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A TRIAL TO DETERMINE AGE OF WOUND AFTER PLASTIC MARK APPLICATION IN COWS AND BUFFALOE'S EARS (With 16 Figs.)

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دراسة لتقدير عمر الجرح الناتج عن استخدام العلامات البلاستيكية
في أذن الأبقار والجاموس

عادل شحاتة ، سهير راشد ، ثابت عبد المنعم ، مجدي سليمان

يعد مشروع التأمين على الماشية من دعائم تشجيع التنمية في مجال الثروة الحيوانية بما يقدمه من رعاية صحية وغذائية وصرف التعويضات المناسبة في حالة نفوق الحيوانات الأمر الذي دفع بالعديد من المنتفعين بالصندوق الي التحايل لابتراز أموال الصندوق بطرق غير مشروعة كاستبدال الحيوانات أو نقل علامات التأمين من حيوان إلى آخر أو وضعها في حيوانات نافقة غير مؤمن عليها رغبة في الحصول على تعويض غير مستحق ويرتبط التحصين دائما بالتأمين ثمتى نفق الحيوان في فترة رد الفعل أجاز الصندوق للمؤمن التعويض كاملا . وتهدف هذه الدراسة الي محاولة حل المشاكل التي يتعرض لها الطبيب البيطري في الحفل للفصل في هذه الحالات . وقد أجري هذا البحث لدراسة التغيرات الباثولوجية المصاحبة لالتئام الجرح الناتج من استخدام العلامات البلاستيكية الخاصة بالتحصين والتأمين في أذن الماشية وعلاقتها بالفترة الزمنية المنقضية من استخدامها . أجريت هذه الدراسة على ١٢ من الجاموس ومثيلها من الأبقار (١٢) المتواجدة بالمستشفى البيطري جامعة أسيوط وقد تم وضع علامتين احداها للتأمين والأخرى للتحصين في كل أذن من آذان الحيوانات جميعها . وتم أخذ عينات من النسيج المحيط بالعلامات جراحيا باستخدام عقار الزيلازين المهدئي بعد ٢٤ ، ٤٨ ، ٩٦ ساعة ، ١ ، ٢ ، ٣ ، ٤ ، ٦ ، ٨ أسابيع وثلاثة وستة شهور من استخدام هذه العلامات . وقد أظهرت نتائج البحث عن وجود علاقة بين التغيرات الباثولوجية ووقت تركيب العلامات مالم يحدث عدوى ثانوية موضعية كما أوردت النتائج اختلاف تداعي هذه التغيرات باختلاف نوعية العلامات ونوع الحيوان . وتعد النتائج التي تم تسجيلها في هذا البحث مرجعا مبدئيا يمكن الاستعانة به في حسم الحالات المتنازع عليها .

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SUMMARY

The sequence of the healing process was studied in 24 cows and buffaloes after application of both insurance and vaccination plastic marks. It had been observed that, during the first three days (24-96 hrs), the most important histopathological changes were haemorrhage, oedema, necrosis and leucocytic infiltration mostly of neutrophil cell type. One week after application of the mark, healing was started in dermis with formation of vascular connective tissue (which was mostly infiltrated with inflammatory cells). Two weeks after application of the mark, regeneration of the epithelium was started. Three weeks after application of the mark, healing of severed auricular cartilage was observed. Complete healing of the dermis and epidermis was observed in cows, four weeks after application of the marks. However in buffaloe epithelization was incomplete at this stage. Six weeks after application of the mark, healing with complete epithelization was observed in both buffaloes and cows. Eight weeks up to six months, connective scar become avascular and contracted, the area was depressed under the surface and was devoid of adnexal structure (hair follicles, sweat and sebaceous glands).

It was concluded that, study the sequence of changes of healing process occurring in the ear of buffaloe and cows after application of the vaccination or insurance mark may be of great help in determination of the time elapsed after its application.

