. 60.87%). It was gathered that almost all the academic staff in group E who were aware of immunohistochemistry obtained the practical experience from universities in developed countries. This experience, they gained during their postgraduate study or post-doctorate fellowship more than a decade ago. This further buttress the fact that the research technique is not new to the academic world of developed countries, unlike in Nigeria. The high number of those without practical experience recorded in group A, than in group E, explains the fact that the practice of the technique in Nigeria is at its barest minimum. Thus, academics with up to 5 years of research experience lack the practical exposure. The few academic staff in group A that claimed to have practical knowledge either gained it from institutions in other countries, human teaching hospitals in Nigeria, or from the only laboratory that conducts the procedure in one of the veterinary schools in Nigeria.

There are a number of probable reasons for the very low application of immunohistochemistry as a research technique in veterinary medical schools in Nigeria. Since a very high number of academic staff were aware of the research technique, the very low percentage of practicability among them can be attributed

to lack of immunohistochemistry laboratory in the study departments, lack of specific antibodies and high cost of the available antibodies, poor technical know-how among the two groups of respondents and lack of appreciation of the need for immunohistochemistry as a basic research technique.

Information gathered from the heads of anatomy departments in the veterinary medical schools in Nigeria indicated the existence of a histology laboratory. The equipment used for tissue processing in a histology laboratory can be used for immunohistochemistry, with the inclusion of other affordable equipment specific for immunohistochemistry. Also, immunohistochemistry slides can be examined with the same light microscope used in studying basic histology slides. Thus, it is possible to upgrade the histology laboratories in the veterinary anatomy departments to histology and immunohistochemistry laboratory.

The high cost of antibodies used for tissue analysis in immunohistochemistry in Nigeria cannot be over emphasised. This is partly due to the cost of isolating the antibodies from the animal species, and partly due to the fact that most of these antibodies are produced elsewhere and imported into the country. Also, it could be attributed to the fact that the demand for the antibodies is very low, as the technique is not highly appreciated in Nigeria, thus, in their quest to recoup the cost of production in a low turn-over income, the producers place high price on the antibodies. Furthermore, as the technique is more practical and widely used in human diagnosis, most of the commercially prepared antibodies are specific to human tissues. Ruiz *et al*. (2005) noted that the lack of specific antibodies for animal tissues have limited the use of immunohistochemistry in veterinary pathology, unlike in human pathology. This may reduce the practical application of immunohistochemistry in veterinary pathology.

The very low technical know-how of the research technique among the two groups of respondents is expected, following the information gathered, that only one veterinary school has a functional immunohistochemistry laboratory in Nigeria. Answers gathered from the respondents showed that most of the respondents from the veterinary school that has the laboratory, and from a nearby veterinary school, have actively conducted the technique several times. Academic staff from these two veterinary schools contributed immensely to the 20.41 % that have carried out the technique several times, a value that is more than halve of the 32.65 % of academic staff that have carried out the technique in all the 10 veterinary schools. The remaining academics that made up this 32.65 % have not carried out the technique more than three times. Also, all the laboratory technologists with high theoretical knowledge of immunohistochemistry where staff of the veterinary medical school with a functional immunohistochemistry laboratory in Nigeria. The very low technical know-how can also be attributed to the non-teaching of immunohistochemistry at either undergraduate or postgraduate level in most of the veterinary medical schools in the country. Only 3 veterinary medical schools in Nigeria teach immunohistochemistry at postgraduate level while 1 school also introduced it at undergraduate level. This result is below average, following the number of accredited+ veterinary schools in the country.

CONCLUSION

Although immunohistochemistry is a routine diagnostic procedure for disease conditions such as cancer, it has not gained a solid stance as a routine research technique in veterinary histology and histopathology. The

authors are optimistic that the introduction of immunohistochemistry as undergraduate and postgraduate course in Nigerian veterinary schools will avail students more knowledge and reasons to inculcate the technique in research.

RECOMMENDATIONS

From the results obtained in the present study, the following recommendations were conferred

1. University administrations should ensure the establishment of immunohistochemistry laboratory in their veterinary medical schools or upgrade the histology or histopathology laboratories to also conduct immunohistochemistry.
2. The National Universities Commission should advice the Government on the need to provide enabling atmosphere for private sector to set companies for the manufacture of reagents and antibodies used in immunohistochemistry.
3. The National Universities Commission should include immunohistochemistry as a core theory and practical course at both undergraduate and postgraduate levels in veterinary medical schools.
4. Academic staff and laboratory technologists that specialize in histology, histopathology and public health should be encouraged to engage in trainings and workshops on the use of immunohistochemistry as a research technique.

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