INTRODUCTION

The type of tissue is considered an important factor from those influencing the process of healing (CTRUKOF and CEROF, 1979). The external ear consists of the external auricular appendage (auricula) with its cartilage covered by skin, its muscles, and the external auditory meatus which is supported by the cartilage and surrounded by epidermis richly supplied with sebaceous glands and specialized apocrine glands (ceruminous glands). The external ear is limited in its deepest aspect by the tympanic membrane. The anatomic details (such as size and position of the auricular appendage, depth and course of the external auditory meatus) in each species are different (JONES and HUNT, 1983).

Repair of tissues involves both fibrous reconstruction and hyperplastic regeneration (SCHILLING, 1968). Although amphibian cartilage is regenerated in a manner resembling histogenesis of cartilage, BLOOM and FAWCETT (1975), WALTER and ISRAEL

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(1979) reported that, regeneration of cartilage is generally poor. The histologic, ultra-structural and histochemical structure as well as the process of chondrogenesis of cartilage was fully studied (SEARLS, 1973; JUNGNEIRA and CORNEIRO, 1980). Healing and regeneration of surgically created superficial and deep traumatic lesions involving the auricular cartilage in dogs were studied by BAYOUMI, *et al.* (1984). The process of healing was fibrous tissue substitution followed by metaplasia to cartilage (FROST, 1979). Such fibrous tissue originate from the multiplication of undifferentiated connective tissue cells of the perichondrium and to a lesser extent of the endostium (JONES and HUNT, 1983).

Concerning the veterinary medical authorities complain, from the adulteration caused by some owners, as they substitutes non vaccinated or non insured dead animal instead of vaccinated or insured one for obtaining the substituant value of insurance.

The present study was carried out to resolve those adultration by investigating the healing process occur in cattl's and buffaloe's ears in relation to time elapsed after application.

MATERIAL and METHODS

In 12 cattle and 12 buffaloes both vaccination and insurance plastic marks were applied in the tip of the two ear of the same animal.

After different periodes (24, 48, 96 hrs., 1, 2, 3 and 4 weeks, 2, 3, 4, 5 and 6 months) the marks with the surrounded skin and conical cartilage pieces were excised under the effect of xylazine in recumbent position. After removal of the skin and cartilage specimens including the marks, the skin edges over and under the ear cartilage were coaptated with No. 1 silk, in interrupted suture pattern.

The skin and conical cartilage pieces were fixed in 10% neutral buffered formalin and carnoy's fixative and embedded in paraffin wax. Sections were cut at 5 micron thickness, and stained with haematoxylin and eosin (H & E) and examined.

RESULTS

Twenty four hours after application of vaccination mark in both cows and buffaloes the area was oedematous and hyperemic. However, the reaction was extensive in buffaloe than in cows. Not infrequently application of the vaccination mark was accompanied by some haemorrhage especially in buffaloe Fig. 1, and cows, the most important pathological changes observed after 24 hours were necrosis, oedema

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and hyperemia Fig. 2. The necrotic changes involved both the epithelial covering (epidermis) and the connective tissue of the Dermis. A very mild neutrophil cells reaction was observed in the necrotic areas in cows. However, this reaction was extensive in buffaloes. Oedema and hyperemia were observed in the adjacent tissue. In buffaloes hyperemia and oedema were extensive, moreover, the collagen fibers of the dermis showed hyaline degeneration which was accompanied by severe oedema and neutrophil cells reaction. Not infrequently in buffaloes the necrotic area was surrounded by haemorrhage. Macrophages contain haemosiderine pigments were sometimes seen in the area.

Twenty four hours after application of the insurance mark, the same changes mentioned above were observed both grossly and microscopically. However, microscopic studies in such cases revealed that the necrotic changes sometimes extend to involve the auricular cartilage Fig. 3. The inflammatory reaction was extensive e.g. severe oedema, hyperemia and heavy neutrophil cells infiltration. Haemorrhage in the area was observed in both cow and buffaloes.

Forty eight hours after application of the vaccination mark, grossly the cardinal signs of inflammation was more prominent N.B. Haemorrhage was frequently observed at both sides of the mark which were swollen and oedematous. Microscopically the neutrophil cells infiltration was very extensive than in the above mentioned period Fig. 4. This neutrophil was especially extensive around the blood vessels. The vascular changes were less prominent. The collagen fibers of the dermis showed hyaline necrosis. Few red blood cells were seen in the necrotic area. However, in buffaloes haemorrhage was a constant feature and the area sometimes was infiltrated with massive amount of fibrin and neutrophil cells.

Forty eight hours after application of insurance mark, necrotic changes were associated with extensive suppuration and the adjacent tissue showed prominent oedema and vascular reaction. The necrotic changes and suppuration involved both epidermal epithelium and connective tissue of the dermis. The auricular cartilage was also sometimes involved. In buffaloes at this period the same changes were also observed and was extensive and involved a wide area. At this time the process of healing was not started in both cow and buffaloes.

96 hours after application of both marks in cows and buffaloes the same changes described above (48 hours) were observed and healing process was not yet started.

One week after application of vaccination mark in cow and buffaloes the cardinal sign of inflammation was grossly completely disappeared. A dark red scar was observed adjacent to the mark. Microscopically necrotic changes at this period were still clearly observed. Heavy population of neutrophil cells were constantly observed in the area of necrosis and in vicinity of it. The blood vessels were dilated and filled with granular proteinous substance. Healing of the dermis started at this stage

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by proliferation of a new vascular connective tissue, which was constantly infiltrated with inflammatory cells (neutrophil and mononuclear cells). This inflammatory cells infiltration was more heavy in buffalo than in cow.

One week after application of insurance mark in cows, the cardinal signs of inflammation were completely disappeared. The area has whitish gray colour and raised above the surface.

Microscopically at this stage healing occurs by vascular connective tissue which was very rich in capillary blood vessels. Healing of cartilaginous tissue begins also at this stage. The area of severed cartilage was filled with regularly spaced invasions of capillary buds. Fibroblastic proliferation was also observed in the area of severed cartilage in addition to this immature undifferentiated triangular cells with rounded and hyperchromatic nuclei could be seen. Regeneration of epithelium at this stage was complete Fig. 5.

One week after application of insurance mark in buffalo grossly the area was dark red in colour sometimes blood accumulates in the vicinity of the mark, the edges surrounding the mark were swollen and oedematous.

Microscopically, extensive necrotic changes were observed and involved epidermis, dermis and the cartilaginous tissue. Both the necrotic tissue and the adjacent healthy tissue were infiltrated with a heavy population of neutrophil, macrophages cells and abundant amount of fibrin. In the dermis the healing process was started by proliferation of network of capillary buds and fibroblast cells, this newly vascular connective tissue was infiltrated with neutrophil and macrophages cells Fig. 7.

Two weeks after application of the insurance mark in buffalo the area was of whitish colour and was greatly swollen. Microscopically the picture was similar to those observed in buffalo with vaccination mark in some cases epithelization is complete Fig. 8.

Two weeks after application of the vaccination mark in both cow and buffalo, grossly the area was of whitish colour.

Microscopically, healing of the area by connective tissue which was avascular due to contraction of occlusion of most of its thin walled blood vessels. This connective tissue was mildly infiltrated by neutrophil cells. Injured cartilage at this stage was started healing by highly cellular C.T. Haemorrhage in the area was extensive and the blood vessels in the area were thrombosed and showed organization and recanalization. Abundant amount of bacterial colonies were seen in the area.

Two weeks after application of vaccination mark, grossly the cardinal signs of inflammation were completely disappeared. The area was of whitish gray colour. The edges adjacent to the mark were raised the dark red scap was fallen.

Microscopically in both cows and buffalo at this stage, the picture was nearly the same, remnant of necrotic tissue could be observed. The dermis was healed by connective scar which was very rich in blood vessels in buffalo but was slightly avascular in cows. In both cases the connective tissue was infiltrated by inflammatory neutrophil, which was especially abundant around the blood vessels. Although in some cases in cows the process of epithelialization was complete, however, regeneration of the epithelium in buffalo was incomplete, Fig. 9. In both cows and buffalo the injured cartilage was healed by fibrous connective tissue with formation of chondroblast cells and the area was highly cellular.

Three weeks after application of the insurance mark in both cows and buffaloes, the area was whitish in colour and in cow it was depressed under the surface.

Microscopically in cow the changes were the same as those mentioned before, however, the process of regeneration of the epithelium was complete and the surface was slightly depressed. In buffalo a similar changes were observed but epithelization was incomplete, the inflammatory cellular infiltration was more prominent than in cows.

Four weeks after application of the vaccination mark in cow, the area started to retain its normal colour but it was depressed under the surface and devoid of hair. In buffalo the area was of whitish colour.

Microscopically the changes were similar to those observed in the aforementioned group, however, the regenerated epithelial covering was thin and has no keratin substances Fig. 10. In buffalo epithelization was incomplete and the inflammatory cellular infiltration was still observed around the blood vessels.

Four weeks after application of the insurance mark in cows, grossly the area mildly swollen and of grayish white colour. Microscopically the necrotic tissue still observed in the area which was mild, infiltrated with neutrophil cells and fibrin. Prevascular leucocytic reaction was prominent. The process of healing was not started. Fibrinoid necrosis of the connective tissue of the deeper parts of the dermis was observed.

Four weeks after application of the insurance mark in buffalo, although healing was completed but necrotic tissue still observed (e.g. healing under scab) Fig. 11. The healed area was devoid of a specialized structure.

Six weeks after application of the vaccination mark in cow, the area was nearly of normal colour but the surface was uneven and devoid of hair.

Microscopically, complete healing occurred at this period. But the epithelial covering was very thin and the surface of the scar was uneven and the area was devoid of specialized structure. In buffalo at this period the area was of whitish colour

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and microscopical examination revealed the feature as mentioned above, but sometimes epithelialization was incomplete in minute focal areas, Fig. 11.

Six weeks after application of the vaccination mark in both cow and buffalo, the process of healing was completed by connective scar (dermis) and complete epithelialization (epidermis), Fig. 12.

Eight weeks after application of the vaccination mark grossly, the area was depressed under the surface and was grayish white in colour.

Microscopically epithelialization was complete and the area devoid of specialized structures. Most of the inflammatory cells infiltrating the scar were degenerated. Some of collagen fibers of the scar were compressed and atrophied, Fig. 13.

Eight weeks after application of the insurance mark in cow and buffalo, healing was completed by scar tissue the still. The superficial layer of scar tissue was infiltrated with few inflammatory cells. Reminent of necrotic tissue were also observed, and the area was devoid of specialized structures.

Three months after application of a vaccination mark in both cows and buffalo, grossly the area was depressed under the surface and was of grayish white colour and devoid of hair. Microscopically the scar was contracted and devoid of specialized structure. Regeneration of the epithelium was complete, and there was a mild degree of hyperkeratosis.

Three months after application of insurance mark in buffalo and cows, the area grossly was mildly swollen, yellowish gray in colour and was devoid of hair. Microscopically, small focal areas of necrosis were still observed in both cows and buffalo. Regeneration of the epithelium was complete in both cow and buffalo. The healed scar showed inflammatory cellular infiltration which was abundant in cow and consists of neutrophil cells. But in buffalo the inflammatory cells was perivascular and consists of mononuclear cells infiltration. In cow healed scar contained widely dilated blood vessels. Hyper keratosis was observed in buffalo.

Six months after application of the vaccination mark, grossly the area was depressed under the surface and was devoid of hair.

Microscopically, healing was completed by scar tissue which highly fibrous and less cellular. Regeneration of the epithelium was complete. The regenerated epithelium was devoid of specialized structures.

DISCUSSION

It has been observed that studying of the healing process in the ears of cow and buffalo at a different period after application of insurance and vaccination marks may help to determine the periods at which these marks were applied.

Twenty four up to ninety six hours after application of both marks, haemorrhage, oedema, necrosis, and neutrophil cells infiltration were the most important pathological changes observed at this period. However, the intensity of inflammatory process varies from buffalo (more severe) to cow (mild in nature). Also the reactions accompanied application of the insurance mark was more severe than those changes accompanied the application of the vaccination mark. In the former necrotic changes commonly involved the auricular cartilage, however, in the latter, necrotic changes in most cases did not involve the auricular cartilage. On week after application of both marks, inflammatory changes begin to disappear with the appearance of a vascular connective tissue in the dermis. This connective tissue was highly cellular and infiltrated by abundant amount of neutrophil cells. Haemorrhage disappeared and a macrophage cells with hemosiderin pigment were observed in the area. At this stage we must point to many facts, firstly healing process was more rapid in cow than buffalo. Secondly healing was more rapid in the ears with vaccination mark than those with insurance mark. We must also noted that if infection set up, healing process did not yet started at this period. In such cases the inflammatory reaction including hyperemia, oedema and leucocytic infiltration were more intense or sometimes suppuration could be observed in some cases. The necrotic changes involved both the entire epithelium of the epidermis, the collagen fibers of the dermis and sometimes the auricular cartilage. Colonies of bacteria could be also observed in such cases.

Two weeks post application of both marks, the inflammatory process was more or less completely disappeared. The vascular connective tissue become less cellular and less vascular and was mildly infiltrated with neutrophil cells. Remnant of necrotic tissue was still observed on the superficial surface of the area. Regeneration of the epithelium of the epidermis was incomplete in buffalo. However, it was complete in some cases of cows at this stage. Healing process was more advanced in cows than in buffalo.

Three weeks after application of both vaccination and insurance marks in cows and buffaloes, the histopathological picture was nearly the same as the preceeding stage except that the process of epithelization was complete in cows with insurance mark. However, it was incomplete in buffalo and cow with vaccination mark and buffalo with insurance mark. Moreover, the healed vascular connective tissue become avascular due to contraction of its thin walled blood vessels. The number of neutrophil cells infiltrating the connective tissue was considerably decreased. Healing of the

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injured auricular cartilage begin at this stage by proliferation of fibroblast and chondroblast cells from the periconderium (JUNQUEIRA and CORNEIRO, 1980 and BAYOUMI, et al. 1984).

Four weeks after application of the two types of marks in cow, the area began to retain its normal colour and although epithelization was complete but the surface epithelium was thin and uneven. Keratinization was absent and the area devoid of a specialized structure. In buffaloe at this stage epithelization was incomplete and the inflammatory cellular reaction was still observed around the blood vesseles.

At this stage in one ear of cow with insurance mark, the area was mildly swollen and the necrotic changes were extensive. The area was infiltrated with neutrophil cells and fibrin, prevascular leucocytic reaction was prominent. The process of healing was not started yet. This delayed healing in our openion might be due to secondary infections during application of insurance mark (it is bigger than vaccination mark). This openion was based on the fact that bacterial colonies were observed in the tissue in such cases. Healing under scab was observed in buffaloe (with insurance mark) at this stage with complete epithelization.

Six weeks after application of the vaccination mark in both cow and buffaloe the process of healing was complete in both the epidermis and dermis. However, the surface was uneven and of whitish colour. The area was completely devoid of specialized structures. Defect in the process of epithelization was seen in minute focal areas.

Six weeks after application of insurance mark in both cattle and buffaloe the process of healing was complete in both dermis and epidermis with complete absence of specialized structures.

Eight weeks after application of vaccination and insurance marks in both cow and buffaloe, contraction of scar tissue lead to depression of the area under the surface. In one ear of a cow with insurance mark, the healing was considerably delayed and this in our openion was due to secondary infection during application of both marks in cow and buffaloe, the healed scar tissue was severly contracted and the area was depressed under surface and devoid of specialized structures. Delayed healing observed in some cows and buffaloe with insurance mark at this stage was due to secondary infection mentioned before.

From the result of the present study we can conclud that the sequence of changes occuring in the healing process in the ears of cow and buffaloe after application of the plastic marks might be of great help in determining the time at which the marks were applied. Consequently it is of great help in discovering the adultration caused by some owners as, they substitutes non vaccinated or non insured dead animal instead of vaccinated or insured one for obtaining the substituant value of insurance.

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